

Contribution of Protein Binding to the Pharmacokinetics of the Ghrelin Receptor Agonist TZP-101 in Healthy Volunteers and Adults with Symptomatic Gastroparesis

Two Randomized, Double-Blind Studies and a Binding Profile Study

William Wargin,¹ Helmut Thomas,² Lilian Clohs,² Carl St-Louis,² Niels Ejksjaer,³ Maria Gutierrez,⁴ Laura Shaughnessy² and Gordana Kosutic²

1 PK-PM Associates, LLC, Research Triangle Park, North Carolina, USA

2 Tranzyme Pharma, Inc., Durham, North Carolina, USA

3 Aarhus University Hospital, Aarhus, Denmark

4 Comprehensive Phase One, Miramar, Florida, USA

Abstract

Background and objective: TZP-101 is a selective, small molecule ghrelin receptor agonist in clinical development for the treatment of gastric motility disorders. The objectives of this study was to assess pharmacokinetic parameters of TZP-101 after multiple- and single-dose administration to healthy subjects and patients with gastroparesis, respectively, and to determine the contribution of protein binding to its pharmacokinetic behaviour.

Methods: Pharmacokinetics following 30-minute intravenous infusions of single (160–600 µg/kg) doses of TZP-101 in patients with gastroparesis and multiple (80–600 µg/kg/day) doses of TZP-101 in healthy subjects were characterized. TZP-101 protein binding was measured in human, dog, rat, rabbit and monkey plasma using equilibrium dialysis.

Results: TZP-101 pharmacokinetic profiles were less than dose proportional in both healthy subjects and patients, most likely because of concentration-dependent protein binding. A small volume of distribution (99–180 mL/kg following single doses) and long half-life (10–20 hours) were concentration independent in both healthy subjects and patients. Systemic clearance increased with increasing dose. Incidence of adverse events was not related to dose or treatment (active vs placebo). TZP-101 binding to human plasma proteins (primarily α_1 -acid glycoprotein) was $\geq 99\%$ between 5 and 15 µmol/L (2.7 and 8.1 µg/mL) and was significantly higher than in other species.

Conclusions: The pharmacokinetic parameters of TZP-101 in patients with gastroparesis and healthy subjects are comparable and display a similar trend toward increased clearance at higher dose levels resulting in little accumulation of TZP-101 at high dose levels and after multiple dosing. Significant protein binding indicates that the fraction of free drug rather than the total plasma concentration should be taken into consideration for human risk assessment based on animal safety data. Furthermore, the concentration of unbound drug should be considered when optimizing the clinical dose.

Background

Ghrelin is the natural ligand for the growth hormone secretagogue receptor (GHSR-1a) and is locally produced in the gastric mucosa.^[1] In addition to stimulating GH secretion, the ghrelin receptor pathway mediates multiple gastrointestinal (GI) functions, including motility.^[2,3] Ghrelin stimulates neuronal signaling in the GI tract to promote gastroprokinetic responses in individuals with compromised gastric function.^[4-6] Therefore, ghrelin receptor agonists may have potential benefit in the treatment of acute and chronic delayed GI motility disorders such as gastroparesis and postoperative ileus.

Treatment options for gastroparesis, a chronic disorder of gastric motility with increased prevalence in patients with diabetes mellitus,^[7] remain limited, particularly for patients with severe symptoms.^[8,9] Postoperative ileus, which frequently occurs following abdominal surgery and is exacerbated by opioid use for pain management, is a transient condition that contributes to patient morbidity and prolonged hospital stays.^[10,11] Treatments that are effective when used chronically or episodically are needed for management of these dysmotility disorders.

TZP-101 [(4R,7S,10R,13R)-7-cyclopropyl-13-(4-fluorobenzyl)-3-oxa-6,9,12,15-tetraaza-4,9,10-trimethyl-4,5,6,7,10,12,13,15,16,17,18-undecahydro-1,2-benzocyclooctadecene-8,11,14-trione] is a selective, small molecule ghrelin receptor agonist in clinical development as a treatment for gastric dysmotility disorders. This macrocyclic peptidomimetic represents the first of a new class of ghrelin receptor agonists that do not dupli-

cate any portion of the sequence of ghrelin, nor include the octanoyl moiety, or surrogate group, required for activity of the endogenous hormone. TZP-101 has enhanced metabolic stability and high affinity (equilibrium dissociation constant [K_i] 22 nmol/L) for the human type 1a GHSR compared with ghrelin,^[12] and shows prokinetic activity in animal models of dysmotility.^[13] TZP-101 is well tolerated in healthy subjects and patients with gastroparesis when administered as single doses ranging from 20 to 600 $\mu\text{g}/\text{kg}$.^[14,15] TZP-101 produced significant reductions in solid meal half-emptying and latency times in patients with diabetes and symptomatic gastroparesis.^[15] A single-dose pharmacokinetic study in healthy subjects showed that the pharmacokinetic profile of TZP-101 was not dose proportional at doses exceeding 160 $\mu\text{g}/\text{kg}$.^[14]

The objectives of the present study were to assess the pharmacokinetic parameters of TZP-101 after multiple- and single-dose administration to healthy subjects and patients with gastroparesis, respectively, and to determine the contribution of protein binding to the pharmacokinetic behaviour of this agent.

