Field efficacy and safety of protamine zinc recombinant human insulin in 276 dogs with diabetes mellitus

C.R. Wardab, K. Christiansenb, J. Lib, W.L. Brysonb, K.A. Jerrentrupc, C. Krohc

a Department of Small Animal Medicine and Surgery, University of Georgia, Athens, GA
b Boehringer Ingelheim Animal Health USA Ltd., St. Joseph, MO
c Boehringer Ingelheim Vetmedica GmbH, Ingelheim am Rhein, Germany

1. Introduction

Diabetes mellitus (DM) is a common disease in dogs with a reported worldwide prevalence of 0.3%–0.6% [1–3]. Similar to human type 1 DM, DM in dogs requires insulin supplementation ideally alongside dietary modification, exercise, and weight control [4]. Treatment goals for dogs include control of clinical signs, maintenance of good physical condition, and avoidance of hypoglycemia and ketosis [4]. Insulin therapy has been shown to be effective in controlling clinical signs and elevated blood glucose (BG) associated with DM in dogs. Insulins differ in the insulin source material, repository form, and concentration. Insulin types most often used to treat canine DM include porcine lente and neutral protamine Hagedorn (NPH), although the use of protamine zinc recombinant human insulin (PZIR), detemir, and glargine insulins has also been reported [4–7]. Insulins licensed for veterinary use in the USA are limited to porcine lente and PZIR. The lack of large controlled studies in dogs has left the practitioner without a clear choice in optimal insulin therapy, relying instead on product familiarity or licensing requirements. Regardless of which insulin is chosen, previous studies indicate that optimal DM control requires twice-daily (BID) injection [4,8].
Treatment of DM is time and labor-intensive. In a recent worldwide study, 20% of pets diagnosed with DM were euthanized at or within the first year after diagnosis [9]. The impact on the owner’s lifestyle was cited as a reason for euthanasia in 32% of cases. The need to give insulin BID can have a negative impact on the owner’s quality of life, thereby making once-daily (SID) insulin therapy an appealing alternative.

PZIR (ProZinc, Boehringer Ingelheim, Germany) insulin was licensed in the USA for cats in 2009 and in dogs in 2019. Its source material is recombinant human insulin, which is precipitated with protamine and zinc. It is generally considered to be long-acting insulin due to prolonged absorption from subcutaneous tissue since the insulin/zinc/protamine complexes cause a slow release of insulin monomers or dimers into the systemic circulation [10]. In a small study, it has been shown to control hyperglycemia and clinical signs associated with DM in dogs [11]. The purpose of this study was to examine the efficacy and safety of PZIR in a large population of naïve and pre-treated diabetic dogs under conditions experienced by the general practitioner with the hypothesis that SID PZIR would be safe and efficacious for the treatment of canine DM.

2. Materials and methods

Seventeen general practice veterinary clinics in the United States participated in the study between September 2013 and July 2016. Written owner consent was obtained before the entry of each dog into the study.

2.1. Inclusion criteria

All dogs enrolled were client-owned and diagnosed with DM based on a fasting BG > 250 mg/dL, glycosuria, and at least one clinical sign consistent with DM (PU/PD and/or body weight (BW) loss). Dogs could be newly diagnosed and naïve to insulin treatment or previously diagnosed and on insulin but considered poorly regulated on their current insulin treatment. Assessment of poor control was based on the persistence of ≥1 clinical diabetic sign, fasted hyperglycemia (>250 mg/dL in naïve diabetics, and >250 mg/dL before insulin injection in previously treated/fasted diabetic dogs) and glycosuria.

2.2. Exclusion criteria

Dogs with suspected concurrent diseases such as pancreatitis, or hyperadrenocorticism, or those having potentially life-threatening diseases were not enrolled. Pregnant or lactating dogs, as well as those in estrus/diestrus or those intended for breeding were not included. Dogs treated with topical or systemic steroids within 30 d (short-acting) or 60 d (long-acting) or gestagens within 6 mo prior to study start were excluded. The administration of low doses of steroids for diagnostic purposes was permitted prior to enrollment (eg, low dose dexamethasone suppression test). Dogs with clinical and laboratory signs of diabetic ketosis or ketoacidosis (DK or DKA) requiring hospitalization and treatment with regular insulin on d −1 of the study were also excluded from participation.

