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Enterically-delivered Insulin Tregopil Exhibits Rapid Absorption Characteristics and a Pharmacodynamic Effect Similar to Human Insulin in Conscious Dogs

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Abstract

Aims: Current therapy fails to emulate rapid (first-phase) insulin release in relation with a meal, a key defect in types 1 and 2 diabetes. We aimed to quantify the pharmacokinetic (PK) and pharmacodynamic (PD) profile of insulin tregopil, an enterically-absorbed insulin analog that restores the normal distribution of insulin between the hepatic portal and peripheral circulations.

Materials and Methods: The PK and PD profile of insulin tregopil were studied in overnight-fasted, catheterized, conscious canines using four approaches: 1) equimolar intraportal infusions of tregopil vs. human insulin, 2) escalating doses of oral tregopil, 3) identical, consecutive enteric doses of tregopil, and 4) comparison of oral tregopil to inhaled and subcutaneous human insulin administration.

Results: Equimolar intraportal infusions of tregopil and human insulin resulted in very similar pharmacokinetic profiles and pharmacodynamic profiles were nearly identical. Enteric delivery of tregopil brought about rapid absorption with $t_{\max}=20$ min in most cases. Median $t_{\max}$ was 20 minutes for oral tregopil and inhaled insulin and 88 minutes for subcutaneous human insulin. Time needed for arterial plasma insulin levels to return to baseline was approximately 90, 210, and 360 minutes for oral tregopil, inhaled insulin, and subcutaneous insulin, respectively.

Conclusions: Enterically-delivered tregopil is rapidly absorbed and restores a portal-to-peripheral vascular distribution. These characteristics should improve post-prandial hyperglycemia in types 1 and 2 diabetes.

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CONFLICT OF INTEREST

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Keywords

oral insulin; type 2 diabetes; type 1 diabetes

INTRODUCTION

Despite being one of the earliest and most significant pathophysiologic abnormalities in the natural history of type 1 (1) and type 2 diabetes (2, 3) (T1DM and T2DM), the loss of first-phase insulin release is not corrected by current therapy. In the physiologic state, a burst of insulin secretion is observed within ten minutes in response to an increase in plasma glucose concentrations.(4) This first-phase insulin response promptly inhibits hepatic glucose production (HGP) and is critical to restraining post-prandial hyperglycemia.(5, 6) Unfortunately, current therapy fails to reproduce two key elements of the first-phase insulin response: rapid absorption into the plasma and physiologic distribution between the hepatic portal and peripheral circulations.

Virtually all patients with T1DM and many patients with T2DM rely on subcutaneously delivered, rapid-acting insulin analogs to suppress HGP and promote glucose disposal during and after eating. Whereas physiologic first-phase insulin release results in a rapid rise in the plasma insulin concentration within 10 minutes post-prandially, 45–120 minutes are required to reach a maximum concentration after subcutaneous delivery of rapid-acting insulin analogs.(6–8) Because insulin arrival at the liver is slower than normal, modulation of HGP is delayed resulting in larger postprandial hyperglycemic excursions.(4).

An additional drawback to subcutaneous insulin delivery is that individuals with diabetes deliver the hormone directly into the peripheral circulation rather than more physiologically into the hepatic portal circulation. As a result, patients relying on subcutaneous insulin delivery “over-insulinize” the peripheral circulation while “under-insulinizing” the liver. This iatrogenic peripheral hyperinsulinemia appears to contribute to hypoglycemia,(9) weight gain,(10) and insulin resistance.(11).

Enteric insulin delivery has potential to both restore an appropriate balance of insulin between the liver and peripheral tissues and to exhibit rapid absorption kinetics. As described in detail elsewhere,(12) tregopil (IN-105 in previous studies) is a novel insulin analog modified for oral delivery by the addition of a single short-chain amphiphilic oligomer at the Lys-β29 residue of recombinant human insulin. In a dose finding study, tregopil given at mealtimes to participants with T2DM resulted in a rapid time to peak concentration (t_{max}) of 30 min.(12) In the present study, we sought to further characterize tregopil’s pharmacokinetic (PK) and pharmacodynamic (PD) properties in the conscious, catheterized canine model. Specifically, these studies aimed to 1) compare the bioeffectiveness of tregopil vs. human insulin when delivered in equimolar doses into the portal vein, 2) determine the dose-response relationship for escalating doses of liquid tregopil, 3) establish the reproducibility of tregopil’s pharmacodynamic response, and 4) in an exploratory analysis, compare the PK/PD profile of orally-delivered tregopil against inhaled human insulin and subcutaneously injected human insulin.

