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ARTICLE

Safety, Pharmacokinetics, and Pharmacodynamics of TD-0714, a Novel Potent Neprilysin Inhibitor in Healthy Adult and Elderly Subjects

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TD-0714 is an orally active, potent, and selective inhibitor of human neprilysin (NEP) in development for the treatment of chronic heart failure. Oral administration of TD-0714 in rats resulted in dose-dependent and sustained increases in plasma cyclic guanosine monophosphate (cGMP) over 24 hours consistent with NEP target engagement. Randomized, double-blind, placebo controlled, single ascending dose (50–600 mg TD-0714) and multiple ascending dose (10–200 mg TD-0714 q.d. for 14 days) studies were conducted in healthy volunteers. TD-0714 was generally well-tolerated and no serious adverse events or clinically significant effects on vital signs or electrocardiogram parameters were observed. TD-0714 exhibited dose-proportional pharmacokinetics (PKs) with high oral bioavailability, minimal accumulation after once daily dosing, and negligible renal elimination. Pharmacodynamic (PD) responses were observed at all dose levels studied, as reflected by statistically significant increases in plasma cGMP concentrations. The increases in cGMP were significantly above the baseline (~ 50–100%) on day 14 for the entire 24-hour interval indicating that sustained cGMP elevations are achieved at steady-state. Maximal steady-state cGMP response was observed in plasma and urine at doses ≥ 50 mg. The TD-0714 PK-PD relationship and safety profile were similar in elderly vs. younger adult subjects. The TD-0714 PK and PD profiles support further clinical development of TD-0714 and suggest the potential for once-daily administration and predictable exposure in patients with cardiorenal diseases regardless of their renal function.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?
☑ Neprilysin inhibitors (NEPis) in combination with other pathway inhibitors offer therapeutic advantage in patients with heart failure.

WHAT QUESTION DID THIS STUDY ADDRESS?
☑ The study evaluates the safety and tolerability of second generation NEPi as well as whether its pharmacokinetic-pharmacodynamic (PK-PD) profile supports further evaluation in patients with heart failure.

WHAT DOES THIS STUDY ADD TO OUR KNOWLEDGE?
☑ The study demonstrates that this novel NEPi is tolerable in healthy volunteers, including elderly subjects as well as elicits a robust PD response at steady-state thereby justifying further evaluation and providing dose recommendations for phase II.

HOW MIGHT THIS CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE?
☑ The study adds to the armamentarium of drugs with potential for significant improvement in heart failure outcomes. Through nontraditional methods of administering microtracer i.v. dose as well as PD assessments in both single ascending dose and multiple ascending dose portions of the study, the study exemplifies how phase I studies can be used efficiently to investigate PK properties of investigational drugs as well as provide clear PK-PD-based dose recommendations for future clinical studies.

Chronic heart failure (CHF) is a complex clinical syndrome that results from functional impairment of ventricular filling or ejection of blood, designated as heart failure with preserved or reduced ejection fraction (HFrEF), respectively.[1] Progression of CHF may, in part, be due to inadequate compensation by protective endogenous neurohormonal systems, which include the natriuretic peptides, atrial natriuretic peptide (ANP), brain natriuretic peptide, and C-type natriuretic peptide.[2] Human neprilysin (hNEP) is the enzyme responsible, at least in part, for degradation of natriuretic peptides.[3] Inhibition of neprilysin (NEP), therefore, leads to elevations in endogenous natriuretic peptide levels, which exert protective cardiorenal effects, via a cyclic guanosine monophosphate (cGMP)-dependent pathway, including vasodilation, diuresis/natriuresis, antiproliferative, antifibrotic, and antihypertrophic effects.[2,4] Inhibition of NEP

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may provide additional cardioprotective effects in addition to inhibition of the renin angiotensin aldosterone system pathway. LCZ696, a combination of an NEP inhibitor (sacubitril) and an angiotensin II receptor blocker (valsartan), has been shown to be more effective than enalapril (angiotensin-converting enzyme inhibitor), on top of standard of care (including beta-blockers and diuretics) treatments for HFrEF.[5]