Subjects and Methods

Study Participants

One randomized, double-blind, multiple-dose, dose-escalation study enrolled healthy men and women aged 18–45 years from a single centre in the US between August and October 2006 (protocol CL-003). In a second randomized, double-blind, single-dose, crossover study, men and

women aged 18–65 years with type 1 or 2 diabetes and a diagnosis of gastroparesis were enrolled at two centres in Denmark and one centre in Sweden from October 2006 to July 2007 (protocol CL-002). Both studies were conducted according to the Declaration of Helsinki, Amendment 5 (October 2000). Independent ethics committees and independent review boards approved the protocols and all subjects and patients provided written informed consent.

Healthy subjects were eligible if they had no evidence of acute or chronic illness, were non-smokers, and had a body mass index (BMI) between 22 and 25 kg/m². Eligible gastroparesis patients were required to have normal upper GI tract endoscopy results, no serious comorbidities, and gastroparesis characterized by delayed gastric emptying and a 3-month history of chronic upper abdominal discomfort with two or more of the following symptoms: postprandial fullness, bloating, epigastric discomfort, early satiety, belching after meals, and nausea and vomiting.

In both studies, eligible women were postmenopausal or permanently sterilized, or used approved methods of contraception. Exclusion criteria included use of any investigational drug within the preceding 30 days and clinically significant drug hypersensitivity.

Clinical Study Procedures

Healthy subjects under fasting conditions received daily 30-minute intravenous infusions of TZP-101 (80, 320 or 600 µg/kg) or placebo for five consecutive days. Patients with gastroparesis received 30-minute intravenous infusions of one TZP-101 dose (160, 320 or 600 µg/kg). For both studies, dose adjustments in subjects were not initiated until a review of the safety data associated with the previous dose level had been completed. Dose selection was based on a previous single-dose safety and pharmacokinetic study in healthy subjects.^[14] In this study, TZP-101 was well tolerated at doses of 20, 40, 80, 160, 320 and 600 µg/kg.

During the multiple-dose study in healthy subjects, blood samples were collected for

measurement of TZP-101 concentrations prior to the start of the infusion and 0.5, 0.75, 1.0, 1.5, 2, 4, 6, 12 and 24 hours after the start of the infusion on dosing days 1 and 5. Additional samples were collected at 48 and 72 hours after receiving the last TZP-101 dose on day 5. Daily samples for trough concentration assessment were taken prior to the start of infusion of study drug on days 2, 3 and 4. Urine samples were collected and assayed for TZP-101 concentrations prior to dosing on day 1 (0 hour) and at intervals from 0–4, 4–8, 8–12 and 12–24 hours after the start of the day 5 infusion. A 72-hour faecal collection for assessment of the amount of TZP-101 eliminated in the faeces began at the start of the infusion on day 5 and finished just prior to discharge on day 8.

In the single-dose study in gastroparesis patients, blood samples for the measurement of plasma TZP-101 concentrations were obtained prior to the start of infusion and 0.25, 0.75, 1.5, 2, 3, 6, 12 and 24 hours after the start of the infusion.

Safety

Vital signs, 12-lead ECGs and adverse events were recorded during the 24-hour post-dose periods in the clinic and at follow-up (3 and 13 days after final dosing day for patients with gastroparesis and healthy subjects, respectively). Haematology and clinical chemistry assessments were made at the follow-up visit. All randomized healthy subjects and gastroparesis patients who received at least one dose of study medication were evaluated for safety.

Bioanalytical Methods

A high-performance liquid chromatographic method using tandem mass spectrometric detection (HPLC-MS/MS) with a lower limit of quantification (LLQ) of 20 ng/mL and effective working range between 20 and 20 000 ng/mL was validated for specific quantification of TZP-101 in human plasma (Tandem Laboratories, Inc., Salt Lake City, UT, USA) as previously described.^[14] An LC-MS/MS method was also used with specificity for quantification of total

TZP-101 in human urine and faeces (Tandem Laboratories, Inc., Salt Lake City, UT, USA). The LLQ for urine samples was 2 ng/mL and the effective working range of the assay was between 2 and 2000 ng/mL. For faecal samples, the LLQ was 20 ng/g and the effective working range of the assay was between 20 and 20 000 ng/g.

Protein Binding

The protein-binding profile of TZP-101 was evaluated in human, cynomolgus monkey, beagle dog, New Zealand white rabbit and Sprague-Dawley rat plasma and in 1 mg/mL α_1 -acid glycoprotein (AAG) and 4% human serum albumin (HSA) [AAG and HSA were purchased from Sigma, St Louis, MO, USA]. Plasma or isolated human protein solutions were spiked with TZP-101 at concentrations ranging from 1 to 100 μ mol/L. Plasma or solutions of purified plasma protein containing TZP-101 were dialysed in RED (Rapid Equilibrium Dialysis) devices (Pierce, Rockford, IL, USA) against an isotonic phosphate buffer at pH 7.4 for 6 hours at 37°C. The buffer (dialysate) and plasma (retentate) samples were prepared for analysis by solid phase extraction, and TZP-101 concentrations were determined in triplicate samples by HPLC with MS detection under standard industry conditions for acceptance criteria.

