

Original Article

Use of aglepristone for the treatment of P₄ induced insulin resistance in dogs

Enrico Bigliardi*, Carla Bresciani, Daniela Callegari, Francesco Di Ianni, Giorgio Morini, Enrico Parmigiani, Ezio Bianchi

Department of Veterinary Science, University of Parma, 43100 Parma, Italy

Insulin resistance (IR) in dogs is suspected when hyperglycemia is present despite administration of insulin doses greater than 1.0 to 1.5 UI/kg. IR is caused by increases in counter regulatory hormones concentrations (glucagon, glucocorticoids, catecholamines and growth hormone). This study was conducted to investigate the use of aglepristone (RU 46534), a P₄ receptor antagonist, for the treatment of IR diabetes mellitus in bitches during the luteal phase. All animals were treated with porcine insulin zinc suspension (Caninsulin) and aglepristone (Alizin) 10 mg/kg subcutaneously at day 1, 2, 9 and 17 from diagnosis. At day 5, no significant variation in glycemia was shown. At day 12 and 20, serum glucose concentrations were significant lower ($p < 0.05$). From day 12 the insulin dose was reduced to 0.8 IU BID. Insulin was reduced in the following weeks and glycemia was controlled.

Keywords: aglepristone, bitches, diabetes mellitus, diestrus, insulin resistance

Introduction

Diabetes mellitus is a common metabolic disorder in canines. Affected subjects are usually more than 5 years of age, and there is a predisposition in females [9]. In dogs, insulin resistance (IR) is suspected when hyperglycemia is still detected despite administration of insulin doses greater than 1.0 to 1.5 IU/kg [5]. Definitions of resistance to exogenously administered insulin vary, but all are based on the dose insulin administered and the resultant blood glucose concentrations [6]. IR is caused by an increase in circulating counter regulatory hormones (glucagon, glucocorticoids, catecholamines and growth hormone). These stress-related diabetogenic hormones increase as a

result of concurrent diseases, endocrine disorders or exogenous administration. Progesterone (P₄) also induces IR by stimulating growth hormone (GH) production in mammary glands [4,6]. Increases in circulating P₄ concentration are associated with exogenous administration and physiologic conditions such as diestrus and pregnancy. In pregnant and non-pregnant bitches, increases in GH are related to increases in circulating P₄ and appears to be secondary to synthesis of GH in mammary glands. It has been clearly demonstrated that P₄ can stimulate mammary GH hypersecretion during the non-pregnant luteal phase [6]. Elevated GH plasma concentrations are characterized by the absence of a pulse pattern and insensitivity to stimulation and inhibition tests, with the exception of inhibition by the progesterone antagonist RU486. In hypophysectomized dogs, no absence or decrease in plasma GH concentrations was detected, which suggested the presence of an extra-pituitary origin. Selman et al. [16] demonstrated that mammary glands were effectively the source of GH concentrations. In human and felines, increased GH concentrations have been observed after progestin treatment [8]. Aglepristone, which is the first P₄ receptor blocker licensed for veterinary use, has been successfully used for pregnancy interruption, pyometra medical therapy and treatment of feline fibroadenomatous mammary hyperplasia [2,3]. Aglepristone binds to uterine P₄ receptors with affinity three times greater than P₄ itself in bitches and nine times greater in queens. Aglepristone blocks receptors, but also displaces P₄ already bound to receptors [11]. Aglepristone does not modify plasma concentrations of P₄, but indirectly induces uterine contractions and cervix dilatation after multiple injections [11]. Polisca et al. [13] demonstrated that aglepristone in dogs reduces the length of P₄ secretion by CL and accelerates the luteolytic process, and that this effect persists for about 6 to 8 days. Several side

*Corresponding author: Tel: +39-3382603449; Fax: +39-521032667; E-mail: enrico.bigliardi@gmail.com

effects have been observed after administration of aglepristone, such as anorexia, restlessness, depression, vomiting, diarrhea decrease in body temperature and local inflammatory reaction after injection [11]. The present study was conducted to investigate the use of aglepristone for the treatment of insulin-resistant diabetes mellitus during the luteal phase.