TD-0714 is an orally active, potent, and highly selective inhibitor of hNEP that is being developed as an investigational compound for the treatment of CHF. TD-0714 is a potent competitive inhibitor of hNEP (K_i = 0.427 nM, dissociation terminal half-life ($t_{1/2}$) determined from k_{off} = 144 minutes)[6] with high selectivity for hNEP over a range of other molecular targets, including human angiotensin-converting enzyme (ACE) and human amyloid precursor protein (internal data). Selectivity over ACE and amyloid precursor protein is important to avoid the adverse effects of angioedema reported previously for ACE-NEP inhibitors, such as omapatrilat.[7] In addition, TD-0714 was > 10-fold more potent compared to LBQ657, which is the active metabolite of sacubitril. The aim of the study described here was to evaluate the safety and pharmacokinetics-pharmacodynamics (PKs-PDs) of TD-0714 to support further evaluation in patients with heart failure. Single ascending dose (SAD) and multiple ascending dose (MAD) studies in healthy volunteers demonstrate that TD-0714 was well-tolerated, including elderly subjects. Furthermore, TD-0714 PK was well-characterized and translated to significantly increased cGMP levels in plasma and urine indicating robust PD response supportive of once-daily dosing.

METHODS

Nonclinical PK-PD study

Male Sprague Dawley rats were administered a single oral dose of TD-0714 (1, 3, 10, or 30 mg/kg) for the assessment of TD-0714 plasma PK and PD (plasma cGMP; *N* = 3 animals per dose per timepoint). NEP activity was evaluated by determining the increase of plasma cGMP following an i.v. bolus of ANP (30 μg/kg) administered 5 minutes prior to each PD sample collection.[6] The cGMP is released extracellularly and into the systemic circulation as a downstream effect of an intracellular signaling pathway initiated by ANP binding to its cell surface receptor protein. ANP is degraded by NEP and, thus, inhibition of NEP activity by TD-0714 results in the potentiation of the ANP signaling cascade (e.g., increased plasma cGMP concentrations). Increases in plasma cGMP concentration are, therefore, indicative of inhibition of NEP activity. A nonlinear mixed effects PK-PD maximum effect (E_{max}) model with an effect compartment was fit to the individual time course of plasma TD-0714 and cGMP concentrations using NONMEM version 7.2 (ICON plc, Dublin, Ireland).

Clinical studies in healthy participants

The studies were conducted in accordance with the protocols and the principles of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (GCP), the United States Code of Federal Regulations (CFR), the principles of the World Medical Association Declaration of Helsinki, Ethical Principles for Medical Research Involving Human Subjects, and all applicable regulatory requirements.

SAD study design

Part A (SAD and food effect study). Healthy subjects were randomized to one of five ascending dose cohorts (50 mg, 100 mg, 200 mg, 400 mg, and 600 mg) to receive a single dose of either TD-0714 or placebo, administered orally. Each cohort enrolled 10 healthy adults (18–55 years of age), randomized in a 4:1 ratio (8 subjects received TD-0714 and 2 subjects received matching placebo). In each cohort, subjects were admitted to the clinical research unit 3 days before dosing (day –3) and began a standardized, controlled daily diet with fixed salt (e.g., sodium and potassium), and protein intake that continued through the end of day 1. TD-0714 was administered orally following an overnight fast of at least 10 hours. Subjects remained fasted, except for water, until after the 4-hour postdose assessments were completed and the 4-hour postdose blood PK sample was collected. Subjects were discharged 72 hours after dosing (day 4). Following a washout period of at least 10 days after the first dose, subjects in the 200 mg cohort were administered a second 200 mg dose of TD-0714 to evaluate the effect of food on TD-0714 PK after a moderate-fat meal.

