Preliminary Study of Protamine Zinc Recombinant Insulin for the Treatment of Diabetes Mellitus in Cats*†

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CLINICAL RELEVANCE

The efficacy of a new protamine zinc formulation based on recombinant insulin (PZIR) was compared with a veterinary-approved beef/pork–source insulin (PZI VET, Idexx Pharmaceuticals) that has been shown to significantly decrease blood glucose in cats with diabetes mellitus (DM). After being examined and weighed and having blood collected for determination of serum fructosamine concentrations, 50 cats with DM and stable glycemic control on PZI VET were switched to PZIR for 30 days at the same dose rate and interval. There was only one reported episode of hypoglycemia, and the cat was withdrawn from the study. In the 47 cats completing the study, there were no significant differences in body weight or serum fructosamine concentrations at days 15 or 30 compared with day 0. The results of this study indicate that PZIR provides glycemic control that is comparable to that of PZI VET when used at the same dose and dosing interval.

INTRODUCTION

A number of insulin formulations have been used for the treatment of diabetes mellitus (DM) in cats, including human recombinant NPH, purified pork–source Lente, beef/pork–source protamine zinc insulin (PZI), and the human synthetic insulin analog glargine.1–6 PZI VET insulin (Idexx Pharmaceuticals) contained beef/pork–source insulin; it significantly improved control of glycemia as demonstrated by decreased 9-hour mean blood glucose concentration, mean blood glucose nadir, and mean serum fructosamine concentration in newly diagnosed diabetic cats and poorly controlled diabetic cats previously treated with other insulin preparations.7 Additionally, cats gained weight and own-

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ers noted improvements in their pets’ clinical signs, such as frequency of urination, water consumption, and appetite. Although PZI VET has been considered by many to be the insulin product of choice for diabetic cats, it is no longer being manufactured because of the lack of availability of an FDA-approved source of bovine-derived pancreatic crystals. Other PZI insulins may be available but are not FDA-approved for veterinary use.

Production of insulin products using recombinant DNA technology has the potential to provide advantages over animal-derived products in terms of cost, safety, and supply. Recently, Idexx Pharmaceuticals developed a protamine zinc product based on recombinant insulin (PZIR) for cats using a formulation identical to that used for PZI VET except that the animal-derived insulin has been replaced by human recombinant insulin. The results of pilot studies carried out in diabetic rats demonstrated that PZIR and PZI VET have similar potency, onset of action, and duration of effect, resulting in a decision to develop it for use in cats. The objective of this study was to demonstrate that cats already well controlled on PZI VET maintained glucose control when switched to PZIR.

Materials and Methods

Study Design

Six private feline specialty clinics in the United States participated in the study. Fifty diabetic cats that had been treated with PZI VET for at least 90 days and that were considered adequately controlled by the clinical investigator were used for this study. Diabetic cats with concurrent acromegaly, hyperadrenocorticism, hyperthyroidism, or a life-threatening illness (e.g., neoplasia) were excluded from the study, as were those that had received glucocorticoids within the past 30 days or megestrol acetate within the past 6 months. The cats were maintained in their home environment, and insulin injections were administered by the owners.

The study was conducted over a period of 30 days. On day 0, a history was obtained, a physical examination was performed, the cat’s weight was recorded, and serum was collected for determination of fructosamine concentrations. Consent for the study was obtained from the owners, who were also asked to maintain the cats on their current diet for the duration of the 30-day study. On the evening of day 0, treatment with PZI VET was discontinued and treatment with PZIR was initiated. PZIR was administered at the same dose rate that PZI VET had been administered before enrolling in the study. All cats in the study were being treated with PZI VET subcutaneously every 12 hours, and, therefore, PZIR was also administered at that dosing frequency.

On day 15, the cats were reexamined and weighed, and serum fructosamine concentrations were determined. Based on the physical examination findings and the owner’s subjective evaluation of the cat’s response to therapy, the dose of PZIR was adjusted at this time if needed. Serum fructosamine was chosen as the con-
firmatory test because it reflects the glycemic state of the animal for the previous 1 to 2 weeks,⁹ giving it greater validity than a single glucose measurement.

