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Ghrelin Agonist (TZP-101): Safety, Pharmacokinetics and Pharmacodynamic Evaluation in Healthy Volunteers: A Phase I, First-in-Human Study

Kenneth C. Lasseter, MD, Laura Shaughnessy, PhD, David Cummings, MD, John C. Pezzullo, PhD, William Wargin, PhD, Rene Gagnon, PhD, Jesus Oliva, and Gordana Kosutic, MD

The authors evaluate the human safety, tolerability, pharmacokinetics, and pharmacodynamics of TZP-101, an agonist of the hGHS-R1a (ghrelin) receptor. Healthy subjects were randomized to either single-dose TZP-101 (20-600 µg/kg) or placebo by 30-minute intravenous infusion. Subjects underwent continuous cardiac monitoring, 12-lead electrocardiograms, and assessment for orthostatic hypotension, injection site tolerability, vital signs, and adverse events during the 24-hour postdose period. Blood and urine samples were collected for pharmacokinetic/pharmacodynamic assessment for 24 hours. Forty-eight subjects randomly received 1 of 6 TZP-101 doses or placebo. TZP-101 was well tolerated, with single episodes each of headache, lower abdominal pain, diarrhea, and dizziness. At the highest dose, 2 subjects experienced bradycardia. All events were self-limited. Mean arterial

blood pressure and heart rate decreased from baseline approximately 45 to 60 minutes after infusion start at higher doses. No other significant changes were observed. Pharmacokinetic analysis revealed less than dose-proportional behavior of drug with low clearance (≈ 7 mL/h/kg), small volume of distribution (≈ 114 mL/kg), and half-life values of ≈ 13 hours, which were independent of dose. Pharmacodynamic analyses suggested TZP-101, at doses as low as 40 µg/kg, expressed activity at the receptor. TZP-101 displayed a promising pharmacokinetic, pharmacodynamic, and safety profile for use in gastrointestinal motility disorders.

Keywords: Receptors; ghrelin; motility; phase I; tolerability
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Ghrelin, a 28-amino acid peptide, is identified as the natural ligand for the growth hormone (GH) secretagogue (hGHS-R1a) receptor. In addition to stimulation of GH secretion, ghrelin has multiple activities, including stimulation of feeding, weight

increase, and gastrointestinal motility.^{1,2} Mechanistic studies in isolated tissues indicate that ghrelin stimulates and coordinates electrical signaling in the gastrointestinal tract to ultimately promote gut motility.³⁻⁶ This was confirmed in a number of pharmacological assays of gastrointestinal motility in rat and dog.⁷⁻¹⁰ A series of recent investigator-initiated trials in humans demonstrated that ghrelin peptide has significant gastroprokinetic effects in healthy volunteers and in patients with gastroparesis.^{2,11-13} These data suggest that a ghrelin agonist could have potential benefit in the treatment of delayed gastrointestinal motility disorders such as postoperative ileus (POI) and gastroparesis. To use these properties for potential therapeutic benefit, a synthetic agonist

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of the human ghrelin receptor, TZP-101, with enhanced metabolic stability and affinity for the GHS receptor, has been developed.

TZP-101 is a novel macrocyclic small molecule. Its structure features an 18-membered macrocycle containing 3 amide bonds and a secondary amine, as well as 4 stereogenic centers. It belongs to the general category of macrocyclic peptidomimetics.

Repeated testing in various membrane and cell-based assays demonstrates that TZP-101 is a potent, selective agonist at the cloned, human ghrelin receptor (*hGHS-R1a*) with a superior profile to that of previous ghrelin agonists based on its comparatively low propensity to cause ghrelin receptor desensitization.¹⁴ The gastroprokinetic activity of TZP-101 (0.03-3.0 mg/kg, intravenous [IV]) has been demonstrated in animal models of gastric emptying in naive rats and in rat models of delayed gastrointestinal transit caused by high caloric intake (ie, gastroparesis), abdominal surgery (ie, POI), pharmacological insults (ie, morphine), and a combination of both morphine treatment and surgical ileus.^{15,16} In summary, these beneficial effects of TZP-101 in reversing delayed gastrointestinal activity of different etiologies suggest that TZP-101 may have utility in the treatment of surgical ileus, gastroparesis, and other disorders characterized by delayed gastrointestinal activity. When tested for safety in a number of single- and multiple-dose toxicity studies, TZP-101 has not disclosed any specific target organ toxicity.

The aim of this first in-human study was to evaluate the safety, tolerability, and pharmacokinetic and pharmacodynamic profile of escalating doses of TZP-101 when administered as a 30-minute IV infusion to healthy volunteers.

SUBJECTS AND METHODS

Selection of Subjects

This study was performed between January and April 2006 at a single clinical center. The study was approved by Independent Investigational Review Board, Inc (Florida), and all subjects were enrolled after signing the approved informed consent. Subjects were eligible if they were men or women between 18 and 45 years of age, were healthy (ie, had no evidence of acute or chronic health disorders), had no evidence of orthostatic hypotension as defined by the American Autonomic Society and the American Academy of Neurology, were nonsmokers, and had a body mass index (BMI) of 22 to 25, inclusive.