2.3. Study design

This was a prospective, baseline-controlled, open-labeled, multi-center field study conducted to investigate the efficacy and safety of protamine zinc recombinant human insulin (PZIR) for the reduction of hyperglycemia and hyperglycemia-associated clinical signs in dogs with DM as a SID or BID therapy for up to 182 d. The study consisted of 2 phases (Fig. 1). During phase 1 (84 d), safety and efficacy were evaluated based on a history and physical examination (PE), including BW and results gained from a CBC, chemistry profile, including fructosamine, urinalysis, and urine culture, as well as, a 9-h blood glucose curve (BGC), in addition to the owner assessment of clinical signs and quality of life (QoL). In phase 2 (98 d), safety and efficacy were further investigated in 3 additional visits on d 112, 154, and 182, which included all the above-listed assessments except for the 9-h BGC, which was optional.

At screening (d −7 to −1), a complete history, PE, and laboratory analysis, including a CBC, clinical chemistry, urinalysis, and urine culture, were completed on each dog to identify concurrent diseases, concomitant treatments, and documentation of demographics (eg, age, breed, sex). The owner assessment included the baseline evaluation of QoL classified as excellent, very good, good, fair, or poor to reflect the effects of DM/diabetes mellitus on the dog. Owners also assessed water consumption and urination classified as excessive, normal, minimal, or unknown. On d −1, dogs were fasted (≥6 h) to obtain a fasted BG level. Afterward, owners fed and administered insulin per previously established protocol in pre-treated dogs; naïve dogs received no insulin. Owners were instructed to entice the dog to consume the regular meal every 12 h. Partial consumption was not documented. If the dog refused to eat before a scheduled visit, the visit was rescheduled. Nine-h BG curves were performed with samples taken at 1, 3, 5, 7, and 9 h post-feeding. PZIR and dosing logs were dispensed to successfully enrolled dogs, and the treatment phase (d 1 to 182 d) with PZIR began the following morning. The insulin starting dose was 0.5–1.0 IU/kg SID administered subcutaneously during or immediately following a meal. For naïve dogs, a starting dose in the lower range was recommended, while for pre-treated dogs, a starting dose in the upper range was allowed based on the dog’s previous insulin dose. Dogs were required to continue their current diet throughout the study and maintain a consistent BID feeding regimen.

In phase 1, dogs were evaluated on d 7, 14, 21, 28, 42, 63, and 84 (Fig. 1). The morning of each study visit, owners fed their dogs and administered PZIR within 1 h of arrival at the clinic, and all data described for phase one were obtained, owner dosing logs were reviewed, and owner assessments completed. Owners were questioned about the occurrence of any Adverse Events (AEs) such as abnormal clinical or behavioral signs. Veterinarians evaluated the overall level of diabetic control for each dog and recommended the same or a new insulin dose considering clinical signs, glucose curves, and fructosamine results. Where possible, dogs were maintained on SID treatment for a minimum of 28 d. In cases where a switch to BID became necessary, the SID dose was decreased by 25% and administered BID. Study veterinarians were instructed to consider a change to BID insulin if clinical
signs were not adequately improved on the current therapy, and the insulin dose could not be increased due to concerns of hypoglycemia (minimum blood glucose $<80–125$ mg/dL). The final decision to switch to BID insulin therapy was left to the treating veterinarian. Insulin dose titration was permitted throughout the study. The last opportunity to change from SID to BID posology was on d 42. Predefined reasons for withdrawal post-enrollment included owner removal of consent, signs of estrus/diestrus, AEs prohibiting further participation in the study, treatments potentially impacting study outcome (eg, steroids, gestagens, other insulins, other anti-diabetic medications), owner non-compliance, welfare reasons as decided by the veterinarian, or other reasons (eg, lost to follow-up). Treatments considered to have no impact on the clinical condition under investigation (eg, antibiotics, vaccines, anti-parasiticides, etc.) were permitted. Dogs were assessed and classified as either showing improvement in at least one lab parameter related to DM (mean BG, min BG, Fructosamine) and one clinical parameter (PU/PD, body weight) or failing to do so on d 84. Dogs successfully completing phase 1 continued into phase 2 for an additional 98 d for a total of 182 d. Dogs in the trial received veterinary care and medication related to the study and treatment of DM free of charge.

2.4. Analytical methods

BG concentrations were measured using a validated veterinary portable glucometer (AlphaTRAK2; Abbott Animal Health). Glycosuria was confirmed by in-house test glucose test strips (Keto-Diastix, Bayer). BG concentrations $<20$ mg/dL were registered as “LO” and assigned 19 mg/dL while concentrations $>750$ mg/dL were registered as “HI” and assigned 751 mg/dL. Test samples for CBC, biochemistry, fructosamine, and urinalysis were sent to a commercial laboratory (IDEXX Preclinical Research Services, CA, USA) and evaluated according to standard laboratory procedures. Fructosamine concentration was determined using the nitro blue tetrazolium reduction method.