TZP-101 and internal standard peaks were integrated using MassLynx™ software version 4.0 (Waters Corp, Milford, MA, USA). Concentrations were calculated using the QuantLynx™ feature of the MassLynx™ software based on quadratic regression of the calibration curves (weighted 1/x) using the peak area ratio of analyte to internal standard. The bound fraction of TZP-101 was calculated from TZP-101 concentrations measured in the buffer (dialysate) and plasma (retentate) compartments at equilibrium. Data are presented as the mean of three determinations.

Pharmacokinetic Analyses

Sample sizes for study participants were selected empirically to adequately characterize the

pharmacokinetics of single and multiple doses of TZP-101.

Plasma TZP-101 concentration-time data were analysed using a two-compartment pharmacokinetic analysis with WinNonlin® Professional version 5.1 (Pharsight Inc., Mountain View, CA, USA). Parameters for analysis of the single-dose study included the observed peak plasma drug concentration (C_{max}), the area under the plasma concentration-time (AUC) curve to the last sampling time at 24 hours (AUC_{last}) and extrapolated to infinity after the single dose (AUC_{∞}), the terminal elimination rate constant (λ_z), the associated half-life ($t_{1/2}$), the volume of distribution based on the terminal elimination rate (V_z), and systemic clearance (CL). The 5-day plasma profile of the multiple-dose study was used to calculate the following parameters, assuming steady-state conditions: the observed maximum and minimum concentrations ($C_{max,ss}$, $C_{min,ss}$), the AUC during a steady-state dosing interval (AUC_{τ}) and during the 24-hour interval (AUC_{24}) on day 1, the accumulation ratio (AUC_{τ}/AUC_{24}), the volume of distribution at steady state (V_{ss}), total CL, λ_z and the terminal phase $t_{1/2}$. Compartmental pharmacokinetic analysis was performed in the multidose study using a two-compartment model and simultaneously fitting the model to the day 1 profile, days 2 through 4 trough samples, the day 5 profile and the terminal-phase samples on days 6–8. All values are presented as mean with standard deviations (SDs).

The fractions of TZP-101 eliminated by the faecal and urinary routes were estimated by comparing the amounts of TZP-101 measured in faeces and urine to the amount of TZP-101 dosed.

Results

A summary of the study design for pharmacokinetic analyses is provided in table I.

Multiple-Dose Study

Twelve healthy women and six healthy men with a mean \pm SD age of 31.1 \pm 7.6 years and

Table I. Summary of study design for pharmacokinetic analyses

| Study design | No. of subjects receiving TZP-101 | Dosing regimen | Analyses |
|---|-----------------------------------|--|--|
| Multiple-dose study in healthy subjects | 14 | 30-min IV infusions of daily TZP-101 doses for 5 days: 80 µg/kg/day (n=3), 320 µg/kg/day (n=3) or 600 µg/kg/day (n=8) | Noncompartmental and compartmental pharmacokinetic analyses; urine and faecal elimination |
| Single-dose study in patients with diabetes mellitus with gastroparesis | 8 | 30-min IV infusions of one TZP-101 dose: 160 µg/kg/day (n=1), 320 µg/kg/day (n=4) or 600 µg/kg (n=3) | Noncompartmental and compartmental pharmacokinetic analyses |

IV = intravenous.

BMI of 23.7 ± 1.2 kg/m² were enrolled. Fourteen subjects received TZP-101 (80 µg/kg/day [n=3]; 320 µg/kg/day [n=3]; 600 µg/kg/day [n=8]) for 5 days. The remaining four subjects received placebo for 5 days as part of the safety analysis. All 18 subjects completed the study. A data safety monitoring group reviewed available safety data and did not modify the dose escalation schedule.

Single-Dose Study

Three women and five men with a mean \pm SD age of 51 ± 16 years, BMI of 25.6 ± 6.5 kg/m² and diabetes for 20 ± 17 years were enrolled and received placebo (for safety analyses) and either 160 µg/kg (n=1), 320 µg/kg (n=4) or 600 µg/kg (n=3) doses of TZP-101 using a crossover design. Gastroparesis symptoms in these patients were moderate to severe. Baseline characteristics have been previously described for these patients.^[15]

Pharmacokinetic Parameters

Figures 1a and b show the plasma concentration versus time profiles for the multiple-dose regimen in healthy subjects and single-dose regimen in gastroparesis patients. The plasma TZP-101 concentration versus time profiles after single or multiple doses tended to increase less than proportionally to the increase in dose level.

