Chapter 10

Treatment of growth hormone excess in dogs with the progesterone receptor antagonist aglépristone

S.F.M. Bhatti[1], L. Duchateau[2], A.C. Okkens[3], L.M.L. Van Ham[1],
J.A. Mol[3], H.S. Kooistra[3]

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[1]Department of Small Animal Medicine and Clinical Biology,
[2]Department of Physiology and Biometrics,
Faculty of Veterinary Medicine,Ghent University, Merelbeke, Belgium
[3]Department of Clinical Sciences of Companion Animals,
Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands

Abstract

Acromegaly or hypersomatotropism in dogs is almost always due to progestin-induced hypersecretion of growth hormone (GH) originating from the mammary gland. The aim of this study was to investigate whether aglépristone, a progesterone receptor antagonist, can be used to treat this form of canine acromegaly. In five Beagle bitches hypersomatotropism was induced by administration of medroxyprogesterone acetate (MPA) for over 1 year. Subsequently, aglépristone was administered. Blood samples were collected before MPA administration, and immediately before, during, and 3.5 and 5.5 weeks after the last administration of aglépristone for determination of the plasma concentrations of GH and insulin-like growth factor-I (IGF-I). In addition, blood samples for the determination of the 6-h plasma profile of GH were collected before MPA administration, before aglépristone administration and 1 week after the last aglépristone treatment.

Administration of MPA resulted in a significant increase of the mean plasma IGF-I concentration, whereas analysis of the pulsatile plasma profile demonstrated a trend ($P = 0.06$) for a higher mean basal plasma GH concentration and a higher mean area under the curve above the zero-level (AUC_0) for GH. Treatment with aglépristone resulted in a significant decrease of the mean plasma GH and IGF-I concentrations. Analysis of the pulsatile plasma profile showed a trend ($P = 0.06$) for a lower mean basal plasma GH concentration and a lower mean AUC_0 for GH 1 week after the last aglépristone treatment compared with these values before aglépristone administration. Three and a half weeks and 5.5 weeks after the last aglépristone administration the mean plasma IGF-I concentration increased again.

In conclusion, aglépristone can be used successfully to treat dogs with progestin-induced hypersomatotropism.

Introduction

Acromegaly is characterized by bony and soft tissue overgrowth due to excessive growth hormone (GH) secretion. The syndrome is known to occur in humans, dogs, and cats. However, the pathogenesis differs among these species. Acromegaly in humans and cats is commonly caused by a somatotroph adenoma of the pituitary gland (Rijnberk et al., 2003) whereas in dogs the GH excess usually originates from an extra-pituitary site (Selman et al., 1994). In dogs, endogenous progesterone secreted during the luteal phase of the oestrous cycle or exogenous progestins such as medroxyprogesterone acetate (MPA) used for oestrus prevention may promote hypersecretion of GH from foci of hyperplastic ductular epithelium of the mammary gland (Eigenmann et al., 1983; Selman et al., 1994; van Garderen et al., 1997). In contrast to the pulsatile secretion pattern of GH in healthy dogs (Takahashi et al., 1981; French et al., 1987, Kooistra et al., 2000), the plasma GH profile in bitches with progestin-induced acromegaly is not pulsatile (Watson et al., 1987). In addition, progestin-induced GH hypersecretion cannot be stimulated with GH-releasing hormone (GHRH) and α-adrenergic agonists, nor can it be inhibited by somatostatin (Watson et al., 1987; Selman et al., 1991). The progestin-induced increase in plasma GH concentrations are associated with increased plasma concentrations of insulin-like growth factor-I (IGF-I) (Selman et al., 1994).

The physical changes of progestin-related hypersomatotropism in dogs tend to develop gradually and consist of prominent skin folds, abdominal distension, and widening of the interdental spaces (Rijnberk, 1996). Due to the insulin-antagonistic action of GH, hyperglycaemia and eventually diabetes mellitus may occur (Eigenmann, 1983a). Ovariectomy is the treatment of choice in female dogs with spontaneous progesterone-induced acromegaly. Plasma GH concentrations rapidly return to normal after ovariohysterectomy (Eigenmann and Venker-van Haagen, 1981). However, in dogs with acromegaly due to progestin administration the detrimental effects of the depot-progestins may continue for a long time after cessation of administration (Eigenmann, 1983b, Rijnberk, 1996).

Progesterone receptor blockers such as aglépristone (RU 46534) and mifepristone (RU 38486) are competitive antagonists of the progesterone receptor (Cadepond et al., 1997; Van Look and Bygdeman, 1989). Aglépristone is the first progesterone receptor blocker licensed for veterinary use and has been used efficiently to terminate pregnancy (Galac et al., 2000; Fieni et al., 2001) and to induce parturition (Baan et al., 2005). Furthermore, it is successfully used for the treatment of fibroadenomatous mammary hyperplasia in cats (Wehrend et al.,

2001; Görlinger et al., 2002; Meisl et al., 2003) and may be a useful adjunct in the medical treatment of endometritis and pyometra in the dog (Trash et al., 2003).