2.5. Study populations

All dogs receiving $\geq1$ dose of PZIR were included in the safety assessment. Dogs meeting all eligibility criteria and passing the d 28 visit were included in the efficacy assessment unless removed for compliance issues on or before d 84 or for AEs unrelated to the study medication or DM. Dogs removed from the study for perceived lack of efficacy on or before the d 28 visit were not included in the efficacy assessment.
due to inadequate time for dose adjustments and insulin equilibration.

2.6. Assessment of efficacy and safety

Clinical parameters used to assess treatment efficacy included owners’ subjective assessment of PU/PD, QoL, and BW measured during PE. To qualify for inclusion in the efficacy analysis, PU and PD each had to be assessed as excessive, and weight loss had to be reported for BW at study start. These parameters were considered improved, if the owner observed a decrease of PU/PD, an increase of QoL category and/or if the veterinarian confirmed BW was stable or increasing. Laboratory glycemic parameters included mean BG, minimum BG gained from a 9-h BGC, as well as serum fructosamine, which were classified good, moderate, poor, or uncontrolled, as seen in Table 1. For the evaluation of safety, veterinarians documented any clinical or laboratory parameters deemed unfavorable or unintended, which were observed during veterinary visits or reported by the owner for dogs that had received PZIR at least once. These were documented as AEs whether or not they were considered to be product related. All AEs were classified as serious or non-serious and coded according to the Veterinary Dictionary of Drug Regulatory Affairs (VEDDRA) and summarized.

2.7. Data analysis

The analysis of an improvement in at least one clinical and one lab parameter related to DM utilized the GLIMMIX procedure of SAS Version 9.4 with a binomial distribution and logit link. The model was an intercept model with a site as a random effect.

For the long-term efficacy assessment, clinical signs of DM, QoL, and fructosamine levels were evaluated up to d 182. Further parameters observed included: percentage of dogs on SID vs BID on d 84 and 182, average insulin dose overall, on SID or BID treatment over time, as well as a subgroup analysis of clinical and laboratory parameters in pre-treated vs naïve diabetic dogs. The analysis of mean and minimum daily BG and mean daily fructosamine were conducted utilizing the MIXED procedure of SAS 9.4. The repeated measures model included the fixed effects of d and pre-study treatment status and their interaction. The random effects included site and subject within pre-study treatment status. The model was a no-intercept model with Kenward-Roger denominator degrees of freedom. For evaluating multiple comparisons, the Tukey method was utilized for the adjustment of $P$-values. The covariance structure utilized was the compound symmetry. Significant differences were determined where the adjusted $P$-value was less than 0.05.

The analysis of hourly BG within d was conducted using the same approach with hour within d and its interaction with pre-study treatment status as fixed effects.

In order to evaluate the safety profile of PZIR, all reported AEs were listed and categorized.

3. Results

In total, 324 dogs were screened, of which 276 met inclusion criteria and were enrolled and evaluated for safety (Safety population). Of those, 52 were removed from the efficacy evaluation (Efficacy population). Those removed were due to early withdrawal from the study prior to or on d 28 ($n = 21$); post inclusion screen failure ($n = 14$); AEs unrelated to treatment of DM ($n = 6$), including neurologic (1), and severe gastrointestinal diseases (2), ophthalmic condition (1), trauma (1), inability to manage dog (1); protocol deviation or owner non-compliance ($n = 9$); or loss to follow up ($n = 2$). A period of 28 d was set as a minimum duration required for insulin equilibration prior to the study start. Reasons for the 21 dogs withdrawn during this period included: 13 dogs for an AE that prohibited further participation, for lack of efficacy or a combination of both (DKA (4), pancreatitis + DKA (3), pancreatitis (2), abdominal mass (2), hyperadrenocorticism (1), dyspnea (1)); 5 dogs for owner withdrawal (surgery, stress, inappropriate expectations, owner-elected euthanasia); and 3 dogs for owner non-compliance. Of the remaining 224 dogs, 126 were insulin naïve and 98 had been pre-treated with insulin and considered poorly controlled by the owner and veterinarian. Pre-study insulins included NPH ($N = 77$), Glargine ($N = 1$), regular ($N = 1$), porcine lente ($N = 17$), PZIR ($N = 1$), and unknown ($N = 1$). The mean age ($\pm$ SD) of the efficacy population was 9 $\pm$ 0.17 yr (range 2–16), and the mean BW was 15.4 ± 12.04 kg (range 15.5–55.9). Breeds included mixed breed (33%), Labrador retriever (9.1%), miniature schnauzer (5.4%), miniature pinscher (5.1%), and others. With respect to sex, 52% were female (4% intact, 96% spayed) and 48% were male (6% intact, 94% neutered). The most commonly recorded diseases in the medical history of the dogs included: urinary tract infection (7.6%), vomiting (4.5%), pancreatitis (3.3%), lethargy (3.3%), and DKA (3.1%). Concomitant diseases and conditions observed during screening included: dental disorder (17.2%), cataract (14.4%), gingivitis (9.7%), heart murmur (5.4%), lipoma (4%), and lens opacity (4%).

