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ABSORPTION KINETICS OF REGULAR, ISOPHANE, AND PROTAMINE ZINC INSULIN IN NORMAL CATS

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ABSTRACT

Absorption kinetics of regular, isophane (NPH), and protamine zinc (PZI) insulin were evaluated in seven clinically normal domestic shorthair cats by measurement of serial serum concentrations of insulin after subcutaneous administration of each insulin preparation. These results were compared to measurements of serial serum insulin concentrations after similar dosages of regular insulin were administered intravenously. Regular insulin administered subcutaneously was better absorbed than NPH and PZI insulins (mean bioavailability index 45.4% vs. 33.0% for NPH and 27.3% for PZI), and resulted in a significantly greater maximal increase in mean circulating insulin concentrations above baseline values (3529 pM vs. 1044 pM for NPH and 344 pM for PZI, $P<0.05$). The mean time interval between insulin administration and time to reach peak concentrations was significantly shorter for regular insulin than for NPH or PZI insulin (0.5 hr vs. 1.6 hr for NPH and 4.1 hr for PZI, $P<0.05$). There was also a significant difference ($P<0.05$) in the mean time interval between insulin injection and return of serum insulin concentrations to baseline values between regular insulin (5.6 hr) and NPH (7.7 hr) or PZI (13.1 hr) insulins. When compared with PZI, NPH insulin showed a significantly ($P<0.05$) greater maximal increase in mean serum insulin concentrations over baseline values. In addition, the interval between insulin administration and time to reach peak concentrations, as well as the time between insulin injection and return of serum insulin concentrations to baseline values, were also significantly shorter with NPH insulin than with PZI. These results suggest that NPH and PZI insulins administered subcutaneously to cats may require a short time to reach peak serum insulin concentrations as well as a relatively short time for circulating insulin concentrations to return to baseline values. If the absorption kinetics are similar to that in this study, most cats with diabetes mellitus would need twice daily injection of NPH or PZI insulin to adequately control the diabetic state.

INTRODUCTION

Treatment with insulin for the control of diabetes mellitus has been used in dogs and cats for many years. Traditionally, information gained from studies of insulin kinetics in human beings has been used to develop treatment regimes of insulin administration for dogs and cats (1-4). Recently, it has become clear, however, that great differences in insulin absorption kinetics exist between species and between individuals within a species (5-7). A study of absorption kinetics of regular and isophane (NPH) insulin in normal dogs showed that NPH insulin reached peak serum concentrations faster and serum concentrations remained above basal values for a shorter period of time than had previously been anticipated (7). Similarly, in a study of a small number cats with overt diabetes mellitus in which blood glucose concentrations were determined following subcutaneous injections of NPH insulin and protamine zinc insulin

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(PZI), there was an earlier peak activity and a shorter duration of action than might have been expected, especially with NPH (8). This often necessitated the use of twice daily insulin administration for adequate blood glucose regulation in the diabetic cats.

In this study, we evaluated the absorption kinetics of three commonly used insulin preparations in seven clinically normal cats by measuring serial serum insulin concentrations after the subcutaneous administration of regular (crystalline) insulin, NPH insulin, and PZI. These results were compared to measurements of serial serum insulin concentrations after similar dosages of regular insulin were administered intravenously to the same cats.

MATERIALS AND METHODS

Seven adult male castrated domestic shorthair cats, weighing 3.8 to 5.6 kg, were used in this study. The cats were determined to be healthy on the basis of results of physical examination and routine laboratory testing (i.e., complete blood count, serum biochemical analysis, and urinalysis). The ELISA test for feline leukemia virus was negative in all cats. The cats were acclimated to their environment for 3 weeks before the study commenced. Throughout the study, the cats were housed in individual cages and given free access to water and food (Feline C/D[a]).

Four separate studies were performed on each cat at 3 to 4 week intervals. In the first two studies, the cats received a beef-pork regular insulin preparation[b] either intravenously (four cats) or subcutaneously (three cats) at a dosage of 0.5 U/kg. Cats that received regular insulin intravenously in the first study period were given subcutaneous insulin in the second study period, and vice versa. For the studies of regular insulin kinetics, blood was collected for determination of serum insulin concentrations before (−30 and 0 min) and 5, 10, 15, 30, 45, 60, 90 min and 2, 3, 4, 5, and 6 hr after administration. In the third and fourth studies, NPH (isophane) insulin suspension[c] or protamine zinc insulin (PZI) suspension[d] was administered subcutaneously to each cat in random order. The cats that received NPH insulin in the third study period were given PZI insulin in the fourth study period, and vice versa. For the studies of NPH and PZI insulin kinetics, blood was collected for determination of serum insulin concentrations before (−30 and 0 min) and 15, 30, 45, 60, 90 min and 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24 hr after administration. All subcutaneous insulin injections were given within a shaved 3 cm^2 area on the dorsal thoracic wall. All blood samples were collected through an indwelling, 18-gauge catheter[e] which was placed in a jugular vein of each cat 18 hr before each testing period.