The presence of progesterone receptors in mammary gland tissue of dogs (Lantinga-van Leeuwen et al., 2000) allows for a targeted endocrine therapy with progesterone receptor blockers in dogs with progestin-induced hypersomatotropism. The aim of this study was therefore to investigate whether the progesterone receptor antagonist aglépristone can be used to treat canine acromegaly.

Materials and methods

Dogs

Five intact Beagle bitches were housed with outdoor access, fed on a commercial dog food once a day, and given water *ad libitum*. The ages and body weights of the dogs ranged from 3 to 9 years (mean 5 years) and 9.0 to 10.3 kg (mean 9.5 kg), respectively. The dogs were accustomed to the laboratory environment and procedures such as collection of blood samples.

Treatments

The five Beagle bitches were treated with the synthetic progestin depot preparation Depo-Promone® (medroxyprogesterone acetate (MPA), Pharmacia Animal Health, Puurs, Belgium). MPA treatment was started during anoestrus and consisted of subcutaneous injections in a dosage of 10 mg/kg body weight at 4-week intervals for a total of 14 (3 dogs) or 15 (2 dogs) injections.

Five days (= day 0) and six days (= day 1) after the last MPA injection (= day -5), aglépristone (Alizin®, Virbac Animal Health, Barneveld, The Netherlands) was administered subcutaneously in a dosage of 10 mg/kg body weight. One (= day 8), two (= day 15), and three (= day 22) weeks later a single aglépristone injection was given in the same dose. Three randomly chosen dogs received the first aglépristone injection after the 14th MPA injection, and the other two dogs after the 15th MPA injection so that these two dogs could serve as control dogs for the three dogs that received the aglépristone injection first.

Blood sample collection

Blood samples for determination of the plasma progesterone concentrations were collected 5 and 12 months after the start of the MPA treatment.

Blood samples for determination of the plasma concentrations of GH and IGF-I were collected before MPA treatment, at days -9, -8, -7, -5, -3, -2, -1, 0 (= immediately before aglépristone treatment and after MPA treatment for over 1 year), at days 1, 3, 5, 7, 8, 11, 13, 15, 18, 20, 22, 25 (= during aglépristone treatment), and at days 46 and 60 (= 3.5 and 5.5 weeks after the last aglépristone injection). On days of treatment (MPA or aglépristone), blood samples were collected prior to the drug administration.

Blood samples for determination of the pulsatile plasma profiles of GH were collected at 15-min intervals between 0800h and 1400h before MPA administration, before aglépristone administration, and 1 week after the last administration of aglépristone (at day 28).

All blood samples were collected by jugular venipuncture after an overnight fast, immediately transferred to ice-chilled EDTA-coated tubes and centrifuged at 4^{o} C for 10 min. Plasma was stored at -25^{o} C until assayed.

Hormone determination

Plasma progesterone concentrations were determined with a previously validated radioimmunoassay (RIA) (Henry et al., 1987). The intra-assay and interassay coefficients of variation were 8.8 % and 7.1 %, respectively. The sensitivity of the assay was 0.005 ng.

Plasma GH concentrations were measured using a commercially available RIA for porcine and canine GH (PGH-46HK; Linco Research, St. Charles MS). The intra-assay coefficient of variation was 7.6 % at a plasma concentration of 4.4 µg/l. The sensitivity of the assay was 1 µg/l.

Total plasma IGF-I concentrations were measured after acid-ethanol extraction to remove interfering IGF binding proteins. Plasma IGF was extracted using a mixture of 87.5 % (v/v) ethanol and 12.5 % 2 M formic acid. Tubes containing 100 µl plasma and 400 µl of the ethanol-formic acid mixture were mixed thoroughly and incubated for 30 min at room temperature. After centrifugation for 30 min at 5500 g at 4° C, a 50 µl aliquot of the supernatant was diluted 1:50 with assay buffer containing 63 mM Na_2HPO_4 (pH 7.4), 13 mM Na_2EDTA, and 0.25 % (w/v) BSA. The extraction efficiency amounted to 92.5 ± 5.7 %. Plasma IGF-I concentrations were measured in a heterologous RIA validated for the dog (Favier et al., 2001). The intra-assay coefficient of variation was 8.6 % at a plasma concentration of 100 µg/l. The sensitivity of the assay was 10 µg/l. IGF-I antiserum AFP4892898 and human IGF-I for iodination were obtained from the National Hormone and Peptide Programme (Harbor-UCLA Medical Center, Torrance CA).

Statistical analysis

To study the effect of MPA administration, the plasma GH and IGF-I concentrations before and after MPA treatment were compared using a mixed model with dog as random effect and period (2 levels: before and after MPA treatment) as categorical fixed effect.