METHODS

Experimental design

The biologic characteristics of insulin tregopil were studied in four parts:

Part 1: Comparison of the Bioeffectiveness of Human Insulin vs. Insulin

Tregopil: The objective of part 1 was to compare the effectiveness of recombinant human insulin and tregopil in altering glucose metabolism when infused at equimolar rates into the hepatic portal vein (the site of entry into the blood after oral delivery). Dogs were fed a chow diet daily and were fasted 18 hours prior to being studied. The experimental protocol consisted of a 40 min control period then two 120 min test periods. Somatostatin was infused through a peripheral vein at 0.8 $\mu\text{g/kg/min}$ to block endogenous hormone production by the endocrine pancreas and glucagon was infused intraportally at a rate equivalent to its normal basal secretion rate (0.57 ng/kg/min). In one group (n=7) human insulin was infused intraportally at 3.6 pmol/kg/min (600 $\mu\text{U/kg/min}$) for 120 min then at 12.0 pmol/kg/min (2000 $\mu\text{U/kg/min}$) for 120 min. In the second group (n=6), insulin tregopil was infused intraportally at the same rates as were used for human insulin. Glucose was infused through a peripheral vein to maintain euglycemia. Steady-state plasma insulin concentrations from the end of each of the two 120 min infusion portal insulin infusion periods are reported as the mean of the insulin concentrations in the last 30 min period. Because somatostatin infusion completely inhibits endogenous insulin secretion, insulin clearance between intraportal infusions of human insulin and tregopil can be calculated and compared by dividing the insulin infusion rate by the arterial plasma steady-state insulin concentration.

Part 2: Determine Dose-Response Relationship for Tregopil—To determine the dose-response relationship for tregopil, dogs received doses of 0.0625 mg/kg (10.4 nmol/kg, n=12), 0.125 mg/kg (20.7 nmol/kg, n=14), or 0.25 mg/kg (41.5 nmol/kg, n=9) via orogastric gavage. Prior to each dose, dogs fasted for 42 hours to ensure the upper GI tract was clear of food and that metabolic parameters had returned to a fasting baseline. Plasma glucose concentrations were clamped at baseline after the enteric tregopil dose using a variable intravenous glucose infusion during the 120 min test period.

Part 3: Establish Reproducibility of Tregopil's Dose Response Relationship—

The reproducibility of the PK and PD profiles for identical, consecutive doses of tregopil in tablet form was determined in dogs following an 18-hour overnight fast. In a single study, dogs received two identical oral doses of 3 mg (22.5 nmol/kg, n=5), 6 mg (45.0 nmol/kg, n=8), or 10 mg (75.0 nmol/kg, n=5) with 120 minutes elapsing between the first and second doses. Arterial plasma glucose concentrations were maintained at baseline via variable intravenous glucose infusion.

Part 4: Compare Pharmacokinetic and Pharmacodynamic Characteristics of Oral Tregopil against Subcutaneous and Inhaled Oral Insulin—

To contextualize the biologic characteristics of tregopil, in a separate, exploratory analysis we compared PK and PD data for the 0.25 mg/kg liquid tregopil dose used in Part 2 against our previous study of discrete doses of inhaled (1 mg, n=3; Nektar Therapeutics, San Carlos, CA) and