Part B (microtracer study). Part B was an open-label, single-dose study to evaluate absolute oral bioavailability and renal elimination of TD-0714. Six healthy subjects were enrolled in a single cohort (see **Table S1**). Subjects received a single, unlabeled, oral dose of TD-0714 (100 mg) on day 1. Subjects received a single 2 minute i.v. infusion of TD-0714 labeled with ^{14}C at a metabolically stable location (10 μg; 0.5 μCi) 3 hours after the oral dose and coinciding with time of maximum plasma concentration (T_{max}) of the oral dose. Following the i.v. infusion, blood and urine samples were collected for PK determination. No PD biomarkers were collected for Part B.

MAD study design

Healthy subjects were randomized to one of five ascending dose cohorts (10 mg, 50 mg, 100 mg, 200 mg, and 100 mg elderly) to receive multiple doses of either TD-0714 or placebo, administered orally. Each cohort enrolled 10 healthy subjects (18–55 years of age), randomized in a 4:1 ratio (8 subjects received TD-0714 and 2 subjects received matching placebo). An additional cohort of 10 elderly subjects (66–80 years of age; 8 subjects received TD-0714 and 2 subjects received matching placebo) were enrolled. In each cohort, subjects were admitted to the clinical research unit 3 days before dosing (day –3). Each subject began a standardized, controlled daily diet with fixed salt (e.g., sodium and potassium) and protein intake, which continued while the subject remained at the clinic. Subjects received a single dose of TD-0714 each day from day 1 to day 14. Subjects were discharged after all visit assessments were completed on day 17. On day 1 and day 14, TD-0714 was administered orally following an overnight fast of at least 10 hours. Subjects remained fasted, except for water, until after the 4-hour postdose assessments were completed and the 4-hour postdose blood PK sample was collected.

Safety and tolerability assessments (SAD and MAD)

The primary end point of this first-in-human study was to evaluate the safety of TD-0714 via assessment of adverse events (AEs; including any serious AEs), physical examinations, vital signs, orthostatic vital signs, 12-lead electrocardiograms, clinical laboratory assessments, urinalysis, and concomitant medication use.

Pharmacokinetic assessments

Blood samples were collected in part A of the SAD study and the MAD study, as detailed in the **Supplementary Material**. For part B subjects, additional PK samples were collected within 5 minutes prior to the start of the infusion, within 2 minutes after the end of the infusion, and then 5, 15, and 30 minutes after completion of the i.v. dose of [^{14}C] TD-0714. Additional PK samples were collected at 5, 10, and 14 hours after administration of the oral TD-0714 dose.

Pharmacodynamic assessments

The PD end point marker cGMP was measured in blood and urine samples obtained throughout day –1 and day 1 in the SAD study and throughout day –1, day 1, and day 14 in the MAD study, as described in the **Supplementary Material**.

Pharmacokinetic/pharmacodynamic analysis

Plasma samples were analyzed for TD-0714 and cGMP as described in the **Supplementary Material**. The PK parameters of TD-0714 were determined at each dose level by noncompartmental analysis using Phoenix WinNonlin version 6.3 (Certara, Sunnyvale, CA).

For PD analysis, cGMP was reported for each time point as change from the time-matched values obtained on day –1 (baseline) to minimize the effects of diurnal variability. Change from time-matched baseline value over the 24-hour period postdose was used to calculate an area under the effect curve ($AUEC_{0–24}$) for each subject. The PD effect of TD-0714 was fit using nonlinear mixed effects methods to an E_{max} dose response model.

RESULTS

Nonclinical PK-PD characterization of TD-0714 in rats

Plasma TD-0714 concentrations in male Sprague Dawley rats following a single oral dose increased in a dose-dependent manner across the dose range from 1–30 mg/kg (**Figure 1a**). Plasma cGMP concentrations did not increase following administration of 1 mg/kg TD-0714 but exhibited a dose-dependent increase following doses ranging from 3–30 mg/kg TD-0714 (**Figure 1b**). Plasma cGMP concentrations remained elevated throughout the 24-hour period after dosing at 10 and 30 mg/kg TD-0714 dose levels.