Pharmacokinetic parameters for days 1 and 5 of the multiple-dose study are summarized in table II. C_{max} and C_{min} values on day 1 or at steady state on day 5 increased less than proportionally to the administered dose. At steady state, the total plasma CL values were low and increased as the dose increased, with mean values of 2.5, 6.4

and 10.1 mL/h/kg for the 80, 320 and 600 µg/kg/day doses, respectively. The mean TZP-101

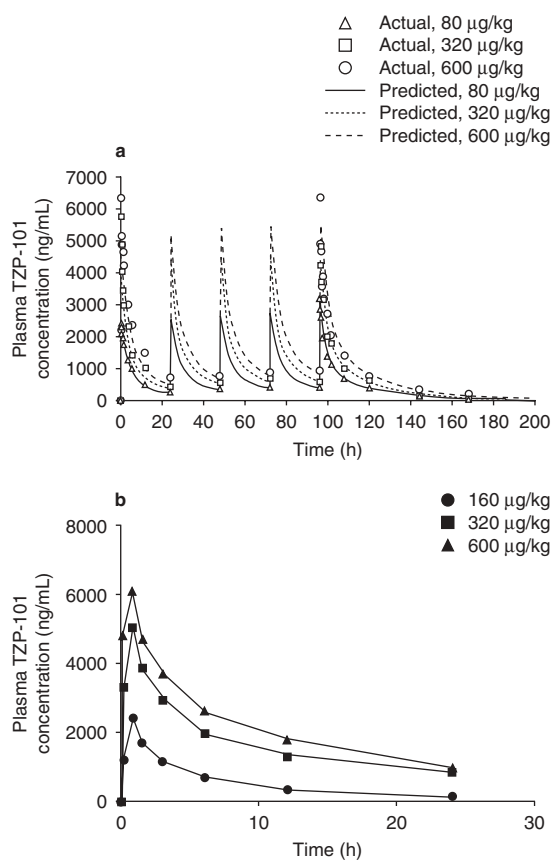


Fig. 1. (a) Mean plasma concentration of TZP-101 vs time following multiple-dose administration of TZP-101 to healthy subjects (80 µg/kg [n=3]; 320 µg/kg [n=3]; 600 µg/kg [n=8]). (b) Mean plasma concentration of TZP-101 vs time following single-dose administration of TZP-101 to patients with gastroparesis (160 µg/kg [n=1]; 320 µg/kg [n=4]; 600 µg/kg [n=3]).

Table II. Pharmacokinetic parameters for TZP-101 in healthy subjects in the multiple-dose study and in gastroparesis patients in the single-dose study^a

| Parameter | Multiple-dose study ($\mu\text{g}/\text{kg}/\text{day}$) | | | Single-dose study ($\mu\text{g}/\text{kg}$) | | |
|---|--|---------------|-----------------|---|------------------------------|------------------------------|
| | 80 (n=3) | 320 (n=3) | 600 (n=8) | 160 (n=1) | 320 (n=4) | 600 (n=3) |
| V_1 (mL/kg) | 33 (0.5) | 74 (14) | 127 (28) | 58 | 51 (4) | 62 (9) |
| C_{max} – day 1 (ng/mL) | 2417 (294) | 5813 (587) | 6665 (1663) | 2490 | 5080 (1510) | 6360 (2330) |
| $C_{\text{max,ss}}$ – day 5 (ng/mL) | 3433 (565) | 5047 (896) | 6364 (672) | NA | NA | NA |
| C_{min} – day 1 (ng/mL) | 246 (33) | 431 (37) | 739 (381) | NA | NA | NA |
| $C_{\text{min,ss}}$ – day 5 (ng/mL) | 361 (81) | 532 (209) | 672 (404) | NA | NA | NA |
| AUC_{24} – day 1 (ng • h/mL) | 17 967 (1272) | 32 675 (4739) | 46 875 (17 619) | 13 700 | 36 800 (7560) | 53 000 (44 300) |
| AUC_{τ} – day 5 (ng • h/mL) | 21 797 (3071) | 33 992 (2838) | 42 746 (13 933) | 15 300 ^b | 54 700 ^b (19 400) | 73 100 ^b (73 200) |
| CL (mL/h/kg) | 2.5 (0.6) | 6.4 (0.8) | 10.1 (3.7) | 10.4 | 6.4 (2.2) | 14.4 (9.3) |
| k_{21} (L/h) | 0.090 (0.029) | 0.113 (0.034) | 0.085 (0.019) | 0.218 | 0.459 (0.345) | 0.528 (0.286) |
| k_{10} (L/h) | 0.110 (0.013) | 0.130 (0.030) | 0.112 (0.023) | 0.179 | 0.158 (0.016) | 0.243 (0.164) |
| k_{12} (L/h) | 0.104 (0.030) | 0.160 (0.098) | 0.092 (0.037) | 0.181 | 0.583 (0.609) | 0.941 (1.02) |
| V_{ss} or V_z (mL/kg) | 71 (2) | 167 (19) | 258 (47) | 119 | 98.9 (49.7) | 180 (83.1) |
| $t_{1/2}$ (h) | 19.4 (4.3) | 16.6 (2.1) | 18.7 (3.5) | 8.9 | 10.6 (3.13) | 10.5 (5.63) |

a Values are presented as mean (SD) with the exception of the single values for the 160 $\mu\text{g}/\text{kg}$ dose in the single-dose study.

b Single-dose AUC values are AUC_{∞} .