subcutaneously-delivered human insulin (2.2 nmol/kg, n=6; Humulin; Eli Lilly, Indianapolis, IN) where similar $C_{\max}$ values were reached. (13) All dogs in this analysis received variable glucose infusions to maintain glucose near euglycemia. Peripheral infusions of somatostatin (0.8 $\mu\text{g/kg/min}$) were given to dogs receiving inhaled and subcutaneous insulin but were not given to dogs receiving oral liquid tregopil. Somatostatin reduces gastrointestinal and hepatic blood flow (14, 15) and slows gastric emptying and intestinal motility, (16) factors that would confound an assessment of tregopil's PK and PD profile. Dogs receiving inhaled and subcutaneous insulin were both briefly anesthetized and intubated to allow for endotracheal insulin delivery or a sham procedure, respectively.(13) Dogs recovered consciousness within 10 minutes of receiving insulin; a period so short insulin sensitivity was not considered to be significantly affected. The dogs receiving oral insulin were not anesthetized. All dogs were studied in the conscious state. At time zero, each dog's insulin bolus was administered based on group assignment and arterial plasma insulin was frequently sampled after insulin delivery.

Animal Care and Surgical Procedures

Conscious dogs of either sex weighing 20–25 kg were studied. Dogs were housed in a surgical facility that met the standards of the American Association for the Accreditation of Laboratory Animal Care guidelines. They were fed a 65–75 kcal/kg/day diet of canned meat and chow (28% protein, 49% carbohydrate, and 23% fat) and the protocols were approved by the Vanderbilt University Medical Center Animal Care Committee. Approximately 16 days prior to an experiment, a laparotomy was performed for the placement of silastic infusion catheters in the jejunal and splenic veins; sampling catheters in the femoral artery, portal vein, and hepatic vein; and ultrasonic flow probes around the hepatic artery and portal vein as described previously (17, 18). All dogs were healthy on the day of study, as evidenced by a leukocyte count $< 18,000/\text{mm}^3$, hematocrit $> 36\%$, good appetite, normal stooling, and healthy physical appearance.

Processing and analysis of samples

Blood samples were collected and processed as described in detail elsewhere (13, 19, 20). Plasma insulin concentrations were determined by radioimmunoassay. The standard curves of human insulin and tregopil were compared and were found to be only minimally different in the range of insulin concentrations studied. In experiments in which oral tregopil was administered endogenous insulin production was somewhat suppressed as indicated by the decline in plasma c-peptide levels making the contribution of endogenous insulin to the total insulin levels less than 30 pmol/L (5 $\mu\text{U/mL}$).

Calculations and Statistical Analysis

Data are presented as means $\pm$ standard error of the mean (SEM) unless indicated otherwise. Net hepatic substrate balances (NHB), non-hepatic glucose uptake, and hepatic sinusoidal hormone concentrations were calculated as described elsewhere (21–23). A positive number for net hepatic glucose balance (NHGB) represents net hepatic glucose output whereas a negative value represents net hepatic glucose uptake. Area under the curve (AUC) was calculated by trapezoidal approximation. Change in AUC (ΔAUC) was determined by

subtracting AUC for the parameter of interest minus the average basal concentration, normalized to equivalent time intervals.

To assess the bioequivalence of intraportal tregopil with human insulin, mean pharmacokinetic (PK) and pharmacodynamic (PD) data were assessed by calculating proportions (human insulin divided by tregopil) and differences (human insulin minus tregopil) for key PK/PD results in Parts 1 and 3. A 90% confidence interval for the ratio of means was calculated using Fieller's theorem,(24) in keeping with FDA guidance.(25) Confidence intervals of proportions and differences were calculated using the GraphPad QuickCalcs Web site: <http://www.graphpad.com/quickcalcs/ConfInterval1.cfm> (accessed June 2017).

The relative bioavailabilities of the 0.25 mg/kg (41.5 nmol/kg) oral dose of liquid tregopil (part 2), the 6 mg (0.27 mg/kg, 45.0 nmol/kg, mean) oral dose of tregopil in tablet form (part 3), and inhaled and subcutaneous insulin doses (part 4) were estimated by comparison to data from a previous study.(26) In the prior experiment, DiCostanzo et al. gave a bolus of human insulin into the portal vein of six dogs over 5 minutes at a rate of 10 mU/kg/min (300 pmol/kg). This study was chosen because the plasma arterial insulin concentration vs. time curves were similar to the tregopil protocols. The estimated bioavailability of oral tregopil relative to an intraportal dose of human insulin is given as:

$$F_{rel} = \frac{AUC_{oral\ tregopil} \cdot D_{HI\ portal}}{AUC_{HI\ portal} \cdot D_{oral\ tregopil}}$$

where $AUC_{oral\ tregopil}$ and $AUC_{HI\ portal}$ are the areas under the curve for the plasma insulin vs. time curves for tregopil and portal insulin of human insulin, respectively. $D_{oral\ tregopil}$ and $D_{HI\ portal}$ are the doses of insulin (in nmol/kg) given in each study.