For all insulin determinations, blood was held on ice after collection, centrifuged within 1 hr, and stored at −70 C until assayed. Serum insulin was measured with a commercial radioimmunoassay kit[f]. This RIA kit was chosen for use in this study because the assay readily detects exogenous porcine and bovine insulin but cross-reacts little, if at all, with endogenous feline insulin. In support of that, baseline serum insulin concentrations were at or near the low end of sensitivity for the assay in all cats studied, and no rise in circulating insulin concentrations could be detected after the intravenous administration of glucose (1 mg/kg) despite a marked rise in serum glucose concentrations. This insulin assay was validated for measuring exogenous porcine and bovine insulin in cat serum using the following procedures. Assay of serial dilutions of two feline serum pools with insulin concentrations of approximately 500

pM and 1000 pM, respectively, resulted in inhibition curves with slopes parallel with the standard curve. Accuracy was determined by adding varying quantities of beef-pork regular insulin to a feline serum pool containing an undetectable concentration or insulin. Linear regression analysis of the resulting data (x = amount of insulin added; y = amount of insulin measured) gave the equation $1.11x + 24.3$, with a correlation coefficient of 0.99. The sensitivity of the insulin assay was 25 pM; the intra-assay coefficient of variation was 6.1%. To avoid between-assay variation in individual cats, each cat had all samples from all four study days run in the same assay.

For the studies of regular, NPH, and PZI insulins injected subcutaneously, the following parameters were analyzed: 1) maximum increase in serum insulin concentrations over baseline values; 2) time interval between insulin administration and point at which serum insulin reached peak concentrations; 3) time interval between insulin administration and the return of serum insulin concentrations to baseline values and 4) the bioavailability index, calculated by dividing the area under the serum insulin curve for each subcutaneous dose by the area under the curve for the intravenous dose of regular insulin (9). All insulin concentrations above baseline values were regarded as exogenous insulin, since both hypoglycemia and exogenous insulin are expected to suppress endogenous insulin secretion.

All results are given as the mean ± SEM. Statistical analyses were performed by Students paired and unpaired t tests; a P value of 0.05 or less was considered significant (10). The areas under the serum insulin curves were calculated by the trapezoidal rule (11). The elimination half-life of regular insulin administered intravenously was calculated by the least squares analysis of the decline of serum insulin concentrations.

RESULTS

After intravenous administration of regular insulin, the mean serum insulin concentration rose significantly ($P<0.001$), peaked at 5 min post-injection (29,680 ± 4,980 pM), and returned to baseline values 5 hr after injection (Figure 1A). The mean elimination half-life was 16.3 ± 1.0 min, with a range of 13.7 to 19.9 min.

After the subcutaneous administration of regular insulin, the mean serum insulin concentration increased significantly ($P<0.01$) by 5 min and reached a peak value (3,370 ± 1000 pM) 30 min post-injection (Figure 1B). The mean serum insulin concentration then gradually fell and was statistically indistinguishable from baseline concentrations 6 hr post-injection (Figure 1B). In individual cats, the maximal increases in serum insulin concentrations over baseline values ranged from 1068 to 8269 pM (mean = 3529 ± 954 pM), the time interval between insulin administration and the point at which circulating insulin reached peak concentrations ranged from 15 to 60 min (mean = 30 ± 5.7 min), the time at which serum insulin returned to baseline concentrations ranged from 5 to 6 hr (mean = 5.6 ± 0.2 hr), and the bioavailability index ranged from 23.9% to 66.1% (mean = 45.4 ± 6.7%).