On day 30, the cats were weighed and received a physical examination and serum samples were collected and analyzed for serum fructosamine concentrations. PZIR was discontinued and PZI VET therapy was reinitiated at the same or an adjusted dose, based on these examinations and the owner’s subjective evaluation of the cat’s response to therapy.

Data Analysis

Values collected on days 0, 15, and 30 for weight, insulin dose, and fructosamine concentrations were analyzed by analysis of variance techniques using SAS PRC MIXED (SAS Institute, Cary, NC), with day as a fixed effect. Values for dose were available only for days 1 and 15. Significance was set at $P \leq .05$. In addition, every cat’s fructosamine value was converted to a diabetic control classification as shown in Table 1, and all class combinations were compared using Fisher’s exact test.

### RESULTS

Of the 50 cats enrolled in the study, 3 were withdrawn for the following reasons: 1 cat reverted to a non–insulin-requiring diabetic state, 1 cat became too fractious to handle, and 1 cat suffered an episode of hypoglycemia on day 14 that required hospitalization. The breeds of the 50 cats included 43 domestic short-, medium-, and long-haired cats, 3 Siamese, and 1 each Birman, Himalayan, Oriental Shorthair mix, and Turkish Angora. Thirty-four were castrated males, and 16 were spayed females. Ages ranged from 3 to 16 years (mean, 10.9 years), and body weights ranged from 9.1 to 20.7 lb (mean, 13.9 lb).

The study completion rate was 94% (47 of 50). Body weight and fructosamine data were available for statistical analysis for all collection periods except for one cat that was not available for body weight or blood collection for fructosamine determination on day 15 and one cat that was not weighed on day 30.

As shown in Table 2, there were no significant ($P > .05$) changes in mean body weight or serum fructosamine concentrations over the 30-day course of the study. There was a small but statistically significant ($P < .05$) increase in the mean dose of insulin administered between days 15 to 30 compared with days 0 to 14 (Table 2). Individually, the dose of insulin administered to 38 of the 47 cats did not change over the course of the study. In the remaining cats, the insulin dose

### TABLE 1. Glycemic Control Classification Scheme Based on Serum Fructosamine Concentrations

<table>
<thead>
<tr>
<th>Glycemic Control Class</th>
<th>Serum Fructosamine Concentration (µmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor</td>
<td>&gt;500</td>
</tr>
<tr>
<td>Moderate</td>
<td>450–500</td>
</tr>
<tr>
<td>Good</td>
<td>&lt;450</td>
</tr>
</tbody>
</table>

### TABLE 2. Variables (Mean ± SD) Used to Assess Control of Glycemia in 47 Cats with Naturally Acquired Diabetes Mellitus Treated with PZIR for 30 Days

<table>
<thead>
<tr>
<th>Day</th>
<th>Body Weight (lb)</th>
<th>Fructosamine (µmol/L)</th>
<th>Insulin Dose (U/dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.8 ± 2.8</td>
<td>445 ± 91.0</td>
<td>4.2 ± 3.5</td>
</tr>
<tr>
<td>15</td>
<td>13.7 ± 2.9</td>
<td>446 ± 95.0</td>
<td>4.4 ± 3.4*</td>
</tr>
<tr>
<td>30</td>
<td>13.7 ± 2.8</td>
<td>432 ± 91.0</td>
<td>NA†</td>
</tr>
</tbody>
</table>

*Value significantly ($P < .05$) different from value obtained on day 0.
†Dose adjustments were made on day 0 (and that dose was administered through day 14) and on day 15 (administered through day 30). On day 30, the animal was placed back on PZI VET.
was increased on day 15 by 1 U or less in nine cats and by 2 U in two cats.

As shown in Table 3, there was an increase in the number of cats achieving good glycemic control (serum fructosamine concentrations <450 µmol/L) over the 30-day course of the study. On day 0, 46.8% (22 of 47) of the cats were considered in good glycemic control, whereas on day 30, 63.8% (30 of 47) of the cats were in good glycemic control. There was a corresponding decrease in the number of cats considered to have moderate glycemic control, defined by serum fructosamine concentrations between 450 and 500 µmol/L. On day 0, 25.5% (12 of 47) of the cats were considered to have moderate glycemic control, whereas on day 30, only 12.8% (6 of 47) of the cats were in the moderate glycemic range.