Materials and Methods

Animals

All bitches included in the study were diabetic subjects under insulin therapy that were referred to our Veterinary Teaching Hospital over a period of two years for insulin-resistant diabetes mellitus that developed during mid-luteal phase of the estrous cycle (25th ~ 30th day of diestrus). The luteal phase was established by vaginal cytology examined upon hematoxylin-eosin staining and blood P₄ concentrations. Criteria for selection of patients were: glycemia persistently > 200 mg/dL over a 12 h period, despite treatment with more than 1.5 IU/Kg BID of insulin; clinical and laboratory findings (P₄ > 2 ng/mL) indicative of diestrus. Diet, training, environmental factors and the insulin protocol administration were similar for all subjects. Exclusion criteria were the presence of other disorders or treatments that can cause insulin resistance. Eight intact bitches of different breeds and ages (9 to 15 years; mean ± standard deviation [SD]: 10.9 ± 1.46 years) met the inclusion criteria, as treatment group (group T; n = 8) and were therefore classified as having a P₄ induced insulin-resistant diabetes mellitus (Table 1). The control group (group C; n = 6) was composed by six diabetic bitches of mixed breeds and ages (8 to 12; mean ± SD: 10 ± 1.41) under insulin therapy in the same phase of the estrous cycle (25th ~ 30th day of diestrus, Table 2). The

study was carried out in accordance with the Italian Legislation on Animal Care (DL 116/92).

Blood sample collection

Blood samples (4 mL) for the determination of P₄ concentration (a commercial available Radioimmunoassay-RIA for canine) and GH (commercially available RIA for canine and porcine GH; Linco Research, USA) were collected before and after treatment with aglepristone by cephalic venipuncture and immediately transfer to ice-chilled EDTA-coated tubes for GH determination, as well as to tubes without anticoagulant for P₄ determination. All samples were centrifuged at 4°C for 10 min, after which plasma was stored at -25°C until assayed. Blood samples for determination of the pulsatile plasma profile of GH were collected at 15 min intervals between 8:00 AM and 14:00 PM before aglepristone administration and five days after the last aglepristone treatment [2]. Serial blood glucose curves were performed on day 0, 5, 12 and 20 after the beginning of treatment.

Therapy

All subjects were treated with porcine insulin zinc suspension (Caninsulin; Intervet, Italy) as reported in Table 1. Bitches in group T were treated with 10 mg/kg subcutaneously (SC) aglepristone (Alizin; Virbac, Italy) on day 1, 2, 9 and 17 in diestrus bitches. The control group received porcine insulin zinc suspension ≤ 1 IU/Kg (Caninsulin; Intervet) as well as saline solution (0.3 mL/kg, SC) on days 1, 2, 9, 17.

Statistical analysis

All values were expressed as the means ± SD. Differences among groups were assessed by ANOVA, and a *p* < 0.05 was considered to indicate statistical significance.

Table 1. Insulin doses, levels of serum glucose (reference rate: 80 ~ 100 mg/dL) and P₄ (reference rate: P₄ > 2 ng/mL) on day 0 in group T

Bitches	Group T (n = 8)		P ₄ (ng/mL)
	Glicemia (mg/dL)	Insulin (IU/kg)	
1	350	1.5	7
2	420	2.28	11
3	370	1.5	8
4	506	1.66	15
5	390	1.5	4
6	650	1.5	9
7	550	3.57	8
8	380	2.33	12
Means SD	452 ± 99.31	1.98 ± 0.68	9.25 ± 3.15

Table 2. Insulin doses, levels of serum glucose (reference rate: 80 ~ 100 mg/dL) and P₄ (reference rate: P₄ > 2 ng/mL) on day 0 in group C

Bitches	Group C		P ₄ (ng/mL)
	Glicemia (mg/dL)	Insulin (IU/kg)	
1	270	1	9
2	230	0.8	12
3	240	1	11
4	210	0.9	14
5	195	0.8	8
6	250	1	9
Means SD	232.5 ± 24.79	0.92 ± 0.089	10.5 ± 2.06

Results

Prior to aglepristone administration, the mean serum P₄ concentrations averaged 9.25 ± 3.15 and 10.50 ± 2.06 ng/mL in group T and group C, respectively ($p > 0.05$) (Tables 1 and 2), while the mean plasma GH concentrations were 2.37 ± 0.17 µg/L in group T and 1.80 ± 0.11 µg/L in group C ($p < 0.05$). Following treatment, the plasma GH concentration in group T was significantly lower 1.7 ± 0.6 µg/L (Tables 3 and 4); however, in group group C it was 1.68 ± 0.07 µg/L, which was not a significant difference (Table 4). At the end of treatment, the mean P₄ serum concentrations were 8.37 ± 2.24 and 9.33 ± 1.79 ng/mL in group T and group C, respectively (Tables 5 and 6). The final P₄ values of group T and C did not differ significantly. In IR subjects (group T), no significant variations in glycemia mean values were observed between day 0 and day 5 ($p < 0.05$). At day 12 and 20, the mean concentration