Hysteresis was observed in the PK-PD relationship. cGMP concentrations observed at the first time point (0.75 hours) were lower compared with later time points, which had nearly the same plasma TD-0714 concentrations (**Figure S1**). An effect compartment was used in the PK-PD model to account for the hysteresis (see **Supplementary Material**). The exposure-response relationship observed between TD-0714 concentrations in the effect compartment and plasma cGMP concentrations is demonstrated in **Figure 1c** with an effect compartment half-maximal effective concentration (EC_{50}) of 93.7 ng/mL. TD-0714 effect compartment concentrations lagged the plasma concentrations by ~ 2 hours (K_{e0} of 0.308/hr, see **Supplementary Material**).

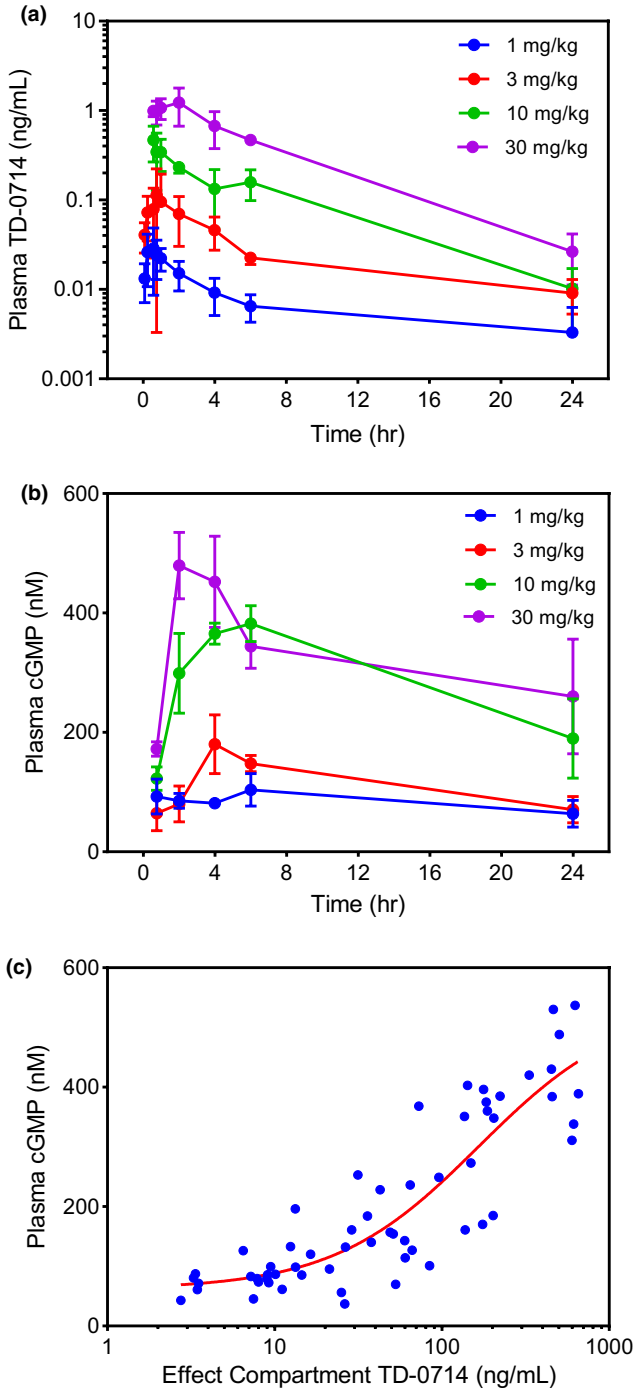

Figure 1 Pharmacokinetic-pharmacodynamic (PK-PD) of TD-0714. Plasma concentration of (**a**) TD-0714 and (**b**) cyclic guanosine monophosphate (cGMP) following a single dose administration of TD-0714 in rats. The error bars represent the SD. (**c**) PK-PD relationship between the estimated TD-0714 concentrations in the effect compartment and plasma cGMP concentrations. Solid blue circles indicate individual estimated concentrations in the effect compartment. The solid red line indicates the PK-PD fit.