Sexually active women were eligible only if an effective method of contraception was being used, or they were surgically sterile or postmenopausal, which was verified with appropriate medical documentation. Contraception was defined as oral contraceptive pills for at least 3 cycles; a double-barrier method of contraception, including both a physical and a chemical barrier method; or Depo-Provera for at least 1 month.

Study Medication

TZP-101 was supplied to the study center as a 1.0-mg/mL sterile solution in 10-mL vials for IV administration. TZP-101 was diluted with commercially available 5% dextrose in water (D5W) to a total volume of 60 mL and infused at a rate of 2.0 mL/min for a total of 30 minutes. Placebo consisted of D5W alone. The preparation of the study drug for the IV infusion was completed by the pharmacist at the study site. Placebo (D5W) for infusion was prepared and labeled similarly to the TZP-101.

Methods

This was a prospective, randomized, double-blind, placebo-controlled, dose escalation phase I trial conducted at a single center according to the Declaration of Helsinki, Amendment 5 (October 2000).

Dose selection. The initial starting dose of 20 μ g/kg TZP-101 for the study was selected based on a combination of data obtained from the safety pharmacology studies, single- and multiple-dose toxicity studies in 2 species, the estimated pharmacologically active dose from nonclinical efficacy models, and emerging clinical data with ghrelin peptide.^{11,17-19} Calculations were made based on the Food and Drug Administration/Center for Drug Evaluation and Research guidance.²⁰ For the initial dose of 20 μ g/kg, a safety factor of approximately 80 was applied to the human equivalent dose, with that dose level being derived from the no observed adverse effect level (NOAEL) of 3 mg/kg in the 14-day dog toxicity study (on a mg/kg basis corrected for the body surface area). The dose escalation steps for each successive cohort depended on the safety results observed in the preceding dose cohort. The dose range for the trial was 20 to 600 μ g/kg.

Study procedures. Subjects received a standardized low-fat meal for dinner the night prior to dosing, then fasted for a minimum of 10 hours and remained

fasting until 4 hours postdosing. On the day of dosing, subjects received a single 30-minute IV infusion of TZP-101 or placebo. All subjects (with the exception of the subjects receiving the 600- $\mu\text{g}/\text{kg}$ dose) received the study dose between 8:35 AM and 9:10 AM. Subjects randomized to 600 $\mu\text{g}/\text{kg}$ received the dose between 7:00 AM and 10:00 AM. Blood samples were collected for determination of TZP-101 concentrations at 0.25, 0.5, 0.55, 0.60, 0.75, 1, 1.5, 3, 4, 6, 8, 12, and 24 hours after the start of the infusion. In addition, blood was collected for determination of endogenous ghrelin, GH, insulin-like growth factor 1 (IGF-1), insulin, glucose, noradrenaline, and adrenaline levels starting just prior to the infusion and 0.5, 1, 2, and 4 hours after the start of the infusion. Retrospectively for the 320- and 600- $\mu\text{g}/\text{kg}$ dose cohorts, multiple blood samples collected up through 24 hours after the start of the infusion were assayed for IGF-1. Beginning with the start of the infusion, urine samples were collected in aliquots for 24 hours and assayed for TZP-101. Subjects underwent continuous cardiac monitoring for 4 hours with multiple (distributed in parallel with the TZP-101 blood level assessments) 12-lead electrocardiograms (ECGs) during dosing and for the 24-hour postinfusion period. Blood pressure measurements were done to assess orthostatic hypotension just prior to the ECG measurement. Subjects were also observed for adverse events and injection site changes, and their vital signs were examined. Approximately 24 hours postinfusion, following completion of all protocol-required testing and when the subjects' clinical condition was judged to be satisfactory, the subjects were released from the clinical research unit. Each subject was scheduled to return to the clinical research unit on postinfusion study days 3 and 14 (± 2) for a follow-up assessment of safety. Dose escalation proceeded only when the Data Safety Monitoring Group (DSMG) determined that the safety data were satisfactory. The planned single doses of TZP-101 studied were 20, 40, 80, 160, 320, and 600 $\mu\text{g}/\text{kg}$. If the safety data were satisfactory for a TZP-101 dose of up to 320 $\mu\text{g}/\text{kg}$, the final dose was to be proposed and agreed on by the DSMG. However, the maximum dose to be studied was not to exceed 600 $\mu\text{g}/\text{kg}$ (to secure adequate safety margins based on the no adverse effect level observed in the TZP-101 14-day toxicity study in dogs). Although this scheme could have been modified based on safety reviews obtained prior to escalation to each dose cohort, this was not necessary during the conduct of this study.