After the subcutaneous administration of NPH insulin, the mean serum insulin concentration increased significantly ($P<0.05$) above the baseline value by 30 min and reached a peak value (962 ± 277 pM) 90 min post-injection (Figure 1C). The mean serum insulin concentration achieved a relative plateau between 1 and 2 hr and then fell to basal concentrations by 8 hr following administration

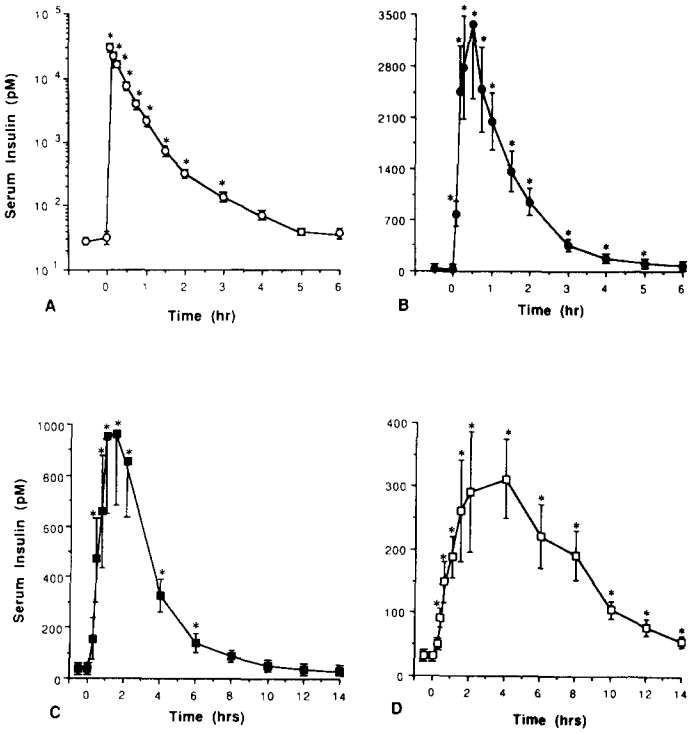

Fig. 1. Mean (± SEM) serum insulin concentrations in 7 normal cats after intravenous administration of regular insulin (A), subcutaneous administration of regular insulin (B), subcutaneous administration of NPH insulin (C), and subcutaneous administration of PZI insulin (D). Notice the change in the scale of the axis for insulin concentrations between the four panels. * $P < 0.05$ as compared to baseline concentrations.

(Figure 1C). In individual cats, the maximal increases in serum insulin concentrations over baseline values ranged from 340 to 2223 pM (mean = 1044 ± 288 pM), the time interval between insulin administration and the point at which circulating insulin reached peak concentrations ranged from 1 to 2 hr (mean = 1.6 ± 0.2 hr), the time at which serum insulin returned to baseline concentrations ranged from 6 to 10 hr (mean = 7.7 ± 0.8 hr), and the bioavailability index ranged from 26.4% to 54% (mean = 33.0 ± 3.6%).

After subcutaneous administration of PZI insulin, the mean serum insulin concentration rose significantly ($P<0.01$) above the baseline concentration by 30 min and reached a peak concentration (303 ± 63 pM) 4 hr post-injection (Figure 1D). The mean serum insulin concentrations declined gradually and was not significantly different from baseline values at 16 hr after administration. In individual cats, the maximal increases in serum insulin concentrations over baseline values ranged from 124 to 787 pM (mean, 344 ± 83.5 pM), the time interval between insulin administration and the point at which circulating insulin reached peak concentrations ranged from 1 to 8 hr (mean, 4.1 ± 1.1 hr), the time at which serum insulin returned to baseline concentrations ranged from 8 to 20 hr (mean, 13.1 ± 1.4 hr), and the bioavailability index ranged from 14.3% to 40.7% (mean = 27.3 ± 3.4%).

Regular insulin administered subcutaneously was better absorbed than NPH and PZI insulins, and resulted in a significantly ($P<0.05$) greater maximal increase in mean circulating insulin concentrations above baseline values. The mean time interval between insulin administration and time to reach peak

concentrations was significantly ($P<0.05$) shorter for regular insulin than for NPH or PZI insulin. There was also a significant difference ($P<0.05$) in the mean time interval between insulin injection and return of serum insulin concentrations to baseline values between regular insulin and NPH (7.7 hr) or PZI (13.1 hr) insulins. When compared with PZI, NPH insulin showed a significantly ($P<0.05$) greater maximal increase in mean serum insulin concentrations over baseline values. In addition, the interval between insulin administration and time to reach peak concentrations, as well as the time between insulin injection and return of serum insulin concentrations to baseline values, were also significantly ($P<0.05$) shorter with NPH insulin than with PZI insulin.

DISCUSSION

The results of this study demonstrate that the characteristics of regular insulin after intravenous or subcutaneous injection to normal cats are similar to previously reported values for normal dogs (7). In both species, regular insulin administered intravenously (at the dose of 0.5 U/kg) produces a very high serum insulin concentration almost immediately post-injection, and, because of this, circulating insulin concentrations remain elevated for a number of hours, in spite of its short half-life. Therefore, depending on the dosage used, regular insulin may have a longer duration of action than the 15 to 30 min that has been previously suggested in cats (12). The clinical significance of this is that frequent intravenous insulin injections could cause an accumulation of insulin in the body resulting in a hypoglycemic crisis in a cat with diabetes mellitus. When a similar dosage of regular insulin is administered subcutaneously, a much lower peak serum insulin level is obtained, and the time interval between insulin administration and return of serum insulin concentrations to baseline values is only an hour or two longer than with the intravenous route.