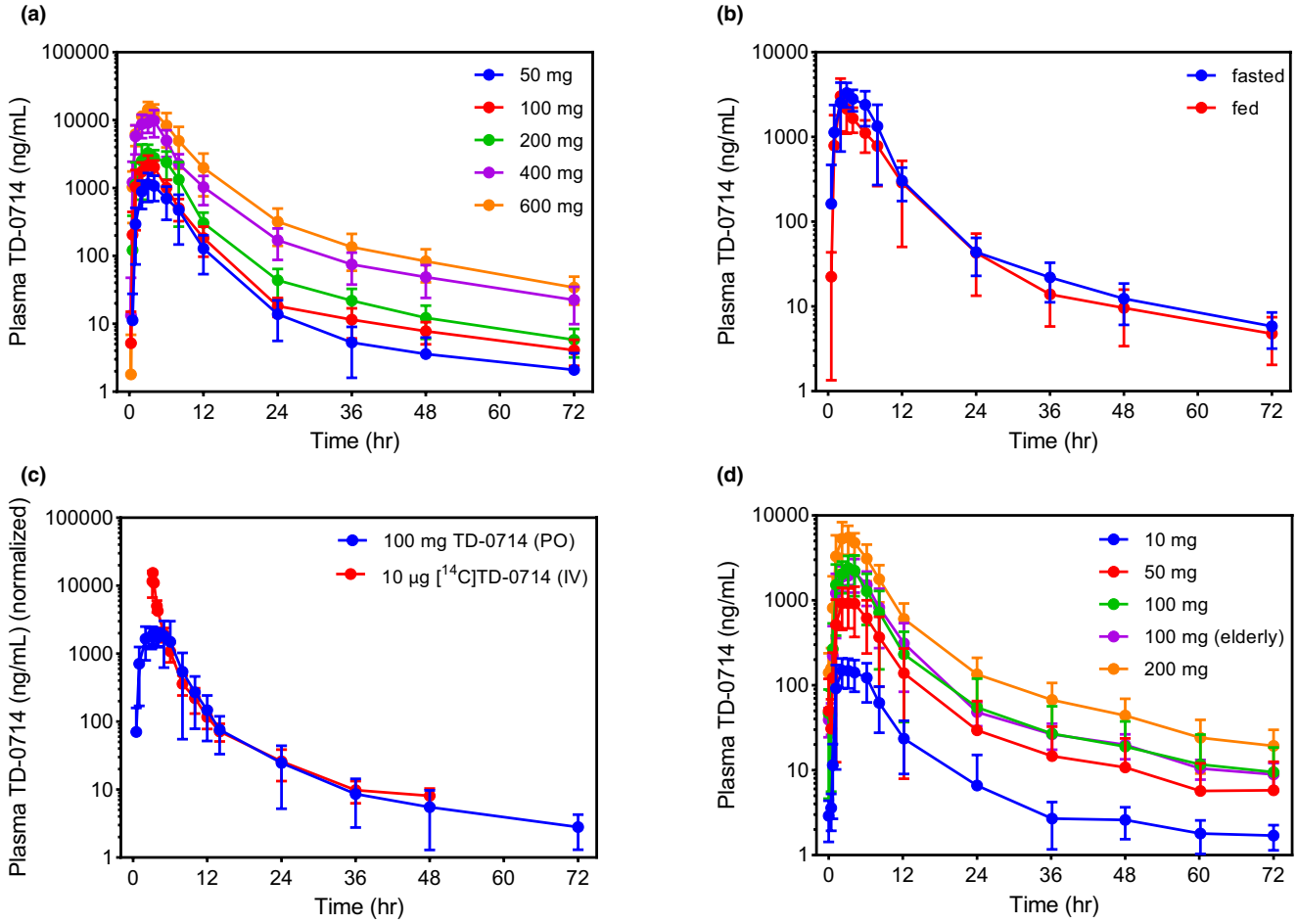

Figure 2 Plasma pharmacokinetic profile of TD-0714 in healthy subjects (mean and SD). (**a**) Plasma concentration-time profiles of TD-0714 on day 1 following administration of a single oral dose of TD-0714 (50–600 mg). (**b**) Plasma concentration-time profiles of TD-0714 following administration of single oral doses of 200 mg TD-0714 under fasted and fed conditions. (**c**) Plasma concentration-time profiles of TD-0714 and [^{14}C]TD-0714 normalized to a 100 mg dose, following administration of a single oral dose of 100 mg TD-0714 followed by a single i.v. infusion of 10 µg (0.5 µCi) of [^{14}C]TD-0714. (**d**) Plasma concentration-time profiles of TD-0714 on day 14 following administration of multiple oral doses of TD-0714 once daily for 14 days (10–200 mg).

Clinical studies

Subject disposition. Fifty subjects were randomized to receive TD-0714 (n = 40) or placebo (n = 10) in part A of the SAD study, whereas 50 subjects were randomized to receive TD-0714 (n = 40) or placebo (n = 10) in the MAD study. Additionally, 6 subjects received a single open label oral dose of TD-0714 followed by an i.v. dose of [^{14}C]TD-0714 in part B of the SAD study. All subjects were included in the safety and PK analyses. Demographic parameters and baseline body mass index were similar in all cohorts, except for mean age in the elderly group in the MAD study (see **Tables S1 and S2**). None of the subjects had a clinically significant medical history relevant for these studies.

Plasma and urine pharmacokinetics after single dose. The mean plasma concentration-time profiles of TD-0714 following administration of single ascending oral doses (50–600 mg) of TD-0714 are presented in **Figure 2a**. Plasma TD-0714 concentrations were detected at the earliest time point (15 minutes) and remained detectable up to 72 hours. A summary of plasma and urine PK parameters following administration of single ascending oral