The following criteria were used as a guide for dose escalation of TZP-101, beginning with 20 $\mu\text{g}/\text{kg}$:

- If there were no drug-related adverse experiences observed or if 1 subject had a drug-related adverse experience that was mild to moderate, the dose of TZP-101 was doubled.
- If 2 subjects had a drug-related adverse experience that was mild to moderate, the dose of TZP-101 was increased by 50%.
- If >2 subjects had a drug-related adverse experience that was mild to moderate, the dose of TZP-101 was increased by 25%.
- If 1 subject had a drug-related adverse experience that was severe in nature, the dose of TZP-101 was increased by 25%.
- If >1 subject had a drug-related adverse experience that was severe in nature, dose escalation of TZP-101 was to stop.

The DSMG remained blinded to treatment assignment throughout dosing and day 3 follow-up period for each dose cohort. However, the review of all safety data was done in an unblinded manner.

Analytical Assays

Following the blood collection for pharmacokinetic and/or pharmacodynamic determinations, the sample tubes were immediately placed on ice. For TZP-101, bioanalysis was performed by Tandem Laboratories, Inc (Salt Lake City, Utah). A validated high-performance liquid chromatography/tandem mass spectrometric (LC/MS/MS) method for the quantitative determination of TZP-101 concentrations in human plasma was used. An aliquot was injected onto the LC/MS/MS system, and TZP-101 and the internal standard (ISTD) NK43233 were extracted from human plasma solid-phase extraction and evaporated to dryness. The residue was reconstituted in an appropriate solvent. The resulting extract was analyzed using a Phenomenex Luna C18(2), 100A, 2 \times 50-mm, 5- μm particle size column and an API 3000 Mass Spectrometer under positive ion mode. For the mass spectrometric detection, the following precursor \rightarrow product ions were monitored: 539.4 \rightarrow 511.4 for TZP-101 and 542.4 \rightarrow 514.4 for NK43233 (ISTD). The interbatch precision and accuracy of the plasma assay, as assessed from the coefficients of variation of the quality control samples analyzed during the test sample analysis, were in the ranges of 3.8% to 6.1% and 0.5% to 2.0% ($n = 22$), respectively, over the concentration range of 15 to 4000 ng/mL. The lower limit of quantification was 5.0 ng/mL, with precision and accuracy at this level, determined from the back-calculated concentrations of calibration standards, of 7.8% and 2.4% ($n = 18$),

Table I Analytical Methods for Pharmacokinetic and Pharmacodynamic Sample Analysis

Parameter	Lab	Methods
Growth hormone	Quest Diagnostics, San Juan Capistrano, California	Immunochemiluminometric assay
Insulin-like growth factor 1	SFBC International, Miami, Florida	Immunoenzymometric assay
Ghrelin	University of Washington Seattle, Washington	Radioimmunoassay
Glucose	SFBC International, Miami, Florida	Enzyme assay
Insulin	SFBC International, Miami, Florida	Radioimmunoassay
Adrenaline	Quest Diagnostics, San Juan Capistrano, California	High-performance liquid chromatography (HPLC), electrochemical detection
Noradrenaline		
TZP-101	Tandem Laboratories, Salt Lake City, Utah	HPLC coupled with tandem mass spectrometry

respectively. This method measured total (bound plus free) TZP-101 concentrations in plasma.

For GH, glucose, insulin, and IGF-1 analyses, a serum sample was prepared by allowing the blood to clot at room temperature for 30 to 45 minutes, followed by centrifugation at 3000 rpm for 15 minutes at 4°C and storage of serum at -70°C. The plasma sample for analysis of adrenaline, noradrenaline, and ghrelin was prepared by collecting the blood in an EDTA tube and incubating for no more than 30 minutes, followed by centrifugation at 3000 rpm for 15 minutes at 4°C and storage of plasma at -70°C. Analytical methods used in the study are summarized in Table I.

Statistical Analysis

The safety analysis of this study was conducted in the safety population, which was defined as all subjects who were randomized and received any dose of study medication (TZP-101 or placebo). Data summaries consisted of descriptive statistics (counts, means, standard deviations, medians, and minimum and maximum values) for continuous variables and frequencies and percentages for discrete variables. Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 8.0. Orthostatic hypotension was defined, according to the American Autonomic Society and the American Academy of Neurology, as a systolic blood pressure decrease of at least 20 mm Hg or a diastolic blood pressure decrease of at least 10 mm Hg within 3 minutes of standing up.

Interim analysis. After all subjects in each cohort were enrolled and had completed the study day 3 follow-up visit assessment, the data were unblinded for the review. The data listings included only the

raw subject data; no derivations or summaries were performed. The DSMG reviewed the safety data, in the context of overall exposure to the study drug, prior to determination of the next dose level to be studied. Five interim safety reviews were held for this dose-escalating safety study.