of blood glucose was significantly lower ($p < 0.05$) than on day 0 (Table 5). In the control group (Table 6), between the animals, blood glucose means concentrations were not significant ($p > 0.05$). On day 20, diabetes mellitus was well controlled in all the bitches (group T; Table 5). This results in group T allowed us to keep insulin dosage less than 1 IU/kg during the following 6 weeks, while before the aglepristone treatment the mean insulin dosage was 1.98 ± 0.68 IU/Kg (Tables 1 and 5). No side effects were recorded during aglepristone administration. The bitches were spayed within two months because they were euglycemic. Six months later they were still euglycemic.

Discussion

Glucose is the main source of energy for all body tissue, except cardiac and skeletal muscle. Blood glucose concentration is a reflection of gastrointestinal absorption, glycogenolysis, gluconeogenesis and glucose consumption by tissues. Glucose production and its metabolism occur as

Table 3. Levels of GH (µg/mL) before and after aglepristone (group T)

Bitches	GH (µg/L) Before aglepristone	GH (µg/L) After aglespristone
1	2.2	1.6
2	2.6	1.7
3	2.1	1.6
4	2.5	1.8
5	2.3	1.5
6	2.4	1.7
7	2.6	1.9
8	2.3	1.8
Means SD	$2.375^a \pm 0.17$	$1.7^b \pm 0.12$

^{ab} $p < 0.05$.

Table 4. Levels of GH (µg/mL) before and after saline solution (group C)

Bitches	GH (µg/L) Before saline solution	GH (µg/L) After saline solution
1	1.9	1.7
2	1.7	1.6
3	1.9	1.7
4	1.8	1.7
5	1.7	1.6
6	1.6	1.8
Means SD	$1.8^a \pm 0.11$	$1.7^b \pm 0.07$

^{ab} $p < 0.05$.

Table 5. Levels of serum glucose (reference rate: 80~100 mg/dL) and P₄ (reference rate: P₄ > 2 ng/mL) at day 0, 5, 12, and 20 (group T)

Bitches	Glucose (mg/dL) Day 0	Glucose (mg/dL) Day 5	Glucose (mg/dL) Day 12	Glucose (mg/dL) Day 20	Insulin (IU/Kg) Day 20	P ₄ (ng/mL) Day 20
1	350	320	280	190	0,8	6,5
2	420	305	200	165	0,8	10
3	370	330	295	195	0,8	7,5
4	506	266	245	185	0,66	12
5	390	315	264	176	0,8	5
6	650	450	280	185	0,8	8
7	550	410	270	175	0,7	7
8	380	290	235	165	0,8	11
Means SD	$452^a \pm 99.31$	$335.75^b \pm 58.28$	$258.625^c \pm 28.67$	$179.5^d \pm 10.39$	0.77 ± 0.052	8.375 ± 2.246

^{ab} $p > 0.05$, ^{ac} $p < 0.05$, ^{ad} $p < 0.05$.

Table 6. Levels of serum glucose (reference rate: 80 ~ 100 mg/dL) and P₄ (reference rate: P₄ > 2 ng/mL) at day 0, 5, 12, and 20 (group C)

Bitches	Glucose (mg/dL)	Glucose (mg/dL)	Glucose (mg/dL)	Glucose (mg/dL)	Insulin (IU/Kg)	P ₄ (ng/mL)
	Day 0	Day 5	Day 12	Day 20	Day 20	Day 20
1	270	255	260	260	1	8
2	230	240	220	250	0,8	11
3	240	220	250	230	1	10
4	210	230	200	210	0,9	12
5	195	190	210	195	0,8	8
6	250	220	230	240	1	7
Means SD	232.5 ^a ± 24.79	225.83 ^b ± 20.08	228.33 ^c ± 21.14	230.83 ^d ± 22.43	0.916 ± 0.08	9.33 ± 1.79

^{ab}*p* > 0.05, ^{ac}*p* < 0.05, ^{ad}*p* < 0.05.