Statistically significant differences in continuous data in part 2 (maximal insulin concentration [C_{max}], insulin AUC, glucose infusion rate [GIR] AUC, and maximal GIR [GIR_{max}]) were assessed in SPSS 24 using the independent-samples Student t-test. Discrete numeric data (t_{max} , time of maximal GIR [$tGIR_{max}$]) were compared using the Mann-Whitney U test. No statistical tests of differences were made in part 4 because of its exploratory nature. Data are presented as means $\pm$ standard error of the mean (SEM) unless otherwise indicated.

RESULTS

Part 1: Comparison of the Bioeffectiveness of Human Insulin vs. Insulin Tregopil

Pharmacokinetic and pharmacodynamic results for equivalent intraportal infusions of tregopil vs human insulin are summarized in table 1 and depicted in figure 1. Arterial plasma insulin concentrations in the final 30 min of each infusion period and AUC in insulin for each period were slightly higher with tregopil compared to human insulin (figure 1A). This fits with whole-body insulin clearance being modestly lower for tregopil relative to human insulin. Arterial and hepatic sinusoidal glucagon concentrations (data not shown) were basal and no different between groups.

Despite slightly higher arterial plasma insulin concentrations with tregopil during period 1, the GIRs required to maintain euglycemia were nearly identical between the two groups (figure 1D, table 1). During period 2 the insulin infusion rate was increased to 12.0 pmol/kg/min again resulting in modestly higher concentrations of tregopil than human insulin, yet the GIR was again no different with the two insulins. At the liver, both insulins caused a similar change in NHGB (figure 1E). Likewise, non-hepatic glucose uptake increased similarly between both groups (figure 1F).

Part 2: Determine Dose-Response Relationship for Tregopil

After orogastric gavage the pharmacokinetic profile for the 0.0625 and 0.125 mg/kg doses were similar, with no statistically significant difference for insulin $C_{\max}$, $t_{\max}$, AUC_{0-120} , or AUC_{0-45} between the two (supplementary table 1, figure 2A). The pharmacodynamic profile (GIR AUC_{0-120} , $GIR_{\max}$, and $t_{GIR\max}$) of the lower two doses differed minimally (figure 2B). When the larger 0.25 mg/kg dose is compared against the lower two doses, however, statistically significant differences were seen for $C_{\max}$, insulin AUC_{0-120} , insulin AUC_{0-45} , GIR AUC_{0-120} , and $GIR_{\max}$. By virtue of the clamp, no significant difference in arterial plasma glucose existed between the groups (figure 2C).

The estimated relative bioavailability of the 0.25 mg/kg (41.5nmol/kg) oral dose of liquid tregopil was 0.82% when compared to an intraportal bolus of human insulin (300 pmol/kg), which had a similar PK profile in the arterial plasma.(26).

Part 3: Establish Reproducibility of Tregopil's Dosing

Similar pharmacokinetic profiles were found between the first and second treatments when 3 and 6 mg tablets were given as shown in figure 3A-B and supplemental figure 1A and C. When individual pharmacokinetic parameters were assessed, the first 3 mg dose was associated with a slightly lower mean $C_{\max}$ (88%) and mean insulin AUC_{0-120} (79%) compared with the second 3 mg dose (supplementary table 2). On the other hand, a higher $C_{\max}$ and insulin AUC_{0-120} was seen with the first 6 mg dose compared with the second (117% and 118%, respectively). There was no difference in $C_{\max}$ and AUC_{0-120} of tregopil between the first doses of 3 and 6 mg. The 10 mg tablet was associated with an increased response relative to the lower doses. Additionally, there was increased variability and significantly higher $C_{\max}$ and insulin AUC_{0-120} with the first dose compared with the second dose (figure 3C, supplementary table 2). A $t_{\max}$ of 20 min was frequently observed with all doses (supplementary table 2).