PZIR resulted in a significant mean BG reduction in the efficacy population over the 84-d time period (Fig. 2), with a significant decrease observed in the overall population beginning d 21 onward ($P = 0.027$). In the naïve population, a significant decrease was observed from d 7 ($P = 0.022$) onward. A further significant decrease was observed on d 42 ($P < 0.0001$), compared to d 7, which remained consistent through the end of phase 1. In pre-treated dogs, the mean BG

<table>
<thead>
<tr>
<th>Categories of control</th>
<th>Mean blood glucose (mg/dL)</th>
<th>Minimum blood glucose (mg/dL)</th>
<th>Fructosamine (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Good</td>
<td>$&lt;200$</td>
<td>$&lt;150$</td>
<td>$&lt;450$</td>
</tr>
<tr>
<td>Moderate</td>
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<td>151–200</td>
<td>451–500</td>
</tr>
<tr>
<td>Poor</td>
<td>301–400</td>
<td>201–250</td>
<td>501–550</td>
</tr>
<tr>
<td>Uncontrolled</td>
<td>$&gt;400$</td>
<td>$&gt;250$</td>
<td>$&gt;550$</td>
</tr>
</tbody>
</table>

Dogs were assigned to categories based on measured mean blood glucose, minimum blood glucose, or serum fructosamine. Improvement in the laboratory category was one assessment of treatment efficacy.
Fig. 2. Median Blood Glucose Measurements. The group median of naive (A) and pre-treated (B) dogs for mean blood glucose at each visit is indicated by horizontal lines in the boxes. The box represents interquartile ranges (25-75%) and lower/upper whiskers the 10%/90%-quantiles. The dots represent 5% and 95% quantiles and the plus signs (+) indicate the minimum and maximum. The open triangles indicate the mean blood glucose at each time point. Time points marked by a star indicate a significant change. In the naive population it marks a significant decrease and in the pre-treated population it marks a significant increase in mean of mean blood glucose compared to baseline (p < 0.05). Time points marked by two stars in the pre-treated population indicate a significant decrease in mean of mean blood glucose compared to day 7 (p < 0.05). Time points with three stars indicate a significant decrease in mean of mean blood glucose compared to baseline and day 7 in both populations.
Minimum Blood Glucose Measurements. The group median of naïve (A) and pre-treated (B) dogs for minimum blood glucose at each visit is indicated by horizontal lines in the boxes. The box represents interquartile ranges (25-75%) and lower/upper whiskers the 10%/90%-quantiles. The dots represent 5% and 95% quantiles and the plus signs (+) indicate the minimum and maximum. The open triangles indicate the mean blood glucose at each time point. Time points marked by a star indicate a significant change. In the naïve population it marks a significant decrease and in the pre-
initially increased at d 7, but returned to pre-treatment levels by d 14 and then decreased significantly from d 7 by d 28 (P < 0.001) with a further significant decrease from baseline and d 7 by d 63 through d 84 (P < 0.0017, Fig. 2). Overall, mean BG decreased from 459.3 ± 112.4 mg/dL at baseline to 330.9 ± 138.7 mg/dL on d 84. Improvement was also noted in mean BG categories that are reported in Table 1. On d −1, 70.5% (158/224) and 22.8% (51/224) of dogs had mean BG levels classified as uncontrolled or poorly controlled, respectively, while 5.8% (13/224) and 0.9% (2/224) had levels classified as moderate or good, respectively. By d 84, the mean glucose had decreased such that 30.0% (66/220), 25.5% (56/220), 25.9% (57/220), and 18.6% (41/220) of dogs had levels classified as uncontrolled, poorly-controlled, moderate, and good, respectively.