Sample size. The sample size in this study was selected empirically. The chosen number of subjects was considered sufficient to adequately characterize safety and pharmacokinetics of escalating doses of TZP-101. Eight subjects (TZP-101: placebo, 6:2) were enrolled for each dose cohort.

Pharmacokinetic analysis. Noncompartmental methods were used to analyze the plasma TZP-101 concentration data. WinNonlin Version 5.1 (Pharsight Corp, Cary, North Carolina) was used for the analysis and generation of descriptive statistics. Noncompartmental parameters included the observed peak plasma drug concentration (C_{max}), the area under the plasma concentration-time curve to the last sampling time at 24 hours and extrapolated to infinity after the single dose (AUC_{0-24h} , $AUC_{0-\infty}$), the terminal elimination rate constant (λ_z), the associated half-life ($t_{1/2}$), the volume of distribution (V_z) based on the terminal elimination rate, and the systemic clearance (CL).

Pharmacodynamic analysis. For each parameter, an area under the concentration-time curve (AUC) from 0 to 4 hours after the start of TZP-101 IV infusion was calculated by the trapezoidal rule. No terminal extrapolation to infinite time was performed. The AUC was plotted against the concentration of TZP-101, and a linear model was tested, using both the AUC and the logarithm of AUC. The Pearson correlation coefficient was calculated and tested for a significant difference from 0. The trend test was performed on all doses from 0 (placebo) to 600 µg/kg.

For IGF-1, analysis was also performed to evaluate the relationship between TZP-101 infusion and IGF-1 levels up to 24 hours after the infusion. The effect was quantified by the IGF-1 AUC from 0 to 24 hours, correcting for baseline (predose) IGF-1. The value of the corrected AUC was compared for the 320- $\mu\text{g}/\text{kg}$ dose versus placebo, as well as for the 600- $\mu\text{g}/\text{kg}$ dose versus placebo, by the Welch *t* test (Student *t* test with equal variances not assumed).

RESULTS

A total of 48 healthy subjects were enrolled in the study and randomized to either 1 of 6 TZP-101 doses or placebo in a dose escalation manner. The mean age of the study subjects was 33.5 years, with a range of 19 to 46 years. Most subjects were women (66.7%). The predominant ethnicity of the study population was Hispanic or Latino (89.6%), and the majority of the population was white (81.3%). Mean height and weight of the study subjects were 165.31 cm and 64.63 kg, respectively, with a calculated mean BMI of 23.57 (range, 22.0-25.1). All 48 subjects completed all procedures that were required by the study protocol, and the data from all 48 were analyzed for safety, pharmacokinetic, and pharmacodynamic properties of TZP-101.

Tolerability and Safety

A total of 9 subjects (18.8%) enrolled in the study experienced at least 1 treatment-emergent adverse event. All treatment-emergent adverse events were temporary, were mild in severity, and did not require any intervention. Of the 14 adverse events experienced by these 9 subjects, 5 were considered by the investigator to be unrelated to study drug. There was no indication of increased frequency of adverse events with increasing dose of TZP-101. Bradycardia was experienced by 2 subjects in the 600- $\mu\text{g}/\text{kg}$ dose group, compared with no subjects in any other dose group, including placebo. One of those 2 subjects also experienced dizziness. One of the 2 subjects had, on ECG, a pre-TZP-101 infusion heart rate of 47 bpm. The subject's heart rate fell to 41 bpm at the 45-, 60-, and 75-minute readings and was accompanied by dizziness. Heart rate had risen to 46 bpm at 4 hours and to 64 bpm at 12 hours after the start of the infusion. The second subject had, on ECG, a pre-TZP-101 infusion heart rate of 55 bpm. The subject's heart rate fell to 45 bpm at the 45-minute reading but had returned to 57 bpm at the 4-hour reading. No other individual adverse events appeared related to dose.

There were no serious adverse events during the study, and no subjects were discontinued from the study due to adverse events. No subjects treated with TZP-101 met the criteria for orthostatic hypotension. In the ECG analysis, there were no notable dose-related trends in mean changes from the baseline in any of the parameters analyzed (PR, QRS, P duration or QTc interval, and PRT axes). The exception was an overall trend toward a dose-dependent decrease in heart rate at 30 through 90 minutes after the start of the infusion. The decrease was most notable for the 320- and 600- $\mu\text{g}/\text{kg}$ doses (Table II).

Pharmacokinetics

Mean plasma TZP-101 concentration-time data are plotted by dose in Figure 1, and noncompartmental pharmacokinetic parameters for TZP-101 are summarized in Table III. The maximum plasma concentration increased with dose, ranging from 497 to 8070 ng/mL over the dose range of 20 to 600 $\mu\text{g}/\text{kg}$. The average $\text{AUC}_{0-\infty}$ increased from 4710 to 72 200 h·ng/mL over the same dose range. Both the C_{max} and $\text{AUC}_{0-\infty}$ tended to increase linearly between 20 and 80 $\mu\text{g}/\text{kg}$, but in a less than linear fashion between 160 and 600 $\mu\text{g}/\text{kg}$. However, the overall mean $t_{1/2}$ was approximately 13 hours and was independent of the administered dose. Systemic CL was low, with average values ranging from 5.1 to 10.6 mL/h/kg over the dose range. V_z values were small, ranging from 0.084 to 0.160 L/kg. Both CL and V_z increased 2-fold over the 20- to 600- $\mu\text{g}/\text{kg}$ dose range.