The pharmacodynamic profile between the first and second treatment with each dose was similar, but not identical (figure 3 D-F, supplemental figure 1B and D). While $GIR_{\max}$ was minimally different with the two lower doses, the 10 mg tablet clearly produced a larger response (figure 3 D-F, supplementary table 3). These findings are in keeping with the PK data. Mean arterial plasma c-peptide was partially reduced by 30 min regardless of treatment order and regardless of doses (figure 3 J-K). The suppression waned as the insulin level dropped.

The estimated relative bioavailability of the 6 mg (45.0 nmol/kg) oral dose of tregopil in tablet form was 0.85% when compared to the 300 pmol/kg intraportal human insulin bolus. (26).

Part 4: Compare Pharmacokinetic and Pharmacodynamic Characteristics of Oral Tregopil against Subcutaneous and Inhaled Insulin

The average insulin $C_{\max}$ was slightly (15%) lower for liquid oral insulin tregopil compared with inhaled insulin and subcutaneous insulin (supplementary table 4). The rapid median $t_{\max}$ was similar between oral insulin tregopil and inhaled insulin (20 min) while the median $t_{\max}$ for subcutaneous insulin was considerably slower (88 min). Although $C_{\max}$ was nearly similar with the 3 delivery routes, when compared to oral insulin tregopil $GIR_{\max}$ was 3.7-fold higher for subcutaneous insulin and 3.0-fold higher for inhaled insulin. Compared with oral insulin tregopil, the time required to reach $t_{GIR_{\max}}$ was over twice as long with inhaled insulin and nearly four times as long with subcutaneous insulin. As shown in figure 4, the time required for the plasma insulin concentration to return to basal levels was considerably shorter with oral insulin tregopil versus inhaled insulin and subcutaneous insulin (approximately 90 min for tregopil vs. 210 min for inhaled insulin, and 360 min for subcutaneous insulin). The time required for the GIR to be reduced back to a rate near zero was likewise similarly shorter with oral insulin tregopil.

DISCUSSION

The results of this study suggest enterically-delivered tregopil can pharmacologically approximate two key components of physiologic insulin release: rapid entry into the circulating plasma ($t_{\max}$ was 20 min in most cases) and appropriate hepatic portal-to-peripheral circulation balance. The data further illustrate four additional characteristics of insulin tregopil.

1. **When tregopil and human insulin were delivered to the hepatic portal vein at equivalent molar rates, their PK profiles were quite similar and their PD profiles were virtually equivalent.** In part 1, there was a tendency towards reduced clearance of tregopil when compared with human insulin, resulting in modestly higher arterial plasma tregopil concentrations. Tregopil's effect on the liver to lower NHGB and on non-hepatic tissues to stimulate glucose uptake was comparable to human insulin. It follows that the GIRs needed to maintain euglycemia were almost equivalent between tregopil and human insulin. Collectively, this implies that while tregopil's clearance rate is somewhat reduced relative to human insulin this effect is pharmacodynamically offset by a small increase in tregopil's levels and effect at target tissues. Because the GIRs for the two groups were not at a steady state at the study's completion, whether or not the PD profiles would have remained nearly equivalent for longer is uncertain.
2. **A threshold amount of enteric liquid tregopil needs to be exceeded to achieve a significant PD effect.** In part 2, 0.0625 mg/kg of liquid tregopil resulted in a minimal rise in the arterial plasma insulin concentrations above baseline and the 0.125 mg/kg dose saw only a marginal increase.

Correspondingly, the GIRs needed to maintain euglycemia rose in a minimal fashion. When 0.25 mg/kg of liquid tregopil was given, however, a robust increase in arterial plasma insulin was seen in parallel with an increase in GIR. Interestingly, although the mass of the 0.125 mg/kg liquid tregopil dose in part 2 was similar to the mass of 3 mg tablet in part 3 (0.136 mg/kg on average), insulin $C_{\max}$ and AUC_{0-120} were approximately 2-fold higher with tablets (part 3) than liquid (part 2). The current study did not elucidate whether gastrointestinal proteases and peptidases degrade liquid vs. tablets differently.