doses of TD-0714 is presented in **Table 1**. Under fasted conditions, TD-0714 exposure (AUC_{0-24} and peak plasma concentration (C_{max})) increased dose-proportionally with increasing doses of TD-0714. The median T_{max} occurred between 2.55 and 3.52 hours postdose, whereas mean $t_{1/2}$ values ranged from 18.7–24.9 hours across the dose range tested. The apparent clearance (CL/F) ranged from 6.30–9.04 L/hr and the apparent volume of distribution ranged from 182–279 L.

Cumulative amounts of TD-0714 excreted in urine were low and corresponded to < 1% of the administered dose. Renal clearance (CL_r) ranged from 0.016–0.035 L/hr, indicating minimal contribution to the overall clearance of TD-0714.

Food effect

The C_{max} of TD-0714, as well as the overall shape of mean concentration-time profiles, were similar under fasted and fed conditions following administration of a single oral dose

Table 1 Summary of TD-0714 pharmacokinetic parameters in plasma and urine following administration of single ascending oral doses of TD-0714 under fasting and fed conditions

Parameter (units)	50 mg TD-0714	100 mg TD-0714	200 mg TD-0714 (fasted)	200 mg TD-0714 (fed)[a]	400 mg TD-0714	600 mg TD-0714
AUC_{0-24}, ng•hr/mL	7,800 (±2,700)	12,900 (±2,890)	22,400 (±5,280)	15,300 (±3,730)	63,600 (±15,600)	100,000 (±35,200)
C_{max}, ng/mL	1,270 (±473)	2,455 (±732)	4,110 (±1,030)	3,270 (±1,510)	11,400 (±3,720)	15,400 (±2,980)
T_{max}, hour	3.00 (2.00, 6.04)	3.00 (2.00, 4.03)	3.52 (2.01, 6.00)	2.00 (2.00, 8.00)	2.55 (1.00, 4.13)	3.00 (2.00, 4.00)
CL/F, L/hr	7.46 (±4.67)	7.73 (±1.70)	9.04 (±2.41)	13.5 (±4.81)	6.30 (±1.65)	6.52 (±2.97)
V_z/F, L	216 (±88)	279 (±87)	246 (±81)	491 (±290)	182 (±58)	194 (±123)
$t_{1/2}$, hr	24.3 (±9.5)	24.9 (±3.9)	18.7 (±1.7)	24.1 (±4.6)	20.0 (±3.2)	19.7 (±4.9)
CL_r, L/hr	0.02[a] (±0.01)	0.03 (±0.01)	0.03 (±0.02)	n.a.[b]	0.04 (±0.01)	0.03 (±0.01)
F_e, %	0.22 (±0.15)	0.30 (±0.12)	0.30 (±0.20)	n.a.[b]	0.57 (±0.20)	0.43 (±0.17)