In order to assess the overall effect of aglépristone on the plasma GH and IGF-I concentrations, a mixed model was fitted with dog as random effect and period (3 levels: immediately before aglépristone, during aglépristone, and 3.5 and 5.5 weeks after the last aglépristone treatment) as categorical fixed effect. The three periods were compared pairwise using Tukey's multiple comparisons technique.

To study the evolution of the GH and IGF-I concentrations during the aglépristone period, a mixed model was fitted with dog as random effect and time since start of aglépristone treatment as continuous fixed effect at a global significance level of 5 %.

The plasma GH and IGF-I concentrations before MPA treatment were compared with the concentrations 3 days after the last aglépristone injection (i.e. at day 25) using a mixed model with dog as random effect and period (2 levels: before MPA treatment and 3 days after the last aglépristone injection) as categorical fixed effect.

To evaluate the effect of withdrawal of aglépristone treatment, the two last measurements during aglépristone treatment (days 22 and 25) were compared with the two measurements after aglépristone treatment (days 46 and 60) using a mixed model with dog as random effect and period (2 levels: days 22 and 25 and days 46 and 60) as categorical fixed effect.

The 6-h plasma profiles of GH were analyzed by means of the Pulsar programme developed by Merriam and Wachter (1982). The programme identifies secretory peaks by height and duration from a smoothed baseline, using the assay standard deviation (SD) as a scale factor. The cut-off parameters G1-G5 of the Pulsar programme were set at 3.98, 2.40, 1.68, 1.24, and 0.93 times the assay SD as criteria for accepting peaks 1, 2, 3, 4, and 5 points wide, respectively. The smoothing time, a window used to calculate a running mean value omitting peaks, was set at 5h. The splitting cut-off parameter was set at 0.5 and the weight assigned to peaks was 0.05. The A-, B-, and C- values of the Pulsar programme, used to calculate the variance of the assay, were set at A=0, B=7.2, and C=5. The values extracted from the Pulsar analysis included the mean of the smoothed baseline, the pulse frequency, and the area under the curve (AUC). The AUC was calculated above the zero-level (AUC_0) as well as above the baseline (AUC_{base}). The difference in variables before MPA treatment,

before aglépristone administration, and 1 week after the last aglépristone injection (i.e. at day 28), were analyzed by the signed rank test with dog as block.

All values are expressed as mean ± SEM or median. Statistical significance was defined at $P \leq 0.05$. Analyses were performed with SAS version 9.1 for Windows (Insightful Corp., Seattle, US).

Ethics of the study

This study was approved by the Ethics Committee of the Faculty of Veterinary Medicine, Ghent University.

Results

During MPA administration none of the dogs showed signs of oestrus and the mean plasma progesterone concentration was low 5 months (0.2 ± 0.2 ng/l) and 12 months (0.2 ± 0.1 ng/l) after the start of the MPA treatment. The mean body weight of the dogs on the day of the last injection of MPA (12.4 ± 0.7 kg) was significantly higher ($P < 0.02$) than that on the day of the first injection of MPA (9.5 ± 0.3 kg) (paired Student's t-test). Signs of acromegaly became apparent in three of the five dogs after 6 months of MPA treatment and consisted of prominent skin folds especially on the head, an increase in the interdental spaces, inspiratory stridor, and snoring.

MPA administration for over 1 year resulted in a higher mean plasma GH concentration (2.3 ± 0.5 µg/l) compared to that before MPA treatment (1.9 ± 0.3 µg/l), although this difference did not reach statistical significance (Figure 1a). However, the mean plasma IGF-I concentration after 1 year of MPA administration (146 ± 25 µg/l) was significantly ($P = 0.003$) higher compared to that before MPA treatment (36 ± 6 µg/l) (Figure 1b).