Pharmacodynamics

The analysis of $\text{AUC}_{0-4 \text{ h}}$ versus TZP-101 concentration indicated that a single 30-minute IV infusion of TZP-101 across the evaluated doses did not affect levels of IGF-1 ($r = 0.12$, $P = .417$), endogenous ghrelin ($r = -0.078$, $P = .600$), glucose ($r = 0.260$, $P = .074$), or noradrenaline ($r = -0.012$, $P = .935$) up to 4 hours after the start of the infusion, as shown in Figure 2B, C, D, and G, respectively. However, 24-hour evaluation of IGF-1 levels following administration of 320 and 600 $\mu\text{g}/\text{kg}$ TZP-101 showed an increase in the IGF-1 plasma levels when compared with placebo ($P = .014$ and $P = .023$ for 320 and 600 $\mu\text{g}/\text{kg}$, respectively; Figure 3). During the observation period, up to 4 hours after the start of the infusion, a statistically significant, dose-dependent, temporary increase in plasma GH level ($r = 0.767$, $P = .001$) was observed in addition to a dose-dependent decrease in insulin ($r = -0.418$, $P = .003$) and an increase in

Table II Mean (\pm SD) Change From Baseline Heart Rate for Up to 4 Hours After TZP-101 IV Infusion

Time Point	TZP-101 Dose Group						
	20 μ g/kg (n = 6)	40 μ g/kg (n = 6)	80 μ g/kg (n = 6)	160 μ g/kg (n = 6)	320 μ g/kg (n = 6)	600 μ g/kg (n = 6)	Placebo (n = 12)
Baseline, mean (\pm SD)	64.5 (4.59)	66.7 (8.76)	62.7 (6.12)	53.3 (3.67)	62.7 (6.44)	62.0 (9.34)	60.2 (8.63)
5 minutes	3.0 (5.14)	0.2 (6.24)	1.5 (1.87)	0.5 (1.05)	1.0 (3.74)	2.2 (7.00)	1.8 (4.75)
15 minutes	1.0 (4.60)	2.3 (3.72)	2.3 (4.72)	-1.0 (1.67)	-1.5 (2.59)	1.7 (6.22)	3.4 (6.30)
30 minutes	1.0 (2.76)	-1.3 (4.41)	1.2 (1.72)	-0.8 (0.75)	-1.7 (7.15)	-3.7 (4.08)	1.0 (4.41)
45 minutes	2.2 (2.32)	-4.2 (3.06)	2.3 (6.35)	-1.7 (3.72)	-4.5 (4.97)	-8.5 (3.02)	-1.6 (3.99)
60 minutes	-1.3 (3.50)	-0.7 (3.67)	3.5 (5.21)	-2.2 (3.06)	-4.5 (6.80)	-7.7 (1.21)	0.3 (6.02)
75 minutes	1.7 (4.27)	-0.7 (3.83)	-1.0 (3.85)	-0.3 (4.08)	-2.5 (7.45)	-8.0 (3.52)	0.6 (4.21)
90 minutes	2.0 (4.98)	-1.8 (5.53)	1.0 (3.85)	-1.2 (2.32)	-3.8 (5.71)	-7.0 (2.53)	-0.6 (4.91)
240 minutes	-1.2 (4.49)	1.0 (3.74)	-0.2 (6.34)	1.7 (4.63)	0.7 (5.47)	1.8 (3.71)	-0.8 (4.59)

Table III Summary of Noncompartmental Pharmacokinetic Parameters for TZP-101

Parameter	Statistics	TZP-101 Dose Level (μ g/kg)					
		20	40	80	160	320	600
C_{\max} , ng/mL	Mean	497	908	1910	3370	6730	8070
	SD	73.1	109	140	379	1840	3070
	%CV	14.7	12.0	7.32	11.3	27.4	38.1
AUC_{0-24} , h·ng/mL	Mean	3510	6370	11 900	21 100	44 500	53 300
	SD	1000	1350	3320	4230	19 900	25 500
	%CV	28.6	21.1	34.6	20.0	44.8	47.8
$t_{1/2\lambda z}$, h	Mean	12.6	12.0	11.5	12.8	15.7	11.9
	SD	2.70	2.84	1.95	0.755	9.00	3.96
	%CV	21.5	23.7	16.9	5.89	57.2	33.2
$AUC_{0-\infty}$, h·ng/mL	Mean	4710	8410	15 300	27 600	71 000	72 200
	SD	1640	2470	5770	6380	50 400	42 800
	%CV	34.9	29.4	37.7	23.1	71.0	59.4
CL, mL/h/kg	Mean	5.05	5.12	5.78	6.05	6.62	10.6
	SD	2.82	1.54	1.82	1.32	4.18	5.03
	%CV	56.1	30.0	31.5	21.8	63.2	47.6
V_z , L/kg	Mean	0.084	0.084	0.092	0.111	0.116	0.160
	SD	0.028	0.012	0.018	0.019	0.033	0.043
	%CV	33.3	14.0	19.4	16.7	27.9	26.6