Data are geometric mean (SD).
AUC_{0-24}, area under the plasma concentration-time curve from 0 to 24 hours; CL/F, apparent oral clearance; CL_r, renal clearance; C_{max}, maximum plasma concentration; F_e, % fraction excreted unchanged in urine; n.a., not applicable; $t_{1/2}$, apparent terminal elimination half-life; T_{max}, time to maximum plasma concentration; V_z/F, apparent volume of distribution during terminal phase.
T_{max} presented as median (minimum, maximum).
N = 8 for all cohorts except.
[a]*n* = 7 for 200 mg (fed) cohort.
[b]Urine was not collected in this cohort.

of 200 mg TD-0714 (**Table 1, Figure 2b**). TD-0714 administration under fed conditions resulted in 25% lower C_{max} and 33% lower AUC_{0-24}, relative to when the same dose was administered under fasted conditions (**Table 1**). However, any differences in exposures were less than the SD of calculated parameters (coefficient of variation = 20–46% for C_{max} and coefficient of variation = 22 to 35% for AUC_{0-24}) and, thus, are not expected to be of clinical significance.

Absolute bioavailability and renal excretion of TD-0714

The plasma concentration-time profiles of TD-0714 and [^{14}C]TD-0714 normalized to a 100 mg dose following administration of a single oral dose of 100 mg TD-0714 followed by a single i.v. infusion of 10 µg (0.5 µCi) of [^{14}C]TD-0714 are shown in **Figure 2c**. Plasma [^{14}C]TD-0714 concentrations were detectable in all subjects from 5 minutes through 45 hours post i.v. infusion (**Figure 2c**). The mean concentration-time profiles of TD-0714 and [^{14}C]TD-0714 were similar and appeared multiphasic.

A summary of plasma and urine TD-0714 and [^{14}C]TD-0714 PK parameters are presented in **Table S3a** and **Table S3b**. The absolute oral bioavailability of TD-0714 based on individual dose-normalized AUC to infinity (AUC_{inf}) values was ~ 75% (90% confidence interval of 54.57–103.7%).

Table 2 Summary of TD-0714 pharmacokinetic parameters on day 14 in plasma and urine following administration of multiple ascending oral q.d. doses of TD-0714

Parameter (units)	10 mg TD-0714[a]	50 mg TD-0714	100 mg TD-0714 (Non-elderly[e])	100 mg TD-0714 (Elderly[f])	200 mg TD-0714
AUC_{tau}, ng•hr/mL	1,170 (295)	7,300 (4,630)	16,000 (7,710)	16,400 (5,860)	38,000 (13,800)
C_{max}, ng/mL	196 (45.0)	1,083 (501)	2,830 (919)	2,538 (690)	6,378 (2760)
T_{max}, hr	4.01 (1.01, 6.00)	3.50 (2.00, 6.00)	2.00 (1.00, 4.00)	2.52 (1.01, 6.00)	3.00 (2.01, 4.01)
CL_{ss}/F, L/hr	9.05 (2.23)	8.81 (3.68)	7.32 (2.86)	6.69 (1.97)	6.25 (3.20)
V_z/F (L)	214[d] (86.6)	283 (134)	236 (127)	200 (66.9)	154 (74.9)
$t_{1/2}$ (hr)	21.0[d] (10.3)	21.6 (2.77)	21.6 (4.43)	20.5 (1.21)	17.4 (1.50)
Ra_{AUC}	1.14[d] (0.23)	1.03 (0.30)	1.04 (0.35)	1.19 (0.30)	1.20 (0.37)
Ra_{Cmax}	1.05 (0.49)	0.94 (0.25)	1.01 (0.32)	1.07 (0.37)	1.06 (0.35)
$CumF_e$ (%)[g]	0.11 (0.29)	0.27 (0.23)	0.30 (0.10)	0.10 (0.05)	0.35 (0.23)
CL_r (L/hr)[g]	n.a.[d]	0.02 (0.02)	0.03 (0.01)	0.009 (0.004)	0.02 (0.01)