a result of interaction of hormones, cytokines and intracellular transport [10]. Several glucose transporters have been identified, such as GLUT-4 in skeletal muscle, cardiac and adipose cells, as well as other glucose transporters (GLUT-1; GLUT-2) independent from insulin action in the brain, liver, kidney, placenta, sperm, adipocytes and erythrocytes. Increasing blood glucose concentrations stimulate insulin secretion, while low blood glucose concentrations suppress insulin secretion and stimulate production of various hormones (glucagon, epinephrine, norepinephrine, growth hormone, cortisol). There is growing evidence suggesting that GH modulates insulin sensitivity via multiple mechanisms due to the influence of crosstalk between GH/Insulin-like growth factor-1 (IGF-1) and insulin signaling, including reduced tyrosine kinase (TK) activity. However, insulin resistance alone appears likely to cause diabetes, once only few bitches in diestrus develop canine diabetes mellitus, which is undoubtedly a multifactorial disease, because there is growing evidence suggesting that GH modulates insulin sensitivity by multiple mechanisms, due to the influence of crosstalk between GH/insulin-like growth factor1 (IGF-1) [14]. Pregnancy is associated with IR in humans and dogs, which occurs in response to suppression of the intracellular transport of glucose and its increasing concentration in blood. P₄, estradiol, growth hormone, placental lactogen and placental cytokines all play important roles in causing insulin resistance [10,17]. There are several causes of resistance to exogenous insulin that do not lead to IR, such as improper handling and administration of insulin. Somogyi effect, which occurs when pronounced hyperglycemia develops in response to severe insulin-induced hypoglycemia, may also cause misdiagnosis of insulin resistance. In diabetic patients, sudden severe insulin-induced hypoglycemia results in development of protective mechanisms involving secretion of catecholamines, glucocorticoids, glucagon, and GH, which lead to pronounced hyperglycemia [6]. Actually, in

subjects showing Somogyi effect, the activity of insulin is high. In our cases, elevated doses of insulin did not induced fatal hypoglycemia. Gestational diabetes mellitus is a clinical condition characterized by a variable degree of glucose intolerance that can develop during pregnancy, parturition and diestrus [7,10]. During pregnancy, this disease is a rare condition [1]. Moreover the basis of this mechanism has not been fully elucidated. It is likely that gestational diabetes mellitus exerts a negative impact on fetuses and can compromise pregnancy and newborn viability [1,10]. A high risk of adverse fetal events, including abortion, small unthrifty pups and overly large pups (macrosomia), has been described in diabetics bitches. Vascular effects of diabetes may reduce placental blood supply, contributing to abortion or poorly-grown pups [12]. Pregnant bitches show greater insulin resistance than non-pregnant diestrous bitches. During the luteal phase basal GH secretion and P₄ concentrations are high. The canine mammary gland expresses genes encoding GH, and its expression is strongly stimulated by P₄. The long exposure to high circulating levels of P₄ during the luteal phase may even result in excess GH with acromegaly and/or diabetes mellitus in bitches [7]. Previous studies identified foci of hyperplastic ductular epithelium of the mammary gland as the site of origin of GH excess induced by progestins. These observations are consistent with the central role of progestins in GH gene expression in canine mammary gland and allow for a target endocrine therapy with progesterone receptor blockers with progestin-induced mammary-derived GH hypersecretion [2]. Some authors have also found that administration of antiprogestin resulted in decreased plasma GH concentrations and normalization of plasma IGF-I concentrations in bitches with progestin-induced acromegaly [2]. The insulin-antagonist action of GH (progestin-induced hypersecretion of GH) may result in hyperglycemia and diabetes mellitus. Hyperglycemia can be reversed after correction of high GH levels [2]. In our cases, the P₄ levels in the luteal phase

resulted in hyperglycemia due to IR [14]. Additionally, treatment with aglepristone (RU 46534) was useful to control the hyperglycemia levels in diestrus bitches, which was clearly demonstrated by the P₄ levels (> 2 ng/mL) at the end of the aglepristone treatment. Our findings are in agreement with those of Watson et al. [18], who found that administration of the mifepristone resulted in a decreased GH plasma concentration in bitches with progestin-induced acromegaly. Polisca et al. [13] showed lower progesterone levels after aglepristone administration, which was likely related to acceleration of the luteolytic process. The gradual decline of progesterone observed in treated dogs suggested that antiprogesterone triggers an anticipated, physiological-like luteolytic process [13]. In our opinion, the results of this prospective study suggest that aglepristone represents an effective therapeutic choice. Nevertheless, the best and definitive treatment for insulin resistance due to progesterone is gonadectomy as soon as possible [15], while the use of aglepristone should be reserved only for cases in which surgery is not possible or authorized by the owners. Ovariohysterectomy promotes a quick drop in P₄ levels, which is of great interest to managing diabetic bitches. In conclusion, the results of the present study demonstrated that aglepristone treatment significantly decreased blood glucose concentration in bitches with progestin-induced insulin-resistance.