In the present study, NPH administered subcutaneously showed an early peak and a fairly rapid decline in serum insulin concentrations, similar to reports of NPH absorption kinetics in clinically normal dogs (7). In most cats, the insulin concentrations fell to baseline values within 6 to 8 hr after administration of NPH insulin, only slightly longer than with subcutaneous regular insulin. In no cat in our study did the serum insulin concentration remain elevated for longer than 10 hr. This data confirms the clinical observation of other investigators that NPH needs to be administered twice daily to achieve adequate blood glucose control in most cats with diabetes mellitus, as in diabetic dogs (2,8,13-15).

The subcutaneously administered PZI in this study showed a slower and more variable climb to peak serum concentrations as compared to the regular and NPH insulins, as well as a more variable period of time for serum insulin concentrations to return to baseline values (8 to 20 hr). This makes the absorption kinetics of PZI rather unpredictable from cat to cat, necessitating serial blood glucose evaluations in all diabetic cats to help one determine whether to administer the PZI on a once-daily or twice-daily schedule. Some cats will require twice daily administration of PZI to adequately control hyperglycemia, while other cats will show a slower absorption and longer duration, making once daily administration possible (8,13-15).

The bioavailability data for the regular, NPH, and PZI insulins administered subcutaneously in this study was quite variable. However, the mean bioavail-

ability index indicated that regular insulin was better absorbed than NPH and PZI insulins, and that NPH insulin was better absorbed than PZI. This difference in bioavailability results in a greater increase in circulating insulin concentrations after administration of regular insulin than NPH insulin, with the lowest serum insulin concentrations found after PZI administration. This may explain the observation that diabetic cats may require higher daily dosages of PZI insulin than NPH insulin to adequately control the diabetic state (13). As expected, there were differences between the 3 insulin preparations studied in the time intervals between insulin administration and point at which serum insulin reached peak concentrations, as well as the time interval between insulin administration and the return of serum insulin concentrations to baseline values. The time to reach peak concentrations and time to return to baseline serum insulin values were shortest for regular insulin, intermediate for NPH insulin, and longest for PZI.

The results of this study can not be used to determine the duration of insulin action in cats but rather the duration of time serum insulin values are increased above baseline concentrations after insulin administration. Duration of insulin action is usually determined by its effect on blood glucose concentrations. We did not measure blood glucose concentrations in the cats of this study for two reasons. First, since cats are quite sensitive to the glucose-lowering effects of exogenous insulin (1,12,13), the cats were allowed to eat *ad libitum* during each study period to help avoid the severe hypoglycemia that would result from the large insulin dosages employed. Second, if hypoglycemia would develop in normal cats, compensatory physiologic responses to the hypoglycemia (i.e., catecholamine and glucagon release) would raise blood glucose concentrations despite persistence of insulin action (16,17). Therefore, blood glucose determinations are of limited usefulness in determining the duration of insulin action in normal cats or normal human subjects.

It should be observed that insulin kinetics in normal cats may differ from that in cats with overt diabetes mellitus. In human patients, absorption kinetics of insulin are known to be altered by factors such as dehydration, acidosis, and hypothermia. We therefore agree with recommendations that a cat with diabetes mellitus be stabilized with the desired preparation of insulin before the effects of the insulin on blood glucose concentrations throughout the day are closely evaluated (14,15). This individual kinetic information can then be used to evaluate the choice of insulin type, dosage and dosing interval. Nevertheless, if the absorption of insulin in diabetic cats is comparable to that found in this study, most cats with diabetes mellitus will need twice daily injection of intermediate-acting or long-acting insulin to adequately control the diabetic state.

ACKNOWLEDGEMENTS/FOOTNOTES

[a] Hills Pet Products, Inc., Topeka, KS 66601
[b] Regular Ilentin I, Eli Lilly and Company, Indianapolis, IN 46285
[c] NPH Ilentin I, Eli Lilly and Company, Indianapolis, IN 46285
[d] Protamine, Zinc & Ilentin I, Eli Lilly and Company, Indianapolis, IN 46285
[e] Venocath-16, Venisystems. Abbott Ireland, Ltd., Sligo, Republic of Ireland
[f] Micromedic Insulin RIA Kit, Micromedic Systems, Inc., Horsham, PA 19044

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