Similar results were observed with minimum BG (over the 9-h BGJC). In the naïve population, PZIR resulted in a significant decrease by d 7 (P = 0.0003), which was maintained over the 84-d period (P < 0.0001), while the pre-treated population showed a significant reduction, compared to baseline, later at d 63 (P = 0.0074, Fig. 3). Overall, minimum BG decreased from 355.8 ± 133.5 mg/dL at baseline to 217.6 ± 138.8 mg/dL on d 84. With respect to the minimum BG improvement categories (Table 1), on d −1, 78.6% (176/224) and 8.9% (20/224) of dogs had minimum BG levels classified as uncontrolled or poorly controlled, respectively, while 4.0% (9/224) and 8.5% (19/224) had levels classified as moderate or good, respectively. These values improved by d 84 with 36.8% (81/220), 8.6% (19/220), 14.0% (31/220), and 40.5% (89/220) of dogs having levels classified as uncontrolled, poorly controlled, moderate, and good, respectively. Figure 4 shows the mean hourly BG response to PZIR obtained over the 9-h BGJC in naïve and pre-treated dogs receiving insulin and food 1 h before initiation of the BGC. In the overall population, a significant decrease in BG was observed between the 1 and 9-h measurement in all animals from d 7 onward and between the 1 and 7 h measurement from d 7 onward except for d 63 (P < 0.05). The lowest BG concentration in the curve was observed at the last blood sampling time (ie, 9 h) in 55% of dogs on d 84.

Fructosamine was measured as an indication of glucose control over the entire 182 d of the study and is represented in Figure 5. In the naïve population, a significant decrease from baseline was observed from d 21 (P = 0.0128) onward after the initiation of treatment with PZIR. Further decreases were observed at d 42 after, which levels stabilized until the end of the study. Although a decreasing trend was noted for fructosamine levels in the pre-treated group, no significant change from baseline was observed. Overall, fructosamine decreased from 525.3 ± 102.5 μmol/L at baseline to 461.2 ± 121.3 μmol/L on d 84 and 457.8 ± 99.8 μmol/L on d 182. With respect to the fructosamine improvement categories (Table 1), on d −1, 33.0% (73/221) and 29.9% (66/221) of dogs had fructosamine levels classified as uncontrolled or poorly controlled, respectively, while 19.9% (44/221) and 17.2% (38/221) had levels classified as moderate or good, respectively. These values improved by d 84, with 15.5% (34/220), 14.5% (32/220), 18.6% (41/220), and 51.4% (113/220) of dogs having levels classified as uncontrolled, poorly-controlled, moderate, and good, respectively. Clinical signs associated with DM and owner-assessed QoL were evaluated until d 182, and compared to baseline evaluations (Table 2). Improvement in PU/PD and QoL was observed in >40% of dogs on d 7 and in approximately 60% of dogs on d 14. On d 84, improvement in PU, PD, and QoL in the percentages of 90% (184/205), 88% (182/206), and 83% (182/220), respectively. There was no apparent change in mean BW (±SD) over time (15.4 ± 12.04 kg at baseline, 15.4 ± 11.81 kg on d 84, and 15.8 ± 12.27 kg on d 182).

A secondary aim of this study was to evaluate minimum PZIR posology and improvement of clinical signs and glycemic parameters with SID vs BID therapy. At study initiation, only 1 dog was assigned, contrary to protocol, to BID therapy. Table 3 represents the percentages of dogs treated SID vs BID, which demonstrated improvement of ≥ one clinical sign (PU/PD/QoL/body weight) and improvement in ≥ glycemic parameter (mean blood glucose, minimum blood glucose, or fructosamine). On d 84, 60% (135/224) of dogs were on SID and 40% (89/224) on BID posology, and out of them, 162 demonstrated clinical and glycemic improvement with 59% (96/162) on SID vs 41% (66/162) on BID. On d 84, 71% (96/135) of SID and 74% (66/89) of BID treated dogs demonstrated clinical and glycemic improvement. Of the efficacy population (n = 224), 187 dogs proceeded into phase 2 of the study. By d 182 (end of phase 2), 57% (107/187) of dogs were still on SID, while 43% (80/187) were on BID dosing.