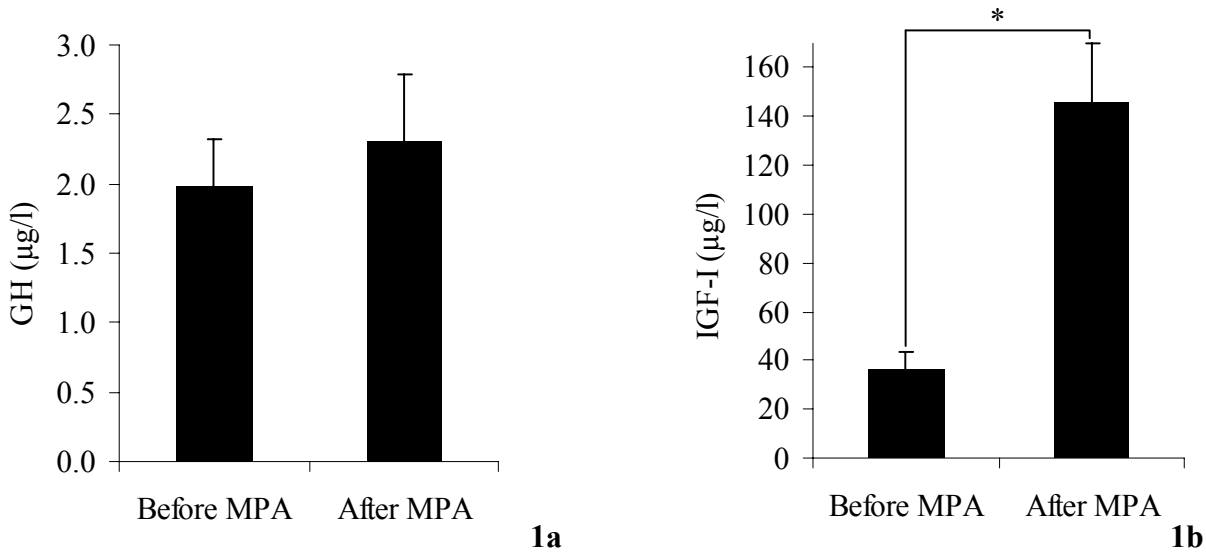

Figure 1. Mean (+ SEM) plasma concentrations of GH **(1a)** and IGF-I **(1b)** in 5 Beagle dogs before administration of medroxyprogesterone acetate (before MPA) and after 1 year treatment with MPA (after MPA). Significant differences between periods are indicated with an asterisk.

Analysis of the pulsatile plasma GH profiles after 1 year of MPA administration revealed a trend (P = 0.06) for a higher mean basal plasma GH concentration and a higher mean AUC_0 for GH compared to these values before MPA treatment (Table 1 and Figure 2).

Table 1. Area under the curve above the baseline (AUC_{base}) for GH (µg/lx6h), AUC above the zero-level (AUC_0) for GH (µg/lx6h), basal plasma GH concentration (µg/l), and GH pulse frequency (peaks per 6h) in 5 Beagle dogs before MPA administration (before MPA), after 1 year of MPA administration (= before aglépristone administration) (before A), and 1 week after the last aglépristone administration (after A).

	AUC_{base} (mean ± SEM)	AUC_0 (mean ± SEM)	GH pulse frequency (median)	Basal GH (mean ± SEM)
Before MPA	1 ± 0.4	11.3 ± 1.1	1	1.7 ± 0.1
Before A	0 ± 0.0	17.0 ± 3.6	0	2.8 ± 0.6
After A	0.3 ± 0.2	8.8 ± 0.7	0	1.4 ± 0.1

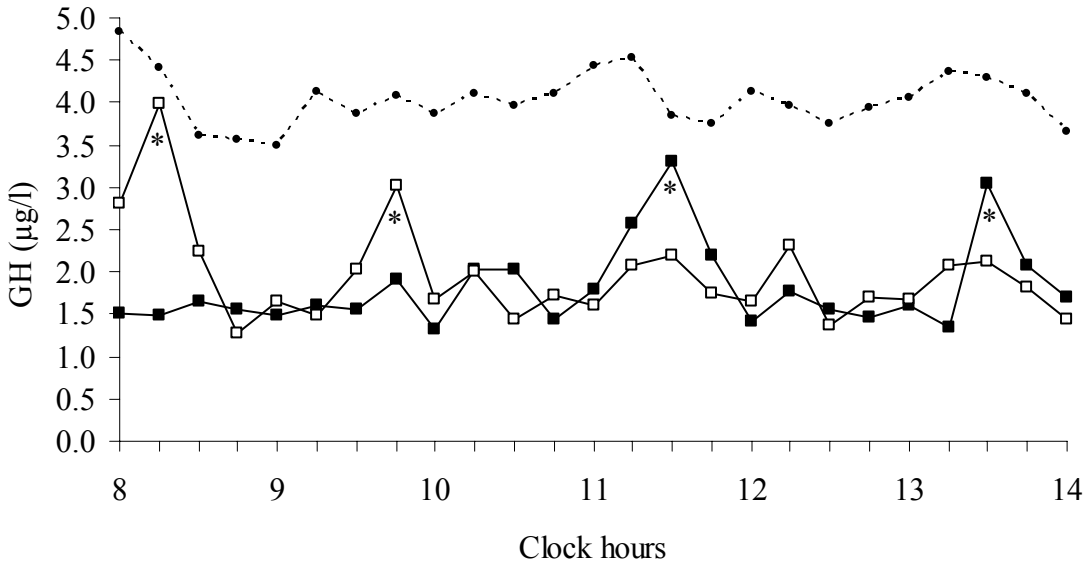

Figure 2. The plasma profiles of GH in a 4-year-old Beagle bitch. Blood samples were collected at 15-min intervals between 0800h and 1400h, before MPA administration (□), after 1 year of MPA administration (= before aglépristone administration) (dotted line), and 1 week after the last aglépristone treatment (■). Significant pulses, calculated by the Pulsar programme, are indicated by an asterisk.