Conflict of interest

There is no conflict of interest.

References

1. **Armenise A, Pastorelli G, Palmisano A, Sontas HB, Romagnoli S.** Gestational diabetes mellitus with diabetic ketoacidosis in a Yorkshire terrier bitch. *J Am Anim Hosp Assoc* 2011, **47**, 285-289.
2. **Bhatti SFM, Duchateau L, Okkens AC, Van Ham LML, Mol JA, Kooistra HS.** Treatment of growth hormone excess in dogs with the progesterone receptor antagonist aglepristone. *Theriogenology* 2006, **66**, 797-803.
3. **Bigliardi E, Ferrari L, Morini G, Quintavalla F.** Feline mammary hypertrophy/fibroadenoma complex. *Rassegna di Medicina Felina* 2007, **3**, 21-25.
4. **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.
5. **Fall T, Hamlin HH, Hedhammar A, Kämpe O, Egenvall A.** Diabetes mellitus in a population of 180,000 insured dogs: incidence, survival, and breed distribution. *J Vet Intern Med* 2007, **21**, 1209-1216.
6. **Hess RS.** Insulin resistance in dogs. *Vet Clin North Am Small Anim Pract* 2010, **40**, 309-316.
7. **Lee WM, Kooistra HS, Mol JA, Dieleman SJ, Schaeffers-Okkens AC.** Ovariectomy during the luteal phase influences secretion of prolactin, growth hormone, and insulin-like growth factor-I in the bitch. *Theriogenology* 2006, **66**, 484-490.
8. **Mol JA, Lantinga-van Leewen I, van Garderen E, Rijnberk A.** Progesterin-induced mammary growth hormone (GH) production. In: Mol JA, Clegg RA (eds.). *Biology of The Mammary Gland*. Vol. 480. pp. 71-76, Kluwer Academic/Plenum, New York, 2000.
9. **Feldman EC, Nelson RW.** *Canine and Feline Endocrinology and Reproduction*. 3rd ed. pp 486-538, WB Saunders, St. Louis, 2004.
10. **Johnson CA.** Glucose Homeostasis during canine pregnancy: insulin resistance, ketosis, and hypoglycemia. *Theriogenology* 2008, **70**, 1418-1423.
11. **Jurka P, Max A, Hawryńska K, Snochowski M.** Age-related pregnancy results and further examination of bitches after aglepristone treatment of pyometra. *Reprod Domest Anim* 2010, **45**, 525-529.
12. **Norman EJ, Wolsky KG, MacKay GA.** Pregnancy-related diabetes mellitus in two dogs. *N Z Vet J* 2006, **54**, 360-364.
13. **Polisca A, Scotti L, Orlandi R, Brecchia G, Maranesi M, Zerani M, Boiti C.** Aglepristone (RU534) administration to non-pregnant bitches in the mid-luteal phase induces early luteal regression. *Theriogenology* 2010, **74**, 672-681.
14. **Pöpl AG, Costa Valle S, González FHD, de Castro Beck CA, Kucharski LC, Martins Da Silva RS.** Estrus cycle effect on muscle tyrosine kinase activity in bitches. *Vet Res Commun* 2012, **36**, 81-84.
15. **Pöpl AG, Mottin TS, González FHD.** Diabetes mellitus remission of inflammatory and progesterone-related conditions in bitches. *Res Vet Sci* 2013, **94**, 471-473.
16. **Selman PJ, Mol JA, Rutteman GR, van Garderen E, Rijnberk A.** Progesterin-induced growth hormone excess in the dog originates in the mammary gland. *Endocrinology* 1994, **134**, 287-292.
17. **Verstegen-Onclin K, Verstegen J.** Endocrinology of pregnancy in the dog: a review. *Theriogenology* 2008, **70**, 291-299.
18. **Watson ADJ, Rutteman GR, Rijnberk A, Mol JA.** Effect of somatostatin analogue SMS 201-995 and antiprogesterin agent RU486 in canine acromegaly. *Front Horm Res* 1987, **17**, 193-198.