Diabetic improvement was also evaluated with respect to naïve or pre-treated status on enrollment (Fig. 6). Of the 224 dogs in the efficacy population on d 84, 56% (126/224) were insulin naïve, and 44% (98/224) were pre-treated with insulin. Of the 162 dogs showing improvement in clinical signs and glycemic parameters on d 84, 62% (101/162) were naïve and 38% (61/162) pre-treated, with 80% (101/126) improvement achieved in naïve- and 62% (61/98) in pre-treated, poorly controlled dogs. Of the naïve dogs that showed clinical and glycemic improvement, 60% (61/101) were on SID and 40% (40/101) on BID treatment, while in the pre-treated dogs showing clinical and glycemic
Fig. 4. Hourly Blood Glucose Measurements. The group median of naïve (A) and pre-treated (B) dogs for mean hourly blood glucose at each visit is indicated by horizontal lines in the boxes. The open triangles indicate the mean of the mean hourly blood glucose at each time point. The box represents interquartile ranges (25-75%) and lower/upper whiskers the 10%/90%-quantiles. The dots represent 5% and 95% quantiles and the plus signs (+) indicate the minimum and maximum. Time points marked by a star indicate a significant change in mean of mean hourly blood glucose compared to the one hour measurement of the respective day ($P < 0.05$).
Fig. 5. Fructosamine Measurements. The group median of naïve (A) and pre-treated (B) dogs for fructosamine at each visit is indicated by horizontal lines in the boxes. The box represents interquartile ranges (25-75%) and lower/upper whiskers the 10%/90%-quantiles. The dots represent 5% and 95% quantiles and the plus signs (+) indicate the minimum and maximum. The open triangles indicate the mean blood glucose at each different time point. Time points marked by one star indicate a significant change. In the naïve population it marks a significant decrease and in the pre-treated population it marks a significant increase in mean fructosamine compared to baseline ($P < 0.05$). Time points marked by two stars in the pre-treated population indicate a significant decrease in mean fructosamine compared to day 7 and days 21 and 28 ($P < 0.05$).
improvement, 57% (35/61) were on SID and 43% (26/61) on BID treatment.

PZIR doses were determined at each visit. The recommended starting dose was 0.5–1.0 U/kg, and a mean (±SD) initial dose of 0.69 (±0.21) U/kg was prescribed SID for naive and 0.66 (±0.19) U/kg for pre-treated dogs. PZIR doses for dogs showing clinical and glycemic improvement on d 84 were 1.27 (±0.35) U/kg per injection SID and 0.81 (±0.28) U/kg per injection BID (1.61 ± 0.56 U/kg/d).

The most common AEs reported for the safety population (n = 276) were lethargy (16.3%, 45/276), anorexia (10.1%, 28/276), clinical hypoglycemia (8.9%, 24/276), vomiting (7.6%, 21/276), and seizures (5.8%, 16/276). Injection site reactions were reported in 7 dogs: all resolved without cessation of therapy. DKA was diagnosed in 11 dogs, pancreatitis in 21, and a combination of both in 4 dogs. Nine deaths and 27 euthanasiasts occurred with 9 dogs euthanized due to owner non-compliance/unwillingness to treat/perceived lack of treatment efficacy/owner request. Other causes for death or euthanasia included (in descending order): pancreatitis, complicated ketosis/ketoadiabetes, neoplasia, ocular disease, clinical hypoglycemia, trauma, unknown/unrecorded, dermatologic disease, dental disease, acute kidney injury, seizure, undiagnosed pain, aggression, and disseminated intravascular coagulation.

4. Discussion

This was the largest field safety and efficacy study conducted over the longest study period to date assessing insulin treatment in diabetic dogs. PZIR significantly lowered glucose parameters and improved clinical signs of DM in 72% of diabetic dogs over the 84-d efficacy trial period. As expected, the effects were most dramatic in the dogs naive to insulin therapy, which showed significant decreases in mean and minimum BG by d 7 with further reductions observed until d 42 when they stabilized until the end of phase 1. PZIR also improved glycemic parameters in dogs pre-treated with insulin; however, the results were less pronounced than those observed in naive animals. This was presumably due to the previously administered insulin influence on baseline glycemic values and clinical signs. Furthermore, dogs pre-treated with insulin were started on a mean PZIR dose of only 0.66 U/kg, which may have been comparatively lower than the previous insulin doses administered to these dogs and also to the successful dose evaluated in this study. This premise would support the initial increases in mean and minimum BG observed at d 7 in this population with significant decreases of glycemic parameters first observed after multiple-dose adjustments at d 63. Too low a starting dose might also explain cases of DKA occurring in this population. Additionally, there may have been selection bias in the pre-treated, poorly controlled group since these dogs were more likely to have had undetected disorders (ie, chronic pancreatitis), which would cause insulin resistance and precipitate entry into the study.