The administration of aglépristone caused no side effects except a short-term skin irritation at the site of the injection in one dog. The mean plasma GH concentration immediately before aglépristone administration (2.3 ± 0.5 µg/l) was significantly higher than that during (1.7 ± 0.3 µg/l; $P < 0.0001$) and 3.5 and 5.5 weeks after (1.8 ± 0.3 µg/l; $P = 0.018$) the last administration of aglépristone (Figure 3a). Also the mean plasma IGF-I concentration immediately before aglépristone administration (146 ± 25 µg/l) was significantly higher than that during (108 ± 27 µg/l; $P < 0.0001$) administration of aglépristone (Figure 3b). In the weeks that aglépristone was administered, analysis of the course of the circulating hormone concentrations indicated a significant decrease in plasma GH ($P = 0.005$) and IGF-I ($P < 0.0001$) concentrations (Figures 4a and b, respectively).

The plasma GH and IGF-I concentrations before MPA treatment did not differ significantly from these concentrations 3 days after the last aglépristone injection (i.e. day 25).

Analysis of the pulsatile plasma GH profiles revealed a trend ($P = 0.06$) for a lower mean basal plasma GH concentration and a lower mean AUC_0 for GH 1 week after the last aglépristone administration (i.e. day 28) compared with these concentrations before aglépristone treatment. The AUC_{base} for GH increased again 1 week after the last aglépristone

injection compared with this concentration before aglépristone administration, although this difference did not reveal statistical significance (Table 1 and Figure 2).

The mean plasma GH concentration at the end of the period of aglépristone administration (i.e. days 22 and 25) (1.5 ± 0.1 µg/l) did not differ significantly from that at 3.5 and 5.5 weeks (i.e. days 46 and 60) after withdrawal of aglépristone (1.8 ± 0.3 µg/l). However, the mean plasma IGF-I concentration at the end of the period of aglépristone administration (88 ± 22 µg/l) was significantly ($P < 0.0001$) lower compared with that 3.5 and 5.5 weeks after withdrawal of aglépristone (124 ± 29 µg/l).

In the two control dogs that did not receive aglépristone after the 14th MPA injection, the mean plasma concentrations of GH and IGF-I before the 14th administration of MPA (1.6 ± 0.1 µg/l and 105 ± 28 µg/l, respectively) were not different from those before the 15th administration of MPA (1.7 ± 0.1 µg/l and 116 ± 26 µg/l, respectively).

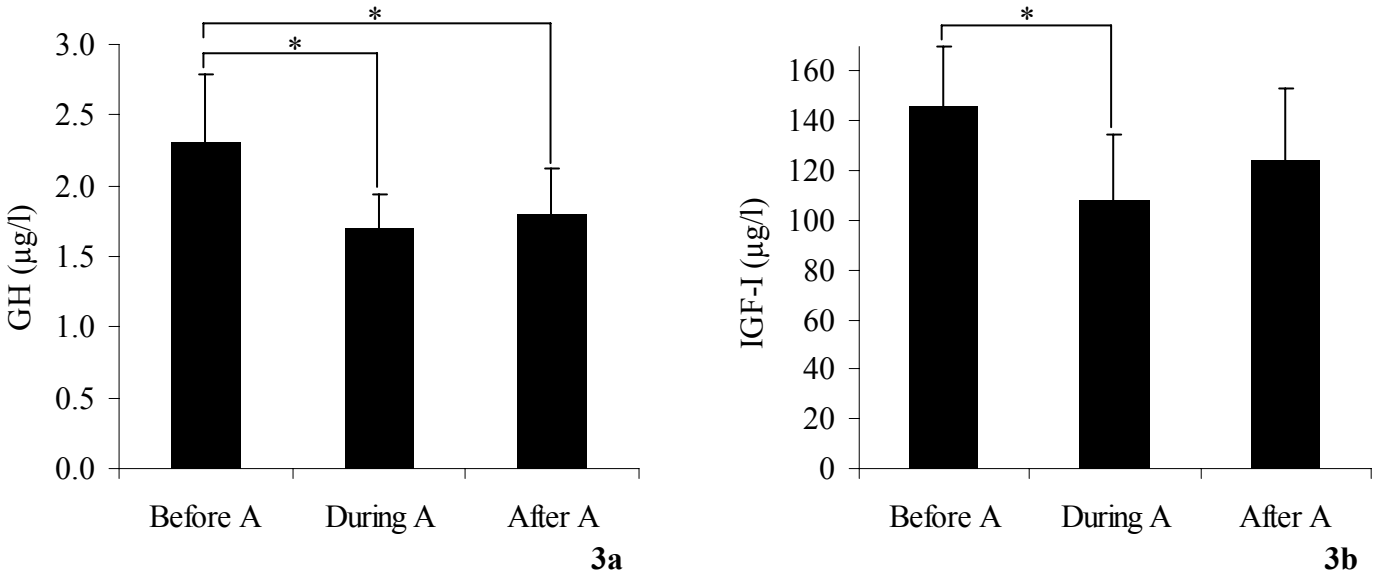

Figure 3. Mean (+ SEM) plasma concentrations of GH **(3a)** and IGF-I **(3b)** in 5 Beagle dogs immediately before aglépristone (before A), during aglépristone (during A), and 3.5 and 5.5 weeks after the last aglépristone administration (after A). Significant differences between periods are indicated with an asterisk.