AUC = area under the plasma concentration-time curve; **AUC₂₄** = AUC during the 24-hour interval; **AUC_τ** = AUC during a steady-state dosing interval; **AUC_∞** = AUC extrapolated to infinity; **CL** = total systemic clearance; **C_{max}** = observed maximum plasma drug concentration; **C_{max,ss}** = observed maximum plasma concentration at steady state; **C_{min}** = observed minimum plasma drug concentration; **C_{min,ss}** = observed minimum plasma concentration at steady state; **k₂₁**, **k₁₂** = transfer rates between the central and peripheral compartments; **k₁₀** = elimination rate from the central compartment; **NA** = not available; **t_{1/2}** = terminal phase half-life; **V₁** = volume of the central compartment; **V_z** = volume of distribution based on terminal elimination rate; **V_{ss}** = volume of distribution at steady state.

$t_{1/2}$ ranged from 16.6 to 19.4 hours and was independent of the administered dose. AUC_{τ} and C_{max} values are plotted versus dose in figure 2 to illustrate the relationship between exposure and dose. The micro-rate constants k_{12} , k_{21} and k_{10} , which represent the transfer rates between the central and peripheral compartments as well as the elimination rate from the central compartment, appeared to be independent of the administered dose.

At the lowest dose level of 80 $\mu\text{g}/\text{kg}$, TZP-101 displayed a lower than expected accumulation ratio ($\text{AUC}_{\tau}/\text{AUC}_{24}$) of 1.2 for a drug with a $t_{1/2}$ of approximately 18 hours that was administered on a daily basis. For several subjects, the steady-state concentrations at the higher dose levels of 320 and 600 $\mu\text{g}/\text{kg}$ were actually lower on day 5 than on day 1, with mean accumulation ratios of 1.0 and 0.9, respectively.

For the single-dose study in gastroparesis patients, noncompartmental analysis yielded C_{max}

and AUC_{24} values that were similar to those measured on day 1 of the multiple-dose study in healthy subjects (table II) and that also increased in a less than dose-proportional manner. The time to reach maximum plasma TZP-101 concentration (t_{max}) generally occurred at the first blood sampling time after completion of the 30-minute infusion (0.75-hour time point) except for two high-dose patients in whom t_{max} occurred during the infusion (0.25-hour time point). Values for V_z were somewhat greater with the high-dose compared with the low- and mid-dose levels of TZP-101, averaging 119, 99 and 180 mL/kg from low to high dose. TZP-101 CL values increased with dose. The $t_{1/2}$ of TZP-101 was similar in the mid- and high-dose groups (~10.5 hours) and shorter with the 160 $\mu\text{g}/\text{kg}$ dose received by a single subject (~9 hours). The $t_{1/2}$ values determined in gastroparesis patients were shorter than those measured in healthy subjects by 40–60%, which was likely the result of the

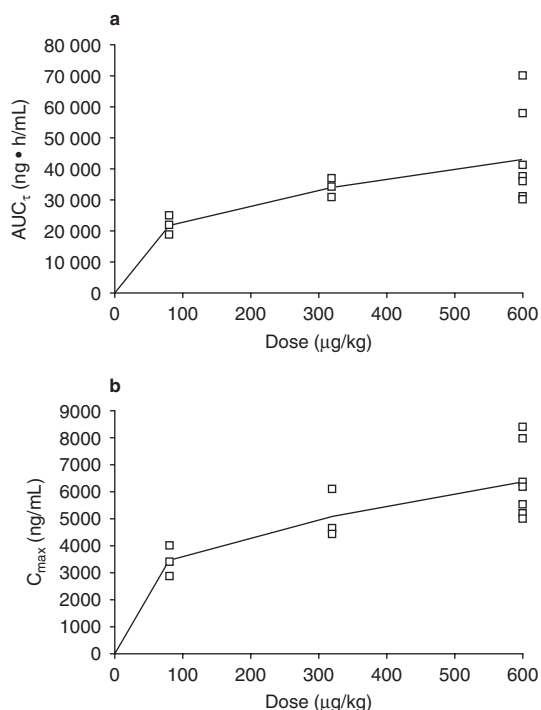


Fig. 2. Relationship between (a) area under the plasma concentration-time curve during a steady-state dosing interval (AUC_t) and TZP-101 dose and (b) maximum plasma concentration (C_{max}) and TZP-101 dose for day 5 noncompartmental pharmacokinetic analyses following multiple-dose administration of TZP-101 to healthy subjects. Values for each subject are shown (80 µg/kg [$n=3$]; 320 µg/kg [$n=3$]; 600 µg/kg [$n=8$]).

truncated blood sampling schedule (24 hours) in patients compared with healthy subjects. There was relatively large variability in the pharmacokinetic parameter estimates, possibly due to the small number of subjects at each dose level.