**DISCUSSION**

Because of the eventual unavailability of PZI VET, the objective of this study was to determine whether cats previously controlled with PZI VET could be successfully switched to PZI R. Although this study was not a direct comparison, the results demonstrate that PZI R provides glycemic control equivalent to PZI VET in stable diabetic cats. For example, over the 30-day course of the study, there were no significant changes in the mean body weight or serum fructosamine concentrations in the 47 cats that completed the study. In addition, although there was a statistically significant increase in the mean dose of insulin administered on day 15 versus day 0, this increase was not clinically relevant because a difference of this magnitude (average, 0.2 U) could be considered to be within the accuracy of the dosing syringe and/or procedure. In 38 of the 47 cats, the dose of insulin did not change over the course of the study. In nine of the remaining cats, the insulin dose was increased on day 15 by 1 U or less. In the two remaining cats, the dose was increased by 2 U. It is interesting to note that the same clinical investigator was treating both of these cats. Of these two cats, one had serum fructosamine values that were in the good range (<450 µmol/L) at the first study visit were but in the moderate range (450 to 500 µmol/L) on days 15 and 30; however, the other cat had fructosamine values that were in the good range throughout the study period. The treatment of DM in cats involves a bit of art as well as science, and variations in the approach and aggressiveness in terms of dose adjustments are common regardless of the type of insulin used.

The animals in this study were considered to be well regulated, having been treated with PZI VET for a minimum of 3 months (much longer, even years, in some patients). It is therefore important to note that when cats were switched to PZI R, almost 20% (8 of 47) demonstrated an improvement in glycemic control as evidenced by their serum fructosamine being classified as good. In addition, although not statistically significant, a tendency for a reduction in mean serum fructosamine concentration was observed between day 0 and day 30. Although PZI R may have been more effective than PZI VET in these

<table>
<thead>
<tr>
<th>Day</th>
<th>Poor (%)</th>
<th>Moderate (%)</th>
<th>Good (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>27.7%</td>
<td>25.5%</td>
<td>46.8%</td>
</tr>
<tr>
<td>15</td>
<td>32.6%</td>
<td>17.4%</td>
<td>50.0%</td>
</tr>
<tr>
<td>30</td>
<td>23.4%</td>
<td>12.8%</td>
<td>63.8%</td>
</tr>
</tbody>
</table>

*One patient was not available for its day 15 visit but did complete a day 30 visit.*
cats, another possible explanation for this improvement is that increased frequency of diabetic monitoring (e.g., physical examinations, owner observations, serum fructosamine measurements) may have allowed for finetuned dosing and therefore better glycemic control.

The safety of PZIR based on the results of this study appears to be excellent. For example, there was only one reported episode of hypoglycemia over the course of the study; despite being successfully treated, that cat was withdrawn from the study and placed back on PZI VET. This cat had a history of variably controlled diabetes with fluctuations in regulation due to various illnesses (e.g., dermatitis, dental disease), but it was considered adequately controlled for the 3 months before enrollment. Although not applicable in this case, hypoglycemia can be a desirable outcome if it is an indication of the onset of diabetic remission. No other adverse events attributable to PZIR were reported; however, eight cats vomited at least once during the study, a common event in cats.

There are many advantages to the use of recombinant DNA technology in the production of insulin products. For example, obtaining pancreatic glands is labor intensive and incurs significant manufacturing and formulating costs because of the inherent variability in source material. In addition, bovine-derived products always carry the concern for transmission of spongiform encephalopathies, although the risk is remote.10

The recombinant PZIR peptide is based on human insulin sequence, which differs from the feline sequence by four amino acids.11 Despite these differences, the results of previous studies indicate that cats are unlikely to develop a clinically significant antibody response to the human-based peptide.12

**CONCLUSION**

PZIR was safe and effective for the treatment of DM in this study of 47 cats and may serve as a viable substitute for PZI VET. Additional studies demonstrating the safe and effective use of PZIR in newly diagnosed and poorly controlled diabetic cats have been conducted and submitted to the FDA as part of their approval process.8

**REFERENCES**