Although clinical and glycemic improvement was observed within the first 1–2 wk after starting treatment with PZIR, glucose parameters continued to decrease for a period of up to 2 mo after treatment initiation. The equilibration period observed in this study could be due, in part, to a low starting dose, compared to the successful dose identified, and to potentially cautious dose increases by the practitioner. A more rapid dose adjustment could have shortened the equilibration time observed, particularly in pre-treated dogs. Still, when initiating treatment with PZIR, an adequate equilibration period is needed to achieve optimal glycemic lowering effects, as for any other insulin. This timeline agrees with recommendations given in the literature, in which clients are advised at the start of insulin therapy that it may take time to establish a stable insulin regimen assuming the absence of insulin-antagonistic disease [4]. In a previous study evaluating PZIR in 20 dogs and another evaluating PZIR in 133 cats, reductions in glucose parameters continued over 45–60 d after treatment initiation, when the full glucose-lowering effects

### Table 2

<table>
<thead>
<tr>
<th>Clinical parameter</th>
<th>D</th>
<th>14</th>
<th>21</th>
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<th>42</th>
<th>63</th>
<th>84</th>
<th>182</th>
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</thead>
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<tr>
<td>Polydipsia</td>
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<td>134</td>
<td>211</td>
<td>64</td>
<td>144</td>
<td>208</td>
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<td>Polyuria</td>
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<td>208</td>
<td>130</td>
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<td>63</td>
<td>140</td>
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<tr>
<td>QOL</td>
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<td>224</td>
<td>38</td>
<td>139</td>
<td>223</td>
<td>62</td>
</tr>
</tbody>
</table>

At each visit, owners were asked to classify their dog’s quality of life (QoL) as excellent, very good, good, fair, or poor. They were also asked to classify polydipsia and/or polyuria as excessive, normal, minimal, or unknown. A change of QoL from poor or fair to very good or excellent, when compared to baseline, was considered an improvement. A change in polydipsia or polyuria category from excessive to normal or minimal, when compared with baseline, was considered an improvement. If drinking or urination status was unknown, the dog was not included in the evaluation.

### Table 3

Clinical and glycemic improvement on Day 84 with differing insulin posology (SID/BID).

<table>
<thead>
<tr>
<th>Insulin posology</th>
<th>Yes</th>
<th>No</th>
<th>Total N</th>
<th>Total %</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SID</td>
<td>96</td>
<td>71</td>
<td>39</td>
<td>29</td>
</tr>
<tr>
<td>BID</td>
<td>66</td>
<td>74</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>162</td>
<td>72</td>
<td>62</td>
<td>28</td>
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</tbody>
</table>

Improvement in ≥1 clinical sign and ≥1 glycemic parameter on day 84.
were achieved [11,12]. In another study involving 10 diabetic dogs treated with glargine and monitored weekly with BGCs, 38 ± 14 d were required to achieve stable insulin doses [7]. Similar results were observed in a study involving porcine lente insulin in which a median of 35 d and a range up to 151 d was needed to achieve dose equilibration after treatment initiation [13]. Although current recommendations suggest full glucose monitoring, including BGC, should be performed between 7–14 d after insulin treatment initiation or change in insulin type or dose [4], the data presented in this study showing that glucose parameters stabilized at d 42 suggest that such monitoring would be of benefit later in therapy and that practitioners may want to delay changing insulin types for at least the first 42 d after treatment initiation.

In addition to mean and minimum BG, fructosamine was evaluated over the entire study duration as an indicator of long-term BG control. Fructosamine was significantly lower in naïve dogs beginning at d 21, with a further decrease observed at d 42. Since fructosamine represents the average BG over the preceding 2–3 wk [14,15], a period of at least 2 wk is expected to see a decrease from baseline. The further decrease observed at d 42 correlates with the mean BG data. A significant reduction in fructosamine was not observed in the insulin pre-treated population. This also correlates with the less pronounced mean and minimum glucose-lowering effects seen in this population of dogs.

With the known variability incurred in monitoring glycemic parameters and the limitations of serum fructosamine concentration in predicting glycemic control [16], the resolution of clinical signs is an important measure of diabetic control [17,18]. PZIR was successful in improving DM-associated clinical signs of PU/PD and in improving QoL in 80%–90% of dogs. This is a remarkable finding since nearly half of those enrolled were pre-treated with insulin. Significantly, clinical signs began to resolve quickly in some (d 7), with 60% of dogs showing improvement by d 14. Quick resolution of clinical signs is of importance to owners since failure to resolve them can result in decreased QoL for both pets and owners and consequently lead to euthanasia [9].

Most insulins used in dogs are optimally administered twice-daily with a meal [8,19]. In this study, a consistent diet-fed BID was recommended to ensure adequate caloric intake. Diet was maintained throughout the study, and thus, changes in glycemic control were not expected to be diet associated. Dogs were fed at the time of insulin administration, and the resulting 9-h BGCs demonstrate effective PZIR reduction of post-prandial hyperglycemia.