Only small amounts of unchanged TZP-101 were recovered in faeces, ranging from 0.24% to 0.31% and averaging 0.29% of the administered dose. The amount recovered in urine was also low, ranging from 0.91% to 1.38%, and averaging 1.17% of the administered dose. The amounts excreted were independent of the administered dose. Thus, negligible amounts of TZP-101 were excreted unchanged, indicating that metabolism is likely to be the major route of elimination and is responsible for clearance of the drug.

Tolerability

In the multiple-dose study, 40 treatment-emergent adverse events considered mild in severity and transient in nature were recorded. None of the events required an intervention or interruption to study drug administration. Of the 40 events, 24 were considered treatment related (20 possible, 4 probable). There was no observed adverse event frequency related to dose or compared with placebo. Safety data have previously been reported for the gastroparesis patients receiving single TZP-101 doses.^[15] Most adverse events were mild and self-limiting and there were no identifiable differences in numbers or types of adverse events for TZP-101 and placebo infusions.

Protein Binding

The protein binding of TZP-101 in plasma of various species as well as in solutions of isolated human plasma proteins was determined by equilibrium dialysis. The fractions of bound TZP-101 in the plasma of various species are presented in figure 3a. TZP-101 was extensively bound ($\geq 99\%$) to human plasma proteins at concentrations at and below 8.1 µg/mL (15 µmol/L). Significantly lower binding of TZP-101 to proteins was observed in monkey, rat, dog and rabbit plasma, with 83%, 85%, 85% and 88%, respectively, bound at 8.1 µg/mL.

The fraction of TZP-101 bound to purified human plasma proteins in isotonic buffer solutions is shown in figure 3b. TZP-101 binding to AAG was high ($\geq 99\%$) at plasma concentrations at or below 5.4 µg/mL (10 µmol/L), with a significant decrease in binding between 8.1 and 13.5 µg/mL (15 and 25 µmol/L). TZP-101 binding was also evaluated in a solution of purified HSA. The fraction of TZP-101 bound to HSA ranged from 28% to 33% and was independent of drug concentration across the concentration range tested (2.7–53.9 µg/mL; 5–100 µmol/L) [figure 3b]. These data indicate that AAG is a major binding protein for TZP-101 in human plasma.

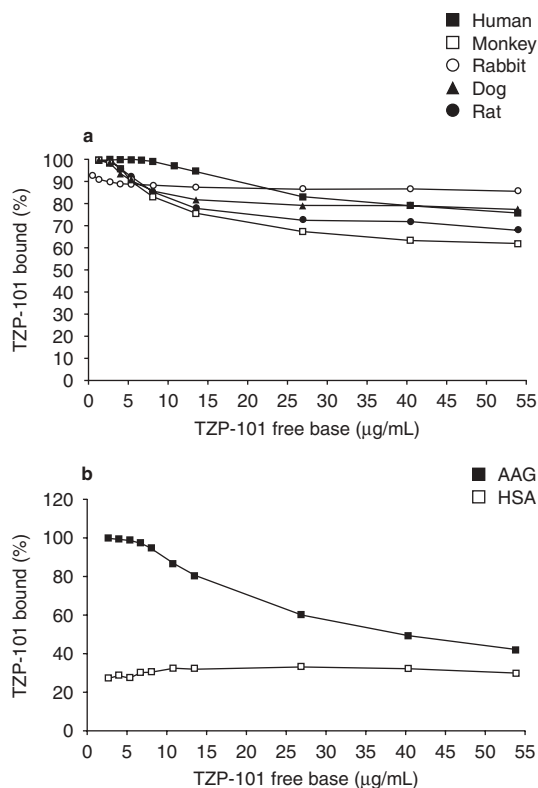


Fig. 3. Fraction of TZP-101 bound to plasma proteins determined by equilibrium dialysis in (a) human, rat, dog, monkey and rabbit plasma and (b) solutions of human α_1 -acid glycoprotein (AAG, 1 mg/mL) and 4% human serum albumin (HSA). Dialysis was against isotonic phosphate buffer pH 7.4 for 6 hours at 37°C. Data are the mean of three determinations.

Discussion

This is the first assessment of steady-state pharmacokinetics of the synthetic ghrelin receptor agonist TZP-101 and the first analysis to compare pharmacokinetic parameters in healthy subjects and patients with diagnosed gastroparesis. The results of the pharmacokinetic analysis are comparable to those reported in a previous study in which healthy subjects received single doses of TZP-101 ranging from 20 to 600 µg/kg.^[14] TZP-101 pharmacokinetic profiles in healthy subjects and in patients with diabetes with gastroparesis are comparable. Key parameters such as C_{max} and AUC were found to change less than proportionally with dose in

the dose range investigated, although dose proportionality has been demonstrated in a previous study up to and including 160 µg/kg.^[14] The high-affinity and concentration-dependent plasma protein binding of TZP-101 could explain this lack of dose proportionality.