3. **There was reasonable PK and PD reproducibility for consecutive doses of tregopil.** Increased intra-subject and inter-subject variability has limited broad acceptance oral insulin to date. In large part, this can be attributed to increased intra-subject and inter-subject variability in the response to oral insulin. In part 3, successive doses of tregopil resulted in similar but not identical PK and PD profiles. The 6 mg dose of tregopil had the best reproducibility, where the ratios between the first and second treatment for $C_{\max}$, arterial plasma insulin AUC_{0-120} , GIR AUC_{0-120} ranged between 0.9–1.2. The 10 mg dose was associated with a larger degree of variability for the arterial plasma insulin profile and for the GIR, with higher insulin concentrations with the first dose, but with an earlier and larger peak GIR for the second dose. While not bioequivalent, these data provide early evidence suggesting tregopil's viability as an adjunctive therapy to reduce postprandial hyperglycemia. Tregopil could feasibly supplement rapid-acting subcutaneous insulin in T1DM and endogenous insulin production $\pm$ exogenous insulin in T2DM. Rapid clearance kinetics and first-pass hepatic extraction of tregopil conceivably would dampen and diminish the effect of variable absorption on glucose uptake in peripheral tissues. Additional studies will be needed, however, to characterize the intra-individual variability in tregopil's PK/PD profile. In this investigation, the dogs receiving enteric tregopil had been fasted for at least 18 hours prior to study. Clearly the dosing time before a meal, interval between meals, meal content, and other clinical factors will influence absorption kinetics significantly(27) and will have to be assessed.
4. **The PK and PD profile of oral insulin tregopil was equally or more rapid than those resulting from inhaled and subcutaneous insulin delivery.** In our analysis comparing the three routes of insulin delivery where roughly similar insulin $C_{\max}$ values were reached, tregopil had a $t_{\max}$ that was the same as inhaled insulin and shorter than subcutaneous insulin. Further, both $t_{GIR\max}$ and the time needed for insulin concentrations to return to basal levels were considerably shorter for oral delivery than the other two delivery routes. The exploratory nature of these studies limits the analysis to some extent, however, as the studies were conducted in different dogs under somewhat different experimental conditions (e.g. dogs receiving inhaled and subcutaneous insulin received somatostatin and brief anesthesia while tregopil dogs did not).

Tregopil's rapid onset and absorption into the hepatic portal circulation are two key strengths that hold promise for clinical translation. For T2DM patients, oral insulin therapy intervenes at the level of one of the earliest pathologic deficiencies in the natural history of

the disease: the loss of first-phase insulin secretion.(3, 5) Restoring early-phase insulin arrival in the plasma would mitigate post-prandial hyperglycemia,(4, 12) which would in turn diminish glucotoxicity and secondary β -cell failure.(28)

For individuals with T1DM, tregopil may reduce postprandial hyperglycemia by rapidly switching the liver from glucose production to glucose uptake at the onset of eating. Present therapy relies on subcutaneous insulin to suppress HGP—a role it is pharmacokinetically poorly suited to accomplish—and stimulate glucose disposal in peripheral tissues. Further, many patients wait until *after* eating to deliver a subcutaneous insulin bolus which exacerbates this PK mismatch and worsens postprandial hyperglycemia.(29) Adding tregopil as an adjunct to rapid-acting subcutaneous insulin could transfer the responsibility of inhibiting HGP from subcutaneous insulin to tregopil. Because tregopil more closely approximates rapid, physiologic insulin release, patients would need smaller subcutaneous prandial insulin doses to minimize postprandial hyperglycemia, potentially decreasing iatrogenic hyperinsulinemia and insulin dosing errors. Moreover, since one-third of tregopil is extracted on first-pass by the liver and because its PD effect is short (90 minutes after a dose), tregopil's contribution to stimulating peripheral glucose uptake is small, lessening its contribution to iatrogenic hypoglycemia.