CV, coefficient of variation.

adrenaline levels ($r = 0.553$, $P = .001$; Figure 2A, E, and F, respectively).

DISCUSSION

This was the first study designed to evaluate the safety, pharmacokinetics, and effects on selected pharmacodynamic parameters of TZP-101 in humans. To evaluate multiple doses of TZP-101, this phase I study was performed in an escalating dose

fashion, with careful monitoring of multiple safety parameters at each dose increase. No acute serious or life-threatening adverse experiences were seen during the course of this study. In fact, almost all previously described adverse experiences were of minimal clinical significance and without consequence. Special attention was paid to cardiovascular findings in this first-in-man study with TZP-101. Multiple ECGs performed on each of the study subjects did not suggest dose- or compound-related

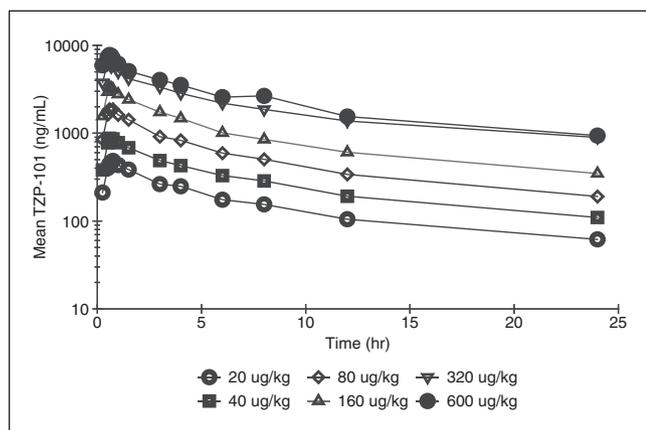


Figure 1. Mean plasma TZP-101 concentration-time profiles after single intravenous (IV) administration of TZP-101 doses of 20, 40, 80, 160, 320, and 600 $\mu\text{g}/\text{kg}$ ($n = 6/\text{group}$).

(when compared with the placebo-treated subject data) trends in mean changes from the baseline in any of the parameters analyzed (PR, QRS, P duration or QTc interval, and PRT axes). The temporary dose-dependent decrease in heart rate coincided closely with the maximum plasma concentration of TZP-101 (around 45 minutes after the initiation of the infusion). The dose-dependent decrease in mean arterial blood pressure observed in this study was previously reported by Nagaya et al for ghrelin peptide when administered as an intravenous infusion both in healthy volunteers¹⁷ and in patients with congestive heart failure.¹⁸ They reported that the decrease in heart rate was not accompanied by an increase in heart rate or elevation in the noradrenaline plasma levels. Nagaya et al speculated that ghrelin may have a direct vasodilator effect on blood vessels and that it inhibits activation of the sympathetic nervous system during hypotension. In our opinion, the temporary decrease in heart rate and mean arterial blood pressure observed in the present study most likely represents an up-regulation of the parasympathetic nervous system and not a down-regulation of the sympathetic nervous system (none of the subjects experienced orthostatic hypotension during multiple assessments done in parallel with the ECGs).

With the respect to pharmacokinetics, both the C_{max} and $\text{AUC}_{0-\infty}$ values increased with increasing dose. The increase was proportional up to the 80- $\mu\text{g}/\text{kg}$ dose, but for the 160- to 600- $\mu\text{g}/\text{kg}$ doses, the increase was less than dose proportional. Previously conducted plasma protein binding studies (data in-house) have shown TZP-101 to be highly bound to $\alpha 1$ -acid glycoprotein. The protein binding is concentration dependent, with the percentage bound ranging from

99.8% to 75.1% over the TZP-101 concentration range of 10 to 100 μM (5931-59310 ng/mL). As a result, the free (unbound) concentration increases in the presence of increasing TZP-101 concentration, resulting in higher concentration of free drug being available for elimination and an apparent increase in the CL and V_z when total TZP-101 plasma concentration is measured. The binding to $\alpha 1$ -acid glycoprotein also has implications for the volume of distribution of TZP-101, retaining the drug in the central compartment and thus limiting the distribution to peripheral tissues. All 36 subjects receiving TZP-101 had measurable drug concentrations in plasma 24 hours postdosing.