TZP-101 is highly bound to proteins in human plasma with $\geq 99\%$ bound at concentrations up to 15 µmol/L. AAG appears to be the major binding protein for TZP-101 in plasma, since the extent (99.8% and 99.7% in whole plasma and AAG, respectively) of binding and the binding profile of TZP-101 over the 5–100 µmol/L concentration range in isolated AAG at a concentration (~25 µmol/L) similar to that found in whole plasma was very similar to the profile observed in whole plasma. In contrast, TZP-101 binding to HSA was independent of concentration and averaged approximately 30%. Differences in the concentrations of unbound TZP-101 across species could have implications for the interpretation of animal safety data and with respect to human risk assessment. Similar interspecies differences in high-affinity binding of drugs to AAG have been previously reported.^[16,17]

AAG is able to bind a broad array of basic, acidic and neutral drugs.^[18] High-affinity drug binding to AAG has been shown to affect the volume of distribution and clearance of drugs after single doses and at steady state.^[19-21] Similarly, the volume of distribution and clearance of TZP-101 appeared to be influenced by binding to AAG. TZP-101 bound to AAG is retained in the central compartment and is not distributed to peripheral tissues. This results in small, dose-independent volume of distribution estimates following single doses, with V_{ss} values following multiple dosing ranging from just 70 to 300 mL/kg. The concentration-dependence of TZP-101 binding to human AAG suggests that increased concentrations of unbound TZP-101 will occur as AAG binding approaches saturation at increasing TZP-101 doses. Hence, higher concentrations of free drug become available for elimination, resulting in increased TZP-101 clearance. This may explain the absence of TZP-101 accumulation at higher doses or following multiple dosing, even though $t_{1/2}$ remains within

approximately a 10- to 20-hour range. These data emphasize that the total TZP-101 concentrations measured in plasma may not be particularly predictive of toxicity since the pharmacologically active component is represented by unbound drug.

Only trace to small amounts of parent TZP-101 were recovered in faeces (~0.3% of the dose) and urine (~1.2% of the dose). Thus, metabolism is likely to be a prerequisite for elimination of TZP-101. The characterization of TZP-101 metabolites is currently ongoing. Plasma protein binding associated with metabolic clearance may hence be the primary determining factor in the pharmacokinetic behaviour of TZP-101 in healthy subjects and gastroparesis patients.

Protein binding to AAG is influenced by genetic variants^[22] and physiological factors such as age^[23] and disease^[24] that can alter AAG glycosylation patterns. In addition, the concentration of AAG in plasma is variable between individuals and is dependent upon disease state and sex, with concentrations in males being somewhat greater than those in females.^[25] Further research is needed to understand if any of these factors influence TZP-101 binding to AAG and the implications for the pharmacokinetic profile of the drug.

Conclusions

The results from the present study indicate that the pharmacokinetic parameters of TZP-101 in patients with gastroparesis and healthy subjects are comparable and display a similar trend towards increased clearance at higher dose levels resulting in little accumulation of TZP-101 at high dose levels and after multiple dosing. Optimization of dosage regimens for TZP-101 in patients with gastric dysmotility should take into consideration the fraction of unbound drug rather than the total plasma concentration. In addition, the AAG binding property of TZP-101 further suggests that interpretation of safety data from animal models requires conversion of total plasma concentrations to concentrations of free drug in order to facilitate a meaningful risk

assessment in humans, since significantly more TZP-101 remains unbound in animal plasma than in human plasma.

Acknowledgements

Funding for the design and conduct of the study and collection and analysis of the data was provided by Tranzyme Pharma, Inc., Durham, NC, USA. Helmut Thomas, Lilian Clohs, Carl St-Louis, Laura Shaughnessy and Gordana Kosutic are employees of Tranzyme Pharma, Inc. Niels Ejskjaer is an advisory board member for Tranzyme Pharma, Inc. William Wargin is a consultant for Tranzyme. Maria Gutierrez has no conflicts of interest that are directly relevant to the content of this study. Patrice Ferriola, PhD provided writing and editing assistance, and was supported by Tranzyme Pharma, Inc.