Tregopil's rapid absorption and clearance kinetics could improve postprandial glycemic control during closed-loop automated control of T1DM. An oral dose of tregopil prior to a meal would quickly reduce HGP, lessening the burden placed on the closed loop system to minimize postprandial hyperglycemia. A recent study by Zisser et al. illustrates this principal. The investigators tested how much a priming bolus of inhaled Technosphere insulin—an insulin analog with similarly rapid absorption kinetics (30)—improved a closed-loop system's post-prandial hyperglycemia compared with no priming bolus. The addition of Technosphere insulin reduced median postprandial glycemic peak by 33 mg/dL and increased the median percentage time in range (70–180 mg/dl, BG) by 21.6% during the 5-hour postprandial period.(12) Because tregopil provides a similar absorption profile and the additional benefit of portal entry into the plasma, future investigations are needed to determine whether its use as an adjunct to closed-loop control can provide an even greater benefit.

Further human studies will also need to clarify whether tregopil should complement or replace subcutaneous prandial insulin. In the fasted dogs we tested, enterically-delivered insulin had fast $t_{\max}$ times (~ 20 min in most cases) and off-times (~ 120 min). Although the rapid $t_{\max}$ would likely blunt early hyperglycemia, patients lacking endogenous insulin production would continue absorbing nutrients beyond the rapid tregopil off-time (31) resulting in late hyperglycemia. If tregopil exhibited similar kinetics in these patients, they would still require subcutaneous prandial insulin to avoid late hyperglycemia. Whether the PK/PD profile is favorably modified by pre-meal dose timing, between-meal intervals, and meal content is an area of active investigation. Clinical studies should analyze the cost-to-benefit ratio of a treatment regimen that might reduce postprandial hyperglycemia at the expense of more complex meal dosing.

Another concern is the limited bioavailability of oral insulin formulations to date, which ultimately increases the cost of production to achieve a needed pharmacologic effect. In the present study, the relative bioavailabilities of tregopil in liquid (0.25 mg/kg) and tablet (6 mg=0.26 mg/kg) forms were 0.82% and 0.85%, respectively. The decreased bioavailability additionally suggests substantial amounts of tregopil remain in the gastrointestinal tract. Although gastrointestinal proteases and peptidases likely degrade much of the intraluminal insulin, no studies have characterized how quickly this occurs with tregopil. Because of several studies have correlated high insulin levels with oncologic risk,(32–35) long-term cancer surveillance seems prudent.

In conclusion, these studies show enterically-delivered insulin tregopil achieves two key therapeutic goals towards mimicking physiologic insulin release: rapid entry into the plasma and a physiologic portal-to-peripheral insulin distribution. These characteristics hold promise for mitigating postprandial hyperglycemia by supplementing subcutaneous prandial insulin therapy in T1DM and endogenous insulin secretion in T2DM (and exogenous insulin in many cases). Clinical studies of tregopil are needed to quantify tregopil's ability to reduce postprandial hyperglycemia and to further characterize intra-individual and inter-individual PK and PD variability in “real-life” settings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Mean plasma concentrations over time of: (A) arterial insulin, (B) hepatic sinusoidal insulin, (C) arterial glucose. D) Mean glucose infusion rate. E) Net hepatic glucose balance. (F) Non-hepatic glucose uptake. Data are presented as mean values bounded by the 90% CI. Po = portal, INS = Human insulin, TREG = tregopil.

Figure 2.
 (A) Arterial plasma insulin concentrations, (B) glucose infusion rates, and (C) arterial plasma glucose profiles after receiving liquid tregopil via orogastric gavage at time zero. Data are presented with mean values and SEM.

Figure 3. Pharmacokinetic and pharmacodynamic profiles from successive enteric doses of tregopil. Following an overnight fast, dogs ingested two identical doses of either 3, 6, or 10 mg tablets with 120 minutes separating the first and second doses (Part 3). Note y-axis scale differs with 10 mg dose for arterial plasma insulin (C) and glucose infusion rate (F). Data are presented as mean values bounded by the 90% CI.

Figure 4.