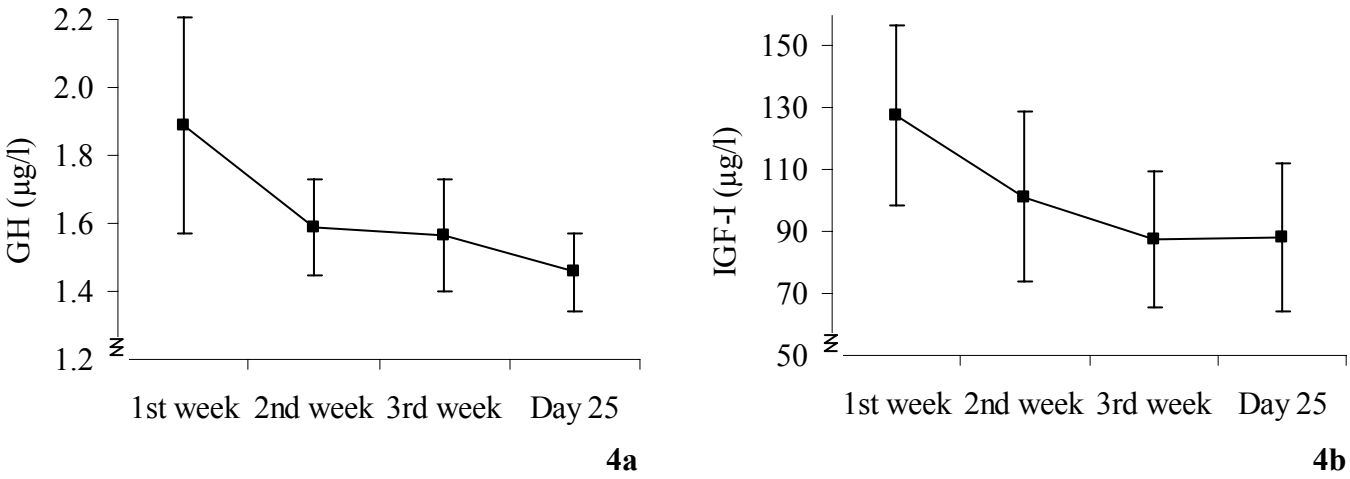

Figure 4. Mean (± SEM) plasma concentrations of GH **(4a)** and IGF-I **(4b)** in 5 Beagle dogs at 1 week, 2 weeks, 3 weeks, and at day 25 during aglépristone administration.

Discussion

In three of the five Beagle dogs, signs of acromegaly became apparent after 6 months of MPA administration. In line with these changes, the mean plasma IGF-I concentrations were raised. Moreover, analysis of the pulsatile plasma profile showed a trend for a higher mean basal GH concentration and a higher mean AUC_0 for GH in the five Beagle dogs. These findings are consistent with progestin-induced hypersecretion of GH (Concannon et al., 1980; Rijnberk et al., 1980; Eigenmann and Rijnberk, 1981; Eigenmann et al., 1983).

Previous studies identified foci of hyperplastic ductular epithelium of the mammary gland as the site of origin of GH excess induced by progestins (Selman et al., 1994; van Garderen et al., 1997). The expression of the gene encoding GH has been demonstrated in canine mammary gland tissue, and sequence analysis has revealed that this gene is identical to the pituitary GH gene (Mol et al., 1995; Mol et al., 1996). Immunohistochemical studies have demonstrated the intracellular co-localization of both the progesterone receptor and GH in progestin-exposed, hyperplastic canine mammary epithelial tissue, whereas immunoreactive GH could not be demonstrated in progesterone receptor-negative epithelial cells (Lantinga-van Leeuwen et al., 2000). These observations are consistent with the central role of progestins in GH gene expression in the canine mammary gland and allow for a targeted endocrine therapy with progesterone receptor blockers in dogs with progestin-induced mammary-derived GH hypersecretion.

To the authors' knowledge, treatment of acromegalic dogs with the progesterone receptor antagonist aglépristone (RU 46534) has not been reported before. The results of the present study demonstrate that progestin-induced elevations in circulating GH and IGF-I concentrations decrease significantly during treatment with aglépristone. At the end of the aglépristone treatment period the plasma GH and IGF-I concentrations did not differ significantly from those before MPA administration. Our findings are in agreement with those of Watson et al. (1987) who found that administration of the antiprogestin mifepristone (RU 38486) resulted in a decrease of plasma GH concentrations and normalization of plasma IGF-I concentrations in bitches with progestin-induced acromegaly.

The mean basal plasma concentrations of GH and IGF-I in the two control dogs that did not receive aglépristone after the 14th MPA injection remained high and did not decrease. This indicates that indeed aglépristone is responsible for the lowering of the plasma GH and IGF-I concentrations in the dogs treated with the progesterone receptor blocker, and that this lowering is not due to, for example, a waning effect of MPA on GH and IGF-I secretion.