References

1. Date Y, Kojima M, Hosoda H, et al. Ghrelin, a novel growth hormone-releasing acylated peptide, is synthesized in a distinct endocrine cell type in the gastrointestinal tracts of rats and humans. *Endocrinology* 2000; 141 (11): 4255-61
2. Edholm T, Levin F, Hellstrom PM, et al. Ghrelin stimulates motility in the small intestine of rats through intrinsic cholinergic neurons. *Regul Pept* 2004; 121 (1-3): 25-30
3. Fukuda H, Mizuta Y, Isomoto H, et al. Ghrelin enhances gastric motility through direct stimulation of intrinsic neural pathways and capsaicin-sensitive afferent neurones in rats. *Scand J Gastroenterol* 2004; 39 (12): 1209-14
4. Murray CD, Martin NM, Patterson M, et al. Ghrelin enhances gastric emptying in diabetic gastroparesis: a double blind, placebo controlled, crossover study. *Gut* 2005; 54 (12): 1693-8
5. Tack J, Depoortere I, Bisschops R, et al. Influence of ghrelin on gastric emptying and meal-related symptoms in idiopathic gastroparesis. *Aliment Pharmacol Ther* 2005; 22 (9): 847-53
6. Binn M, Albert C, Gougeon A, et al. Ghrelin gastrokinetic action in patients with neurogenic gastroparesis. *Peptides* 2006; 27 (7): 1603-6
7. Bytzer P, Talley NJ, Leemon M, et al. Prevalence of gastrointestinal symptoms associated with diabetes mellitus: a population-based survey of 15 000 adults. *Arch Intern Med* 2001; 161 (16): 1989-96
8. Abell TL, Bernstein RK, Cutts T, et al. Treatment of gastroparesis: a multidisciplinary clinical review. *Neurogastroenterol Motil* 2006; 18 (4): 263-83
9. Camilleri M. Clinical practice: diabetic gastroparesis. *N Engl J Med* 2007; 356 (8): 820-9
10. Artinyan A, Nunoo-Mensah JW, Balasubramaniam S, et al. Prolonged postoperative ileus: definition, risk factors, and predictors after surgery. *World J Surg* 2008; 32 (7): 1495-500
11. Traut U, Brugger L, Kunz R, et al. Systemic prokinetic pharmacologic treatment for postoperative adynamic ileus

- following abdominal surgery in adults. *Cochrane Database Syst Rev* 2008; (1): CD004930
12. Ankersen M, Kramer Nielsen K, Kruse Hansen T, et al. Growth hormone secretagogues derived from NN703 with hydrazides as c-terminal. *Eur J Med Chem* 2000; 35 (5): 487-97
 13. Venkova K, Fraser G, Hoveyda HR, et al. Prokinetic effects of a new ghrelin receptor agonist TZP-101 in a rat model of postoperative ileus. *Dig Dis Sci* 2007; 52 (9): 2241-8
 14. Lasseter KC, Shaughnessy L, Cummings D, et al. Ghrelin agonist (TZP-101): safety, pharmacokinetics and pharmacodynamic evaluation in healthy volunteers. A phase I, first-in-human study. *J Clin Pharmacol* 2008; 48 (2): 193-202
 15. Ejskjaer N, Vestergaard E, Hellstrom P, et al. Ghrelin agonist (TZP-101) accelerates gastric emptying in adults with diabetes and symptomatic gastroparesis: an exploratory, randomized, placebo-controlled, double-blind study. *Aliment Pharm Ther*. Epub 2009 Feb 27
 16. Acharya MR, Sparreboom A, Sausville EA, et al. Interspecies differences in plasma protein binding of MS-275, a novel histone deacetylase inhibitor. *Cancer Chemother Pharmacol* 2006; 57 (3): 275-81
 17. Fuse E, Kuwabara T, Sparreboom A, et al. Review of UCN-01 development: a lesson in the importance of clinical pharmacology. *J Clin Pharmacol* 2005; 45 (4): 394-403
 18. Otagiri M. A molecular functional study on the interactions of drugs with plasma proteins. *Drug Metab Pharmacokinet* 2005; 20 (5): 309-23
 19. Fuse E, Tani H, Takai K, et al. Altered pharmacokinetics of a novel anticancer drug, UCN-01, caused by specific high affinity binding to alpha1-acid glycoprotein in humans. *Cancer Res* 1999; 59 (5): 1054-60
 20. Sparreboom A, Chen H, Acharya MR, et al. Effects of alpha1-acid glycoprotein on the clinical pharmacokinetics of 7-hydroxystaurosporine. *Clin Cancer Res* 2004; 10 (20): 6840-6
 21. Hedaya MA, Daoud SS. Tissue distribution and plasma pharmacokinetics of UCN-01 at steady-state and following bolus administration in rats: influence of human alpha1-acid glycoprotein binding. *Anticancer Res* 2001; 21 (6A): 4005-10
 22. Nakagawa T, Kishino S, Itoh S, et al. Differential binding of disopyramide and warfarin enantiomers to human alpha(1)-acid glycoprotein variants. *Br J Clin Pharmacol* 2003; 56 (6): 664-9
 23. Grandison MK, Boudinot FD. Age-related changes in protein binding of drugs: implications for therapy. *Clin Pharmacokinet* 2000; 38 (3): 271-90
 24. Higai K, Azuma Y, Aoki Y, et al. Altered glycosylation of alpha1-acid glycoprotein in patients with inflammation and diabetes mellitus. *Clin Chim Acta* 2003; 329 (1-2): 117-25
 25. Kishino S, Nomura A, Zhai S, et al. Alpha-1-acid glycoprotein concentration and the protein binding of disopyramide in healthy subjects. *J Clin Pharmacol* 1995; 35 (5): 510-4
-
- Correspondence: Dr *Gordana Kosutic*, Tranzyme Pharma, 4819 Emperor Blvd, Ste 400, Durham, NC 27703, USA.
E-mail: gkosutic@tranzyme.com