(A) Arterial plasma insulin concentrations, (B) glucose infusion rates, and (C) arterial plasma glucose profiles after receiving inhaled insulin vs. subcutaneous insulin vs. oral liquid insulin tregopil at time zero. Data are presented with mean values and SEM.

Pharmacokinetic and pharmacodynamics results from equivalent intraportal infusions of human insulin vs. tregopil (Part 1). A positive value for NHGB indicates NHG output and a negative value indicates NHG uptake. NHGB represents insulin's ability to suppress NHG output and stimulate NHG uptake. Data are presented as means $\pm$ SEM.

Table 1.

	Sample mean $\pm$ SEM	Estimate and 90% CI for mean ratios (human insulin/tregopil)	Estimate and 90% CI for mean differences (human insulin-tregopil)
Pharmacokinetic results			
Arterial plasma insulin, baseline (pmol/L)	52.7 $\pm$ 8.9	36.9 $\pm$ 6.4	
Arterial plasma insulin, last 30 min of period 1 (pmol/L)	76.2 $\pm$ 4.8	95.9 $\pm$ 9.8	0.79 (0.65 to 1.00)
Arterial plasma insulin, last 30 min of period 2 (pmol/L)	291 $\pm$ 16.8	336 $\pm$ 30.3	0.87 (0.72 to 1.06)
Arterial plasma insulin AUC0-min120 min (nmol min/L)	8.12 $\pm$ 0.60	10.8 $\pm$ 1.4	0.75 (0.58 to 1.02)
Arterial plasma insulin AUC120-240 min (nmol min/L)	32.5 $\pm$ 2.7	35.9 $\pm$ 6.1	0.91 (0.66 to 1.33)
Fractional hepatic insulin extraction, last 30 min of period 1	0.61 $\pm$ 0.02	0.34 $\pm$ 0.04	1.79 (1.48 to 2.25)
Fractional hepatic insulin extraction, last 30 min of period 2	0.52 $\pm$ 0.03	0.30 $\pm$ 0.03	1.73 (1.44 to 2.13)
Insulin clearance, last 30 min of period 1 (mL/kg/min)	50 $\pm$ 3.5	42 $\pm$ 4.0	1.19 (0.98 to 1.47)
Insulin clearance, last 30 min of period 2 (mL/kg/min)	43 $\pm$ 2.5	42 $\pm$ 4.3	1.0 (0.83 to 1.23)
Pharmacodynamic results			
GIR AUC0-120 min (mg/kg)	217.6 $\pm$ 27.3	241.7 $\pm$ 17.0	0.90 (0.68 to 1.15)
GIR AUC120-240 min (mg/kg)	1226 $\pm$ 139	1300 $\pm$ 84	0.94 (0.73 to 1.18)
NHGB, baseline (mg/kg/min)	1.9 $\pm$ 0.3	1.3 $\pm$ 0.2	
NHGB, last 30 min period 1 (mg/kg/min)	-0.15 $\pm$ 0.18	-0.68 $\pm$ 0.16	0.22 (-0.24 to 0.76)
NHGB, last 30 min period 2 (mg/kg/min)	-1.3 $\pm$ 0.4	-1.6 $\pm$ 0.2	0.81 (0.38 to 1.32)
NHGB, baseline to period 1 (mg/kg/min)	2.1 $\pm$ 0.3	2.0 $\pm$ 0.4	1.1 (0.69 to 1.7)
NHGB, period 1 to period 2 (mg/kg/min)	1.1 $\pm$ 0.4	0.9 $\pm$ 0.2	1.2 (0.4 to 2.5)
Non-hepatic glucose uptake, baseline (mg/kg/min)	2.0 $\pm$ 0.4	1.3 $\pm$ 0.2	
Non-hepatic glucose uptake, last 30 min period 1 (mg/kg/min)	1.7 $\pm$ 0.2	2.1 $\pm$ 0.2	0.86 (0.72 to 1.01)
Non-hepatic glucose uptake, last 30 min period 2 (mg/kg/min)	11.0 $\pm$ 1.8	12.6 $\pm$ 0.6	0.87 (0.63 to 1.13)