In future studies, additional time points may help to better assess the elimination phase of the parent drug and aid in calculating a more precise half-life. The estimated half-life was approximately 13 hours and did not suggest a dose-dependent pattern. This rather long half-life may allow for a decreased frequency of dosing in future clinical settings. Furthermore, only a very small concentration of unchanged TZP-101 was found in urine, suggesting a principal hepatic route of elimination. Characterization of the metabolites was not done in this study. However, as this is necessary for adequate characterization and future development of the compound, the bioanalytical assays are currently under development, and data will be analyzed for metabolites retrospectively. Due to the unbalanced numbers between genders enrolled in the study, we could not confirm earlier animal data showing no gender differences in pharmacokinetic behavior of the compound. The 30-minute infusion of TZP-101 induced a dose-dependent GH release in the healthy volunteers. The elevation of GH persisted for several hours and then returned to baseline values, although TZP-101 had detectable plasma levels for 24 hours after the infusion at all of the administered doses. The GH-releasing effects of ghrelin are thought to be mediated by specific receptors, GHS-R, mainly present in the pituitary and hypothalamus. It has been shown that TZP-101 penetration through the blood-brain barrier is minimal (unpublished in-house data). However, the pituitary gland is a highly vascularized peripheral organ outside the blood-brain barrier, with both arterial and venous blood supplies most likely allowing for TZP-101 delivery to the anterior pituitary gland. IGF-1 did not follow the increase in GH circulating levels during the 4-hour observation period. A similar observation was reported by Nagaya et al.^{17,18} All subjects enrolled in the study were fasted for 12 hours before the dosing. As suggested by previous data,^{21,22} fasting may alter GH responses to ghrelin