BID insulin administration can have a negative impact on both pet and owner QoL [19,20]. The requirement of owners to be available to administer injections at 12-h intervals combined with needle-shy patients can be overwhelming and lead to treatment non-compliance or euthanasia [9]. The PZIR SID frequency and dose selected in this study were based upon prior clinical experience gained from a small field study conducted in dogs [11], as well as a PK/PD laboratory study conducted in 10 healthy dogs [10]. After injection of 0.8 U/kg, the onset of action was observed at 3.5 h (0.5–10 h), time to glucose nadir was observed at 14 h (5->24 h), and duration of action was greater than 24 h (16 to >24 h). These indicated a later onset and prolonged duration of action for PZIR in dogs.

For accommodating field trial conditions in the current study, the glucose curves were limited to 9 h to suit
schedules in most practices. However, the minimum BG level observed in the 9-h BGC was at the latest measurement time point in 55% of dogs, and a continued decrease in the curve suggests a nadir at a later time point. This indicates that an extended curve and/or the use of continuous BG monitoring devices could have been beneficial for the evaluation of the true PZIR nadir and the full duration of action. It is likely that mean glucose values would have been lower, and the percentage of dogs showing improvement for this parameter would be more in line with the positive observations made for fructosamine and clinical signs (44% showing improvement in mean BG vs 70% showing improvement in fructosamine, 96% overall improvement in at least one clinical sign) had later time points beyond 9 h been observed. The yet undetected true nadir prevents a direct comparison to other data in the literature. The suggested prolonged activity of PZIR in this study, in addition to the high rate of improvement observed with SID posology, further supports the use of SID administration in dogs. However, to fully characterize PZIR-action in dogs, further studies (eg, continuous BG monitoring) are needed.

The AEs reported in this study were similar to those reported in previous DM studies and related to the age of the population and existing comorbidities. Lethargy, anorexia, and vomiting were among the most commonly reported AEs. Although hypoglycemia is a common concern with insulin therapy, clinical hypoglycemia was observed in 8.9% of dogs in this study. The rate of clinical hypoglycemia observed for PZIR is thus favorable to those reported for other insulins having rates between 38.6% (porcine lente) and 40% (detemir) [5,13].

One of the aims of the study was to evaluate the minimum posology for PZIR. Although a switch to BID was allowed in the study and veterinarians and owners are accustomed to BID administration, the study confirmed a high SID improvement rate for PZIR overall and also in the naive and pre-treated sub-populations. Significant and meaningful to veterinarians, pets, and owners, on both assessment ds (84 and 182), nearly 60% of dogs were still on SID dosing, and 71% of dogs on SID treatment showed clinical and glycemic improvement on d 84. The observed improvement rate could be life-saving when it comes to the decision on therapy vs euthanasia and suggests the use of PZIR as an SID treatment in cases where BID insulin administration is not an option for the owner. When looking at the SID outcome in this study, it is important to consider that this regimen selection could be underrepresented due to the common practice BID posology associated with the most frequently used insulin types in dogs (Lente and NPH) to achieve adequate glycemic control [4,19]. Additionally, if adequate equilibration time had not been given or dose adjustment was too hesitant, some cases may have been prematurely switched to BID posology, thus impacting the SID/BID outcome. Although many insulins are available on the market, the most common insulins used in dogs are porcine lente and NPH, which are recommended for BID use [4,6,13,18,19]. Of these, porcine lente is FDA-approved for use in dogs, and NPH is used off-label. Given the variability of response to different insulins, PZIR as veterinary-approved insulin for use in both dogs and cats is an important addition to the market to provide choice to prescribing veterinarians, especially in light of the improvement observed with SID posology, as well as the rate of improvement observed in pre-treated, poorly controlled dogs in this study. The study confirms PZIR safely and effectively reduces glycemic parameters and clinical signs in both naïve and pre-treated diabetic dogs. The high percentage of SID treated dogs showing improvement in diabetic parameters and clinical signs confirms the long duration of action of PZIR, which will positively impact the lives of both diabetic dogs and their owners.

5. Conclusions

In this large field trial in diabetic dogs, PZIR safely and effectively improved glycemic parameters and clinical signs in diabetic dogs, many of whom were improved using PZIR once-daily. Although significant decreases in mean blood glucose were seen in naïve dogs after 1 wk of PZIR treatment, it took 42 d before stable glucose improvement was achieved.

CRediT authorship contribution statement


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