The 6-h pulsatile plasma profile of GH represents a more sensitive way of analyzing the effects of different treatments on the secretion of GH than the plasma GH concentration itself. Analysis of the plasma GH profiles revealed that the mean basal plasma GH concentration and AUC_0 for GH tended to decrease at the end of the treatment with the progesterone receptor blocker compared with these values before aglépristone administration. In addition, the AUC_{base} for GH, i.e., the amount of GH secreted in pulses, increased again during aglépristone treatment, although this difference did not reveal statistical significance. Thus, treatment with aglépristone resulted in partial restoration of the normal pulsatile GH secretion. Higher dosages of aglépristone may result in complete normalization of the secretion pattern of GH.

Plasma IGF-I concentrations are generally regarded as more sensitive indicators of the GH status than plasma GH concentrations (Clemmons and Strasburger, 2004). Consequently, the significantly higher plasma IGF-I concentrations at days 46 and 60 compared with those at days 22 and 25 therefore suggest increased GH exposure, despite the fact that analysis of the plasma GH concentrations did not reveal a significant increase. The recurrence of IGF-I hypersecretion after withdrawal of aglépristone treatment is not surprising as all dogs received injections of a depot progestin preparation for a period of 1 year, and the progestin effect of this depot preparation is much longer than the duration of aglépristone treatment in the present study. Similarly, in a cat with fibroadenomatous mammary hyperplasia due to treatment with a depot progestin preparation hyperplasia recurred 8 days after discontinuation

of aglépristone administration (Wehrend et al., 2001). This indicates that treatment with an antiprogestin is required as long as the action of the synthetic progestin is present. Also in our 3 dogs with acromegalic signs, no physical changes were visible during or after treatment with aglépristone.

Due to the insulin-antagonistic action of GH, progestin-induced hypersecretion of GH may also result in hyperglycaemia and eventually manifest diabetes mellitus may ensue (Eigenmann and Rijnberk, 1981; Eigenmann, 1983a). Disappearance of these catabolic abnormalities depends on the functional status of the pancreatic β-cells. If an adequate population of functional β-cells is present at the time the progestin effect is blocked, hyperinsulinemia, carbohydrate intolerance, and hyperglycaemia may be reversible after correction of the hypersomatotropism (Eigenmann, 1983b). If the population of functional β-cells is severely decreased, permanent diabetes mellitus can be anticipated. Because the effects of depot progestins may persist for several months, prevention or reversal of the catabolic effects of progestin-induced GH excess is especially important when, in case of hyperglycaemia, the depot progestin has been administered only recently. The results of the present study illustrate that in these cases aglépristone offers an effective treatment option.

In conclusion, administration of aglépristone significantly decreases plasma GH and IGF-I concentrations in dogs with progestin-induced hypersomatotropism.

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References

Baan M, Taverne MA, Kooistra HS, de Gier J, Dieleman SJ, Okkens AC. Induction of parturition in the bitch with the progesterone-receptor blocker aglepristone. Theriogenology 2005;63:1958-1972.

Cadepond F, Ulmann A, Baulieu EE. RU486 (mifepristone): mechanisms of action and clinical uses. Annu Rev Med 1997;48:129-156.

Clemmons DR and Strasburger C. Monitoring the response to treatment in acromegaly. J Clin Endocrinol Metab 2004;89:5289-5291.

Concannon PW, Altszuler N, Hampshire J, Butler WR, Hansel W. Growth homone, prolactin, and cortisol in dogs developing mammary nodules and an acromegaly-like appearance during treatment with medroxyprogesterone acetate. Endocrinology 1980;106:1173-1177.

Eigenmann JE and Rijnberk A. Influence of medroxyprogesterone acetate (Provera) on plasma growth hormone levels and on carbohydrate metabolism. I. Studies in the ovariohysterectomized bitch. Acta Endocrinol (Copenh) 1981;98:599-602.

Eigenmann JE and Venker-van Haagen AJ. Progestagen-induced and spontaneous canine acromegaly due to reversible growth hormone overproduction: clinical picture and pathogenesis. J Am Anim Hosp Assoc 1981;17:813.

Eigenmann JE. Diabetes mellitus in the bitch. 1. Pathogenesis and clinical picture Tierarztl Prax 1983a;11:361-368.

Eigenmann JE. Diabetes mellitus in the bitch. 2. Therapy Tierarztl Prax 1983b;11:529-536.

Eigenmann JE, Eigenmann RY, Rijnberk A, van der Gaag I, Zapf J, Froesch ER. Progesterone-controlled growth hormone overproduction and naturally occurring canine diabetes and acromegaly. Acta Endocrinol (Copenh) 1983;104:167-176.

Favier RP, Mol JA, Kooistra HS, Rijnberk A. Large body size in the dog is associated with transient growth hormone excess at a young age. J Endocrinol 2001;170: 479-484.