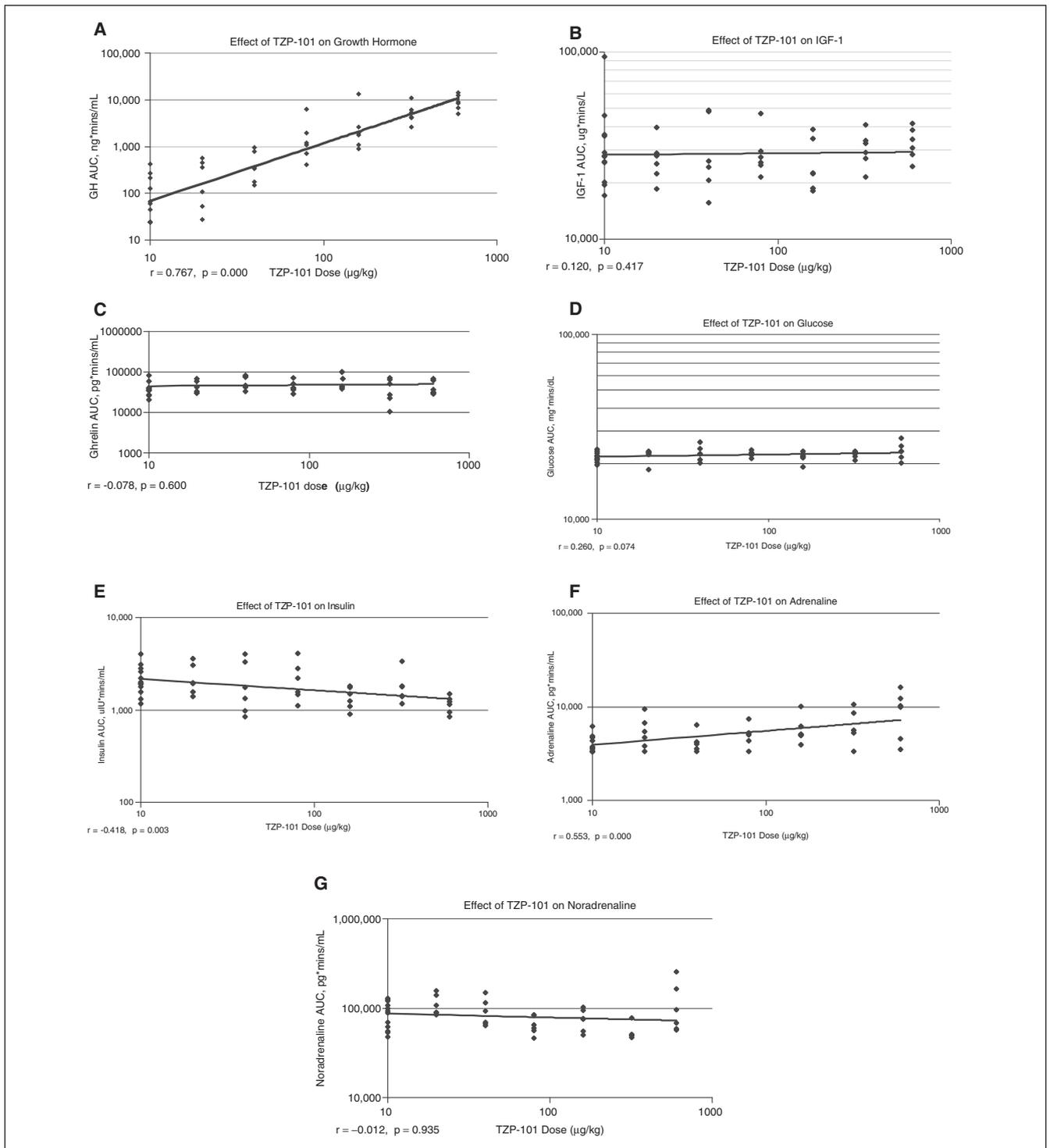


Figure 2. Logarithmic AUC_{0-4h} across logarithmic TZP-101 concentration for (A) growth hormone, (B) insulin-like growth factor 1 (IGF-1), (C) ghrelin, (D) glucose, (E) insulin, (F) adrenaline, and (G) noradrenaline. Up to 4 hours following TZP-101 infusion, there was a significant increase in growth hormone (GH; $r = 0.767, P = .001$) and adrenaline ($r = 0.553, P = .001$) and a significant decrease in insulin ($r = -0.418, P = .003$), but TZP-101 had no effect on IGF-1, ghrelin, glucose, and noradrenaline ($r = 0.12, P = .417$; $r = -0.078, P = .600$; $r = 0.260, P = .074$; $r = -0.012, P = .935$, respectively). AUC = area under concentration-time curve; GH = growth hormone.

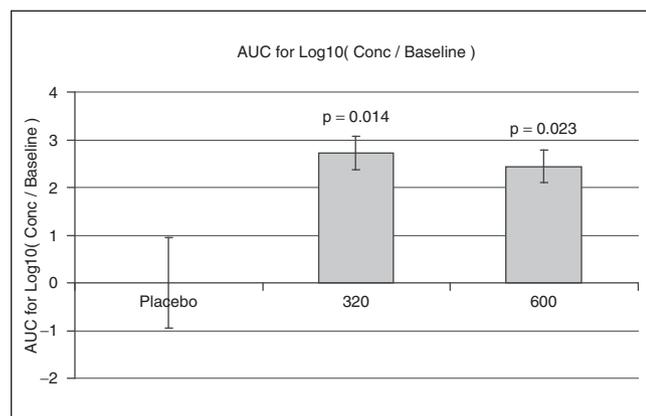


Figure 3. AUC_{0-24h} for IGF-1 following 320- and 600- $\mu\text{g}/\text{kg}$ TZP-101 infusions. The 24-hour evaluation of insulin-like growth factor 1 (IGF-1) levels following administration of 320 and 600 $\mu\text{g}/\text{kg}$ revealed a statistically significant increase in the hormone levels (when corrected for the baseline levels) compared with placebo ($P = .014$ and $P = .023$ for 320 and 600 $\mu\text{g}/\text{kg}$, respectively). AUC = area under concentration-time curve.

and contribute to the uncoupling of the GH/IGF-1 axis in study subjects. As described by Chen et al,²³ fasting induces reduction in both free and bioactive IGF-1, and circulating free IGF-I is considered to be the most important component of the circulating IGF system in the feedback regulation of GH. As 24-hour data collected for the 2 highest dose levels suggested a trend toward the IGF-1 increase, the speculation could be also made that the 4-hour period was not long enough to observe the change in IGF-1 levels.

Our data have shown a significant increase in plasma adrenaline but not noradrenaline level. Considering the presence of GHS-binding sites in the adrenal gland,²⁴ the adrenaline-releasing effect of TZP-101 may be due to direct effects on the adrenal. In the present study, we also observed a trend that TZP-101 induced a dose-dependent decrease in areas under the curves for insulin. The fact that GHRs are also expressed in pancreatic islets²⁵ suggests that ghrelin is involved in the regulation of islet function. Studies performed by others so far have, however, resulted in conflicting results, showing both that ghrelin inhibits insulin secretion^{26,27} and that ghrelin stimulates insulin secretion.^{25,28} More recently, Heijboer and colleagues²⁹ have shown in a hyperinsulinemic/euglycemic clamp rat model study that ghrelin hampers insulin's capacity to suppress endogenous glucose production, whereas it reinforces the action of insulin on glucose disposal due to an increase in insulin sensitivity. However, the mechanism whereby ghrelin and/or TZP-101 affect insulin behavior remains to be established.

CONCLUSION

Single doses of TZP-101 were well tolerated in healthy individuals, and with the possible exception of bradycardia, no adverse event appeared to be dose related. The pharmacokinetic profile of TZP-101 is predictable and long lasting. This makes the drug particularly suitable for applications where single daily dosing is desirable. The good drug properties of the compound and the data suggesting that TZP-101, an agonist for the ghrelin receptor, expresses activity at a receptor level at doses as low as 40 $\mu\text{g}/\text{kg}$ justify further clinical investigation.

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