Fieni F, Martal J, Marnet PG, Siliart B, Bernard F, Riou M, Bruyas JF, Tainturier D. Hormonal variation after early or mid-pregnancy termination in bitches with aglépristone (RU534). J Reprod Fertil Suppl 2001;57:243-248.

French MB, Vaitkus P, Cukerman E, Sirek A, Sirek OV. Secretory pattern of canine growth hormone. Am J Physiol 1987;252:268-272.

Galac S, Kooistra HS, Butinar J, Bevers MM, Dieleman SJ, Voorhout G, Okkens AC. Termination of mid-gestation pregnancy in bitches with aglepristone, a progesterone receptor antagonist. Theriogenology 2000;53:941-950.

Görlinger S, Kooistra HS, van den Broek A, Okkens AC. Treatment of fibroadenomatous hyperplasia in cats with aglepristone. J Vet Intern Med 2002;16:710-713.

Henry M, Figueiredo AE, Palhares MS, Coryn M. Clinical aspects of the oestrous cycle in donkeys (Equus asinus). J Reprod Fertil Suppl 1987;35:297-303.

Kooistra HS, den Hertog E, Okkens AC, Mol JA, Rijnberk A. Pulsatile secretion pattern of growth hormone during the luteal phase and mid-anoestrus in beagle bitches. J Reprod Fertil 2000;119:217-222.

Lantinga-van Leeuwen IS, van Garderen E, Rutteman GR, Mol JA. Cloning and cellular localization of the canine progesterone receptor: co-localization with growth hormone in the mammary gland. J Steroid Biochem Mol Biol 2000;75:219-228.

Meisl D, Hubler M, Arnold S. Treatment of fibroepithelial hyperplasia (FEH) of the mammary gland in the cat with the progesterone antagonist Aglepristone (Alizine®). Schweiz Arch Tierheilkd. 2003;145:130-136.

Merriam GR and Wachter KW. Algorithms for the study of episodic hormone secretion. Am J Physiol 1982;243:310-318.

Mol JA, van Garderen E, Selman PJ, Wolfswinkel J, Rijinberk A, Rutteman GR. Growth hormone mRNA in mammary gland tumors of dogs and cats. J Clin Invest 1995;95:2028-2034.

Mol JA, van Garderen E, Rutteman GR, Rijnberk A. New insights in the molecular mechanism of progestin-induced proliferation of mammary epithelium: induction of the local biosynthesis of growth hormone (GH) in the mammary glands of dogs, cats and humans. J Steroid Biochem Mol Biol 1996;57:67-71.

Rijnberk A, Eigenmann JE, Belshaw BE, Hampshire J, Altszuler N. Acromegaly associated with transient overproduction of growth hormone in a dog. J Am Vet Med Assoc 1980;177:534-537.

Rijnberk A (1996). Growth hormone excess. In: Clinical Endocrinology of dogs and cats, pp 22-26. Ed. A. Rijnberk. Kluwer Academic Publishers, Dordrecht/Boston.

Rijnberk A, Kooistra HS, Mol JA. Endocrine diseases in dogs and cats: similarities and differences with endocrine diseases in humans. GH & IGF Res 2003;13:158-164.

Selman PJ, Mol JA, Rutteman GR, Rijnberk A. Progestins and growth hormone excess in the dog. Acta Endocrinol (Copenh) 1991;125 Suppl 1:42-47.

Selman PJ, Mol JA, Rutteman GR, van Garderen E, Rijnberk A. Progestin-induced growth hormone excess in the dog originates in the mammary gland. Endocrinology 1994;134:287-292.

Takahashi Y, Ebihara S, Nakamura Y, Takahashi K. A model of human sleep-related growth hormone secretion in dogs: effects of 3, 6, and 12 hours of forced wakefulness on plasma growth hormone, cortisol, and sleep stages. Endocrinology 1981;109:262-272.

Trash K, Wehrend A, Bostedt H. Follow-up examinations of bitches after conservative treatment of pyometra with the antigestagen aglépristone. J Vet Med 2003;50:375-379.

van Garderen E, de Wit M, Voorhout WF, Rutteman GR, Mol JA, Nederbragt H, Misdorp W. Expression of growth hormone in canine mammary tissue and mammary tumors. Evidence for a potential autocrine/paracrine stimulatory loop. Am J Pathol 1997;150:1037-1047.

Van Look PF and Bygdeman M. Antiprogestational steroids: a new dimension in human fertility regulation. Oxf Rev Reprod Biol 1989;11:2-60.

Watson ADJ, Rutteman GR, Rijnberk A, Mol JA. Effect of somatostatin analogue SMS 201-995 and antiprogestin agent RU 486 in canine acromegaly. Front Horm Res 1987;17:193-198.

Wehrend A, Hospes R, Gruber AD. Treatment of feline mammary fibroadenomatous hyperplasia with a progesterone-antagonist. Vet Rec 2001;148:346-347.