AUC_{tau}, area under the plasma concentration-time curve from zero to the end of the dosing interval; CL_r, renal clearance; CL_{ss}/F, apparent total body clearance from plasma at steady state; C_{max}, maximum plasma concentration; $CumF_e$, cumulative percent of dose recovered; n.a., not applicable; Ra_{AUC}, accumulation ratio based on AUC; Ra_{Cmax}, accumulation ratio based on C_{max}; $t_{1/2}$, apparent terminal elimination half-life; T_{max}, time to maximum plasma concentration; V_z/F, apparent volume of distribution during terminal phase.
T_{max} are presented as median (minimum, maximum).
N = 8 for all cohorts except as indicated.
[a]*N* = 7; [b]*n* = 4; [c]*n* = 6 for 10 mg cohort.
[d]Not available due to undetectable concentrations in the urine.
[e]Non-elderly subjects aged 19–54 years.
[f]Elderly subjects aged 66–80 years.
[g]$CumF_e$ and CL_r are presented for urine data only. All other pharmacokinetic parameters are for plasma data only. Data are geometric mean (SD).

Less than 1% of both the oral and i.v. doses were excreted as unchanged parent drug in the urine, consistent with renal excretion being a minor elimination pathway.

Plasma and urine pharmacokinetics after multiple doses

Plasma and urine PK were consistent between the single and multiple dose studies (**Figure 2d**). A summary of plasma and urine TD-0714 PK parameters following administration of multiple ascending oral doses of TD-0714 is presented in **Table 2**.

Following administration of single (day 1) and multiple (day 14) oral doses of TD-0714, the AUC_{0-24} and C_{max} of TD-0714 in plasma increased dose-proportionally with increasing doses of TD-0714 from 10 to 200 mg. The median T_{max} values were similar for all doses on both days and occurred between ~ 2 and 4 hours postdose. The mean $t_{1/2}$ values on day 14 were similar across all doses and ranged from ~ 17.4–21.6 hours. The arithmetic mean CL_{ss}/F and apparent volume of distribution values on day 14 were similar across all doses and ranged from ~ 6.25–9.05 L/hr and from ~ 154–283 L, respectively.

Plasma TD-0714 trough concentrations reached steady-state within 3 to 6 days (**Figure S2**) and mean accumulation ratios based on either cumulative exposure or peak exposure of TD-0714 were similar across the 10–200 mg doses, with values ranging from 0.94 (±0.25) to 1.20 (±0.37). Accordingly, accumulation of TD-0714 in plasma following 14 days of once-daily dosing was not considered clinically meaningful.

Following administration of either single or multiple oral doses of TD-0714 (assessed on day 14), cumulative amounts of TD-0714 excreted in urine over 24 hours postdose were low and corresponded to < 1% of the dose administered. The mean CL_r on both days ranged from 0.015–0.028 L/hr across the 50–200 mg dose range, indicating minimal contribution of CL_r to the overall apparent total body clearance of TD-0714 (CL_{ss}/F).

The mean plasma and urine PK parameters of TD-0714 following 100 mg of TD-0714 were generally similar in elderly (66–80 years) vs. non-elderly (19–54 years) adult subjects. Thus, no dose modifications are anticipated to be required for elderly subjects with varying levels of renal function to achieve comparable exposures.

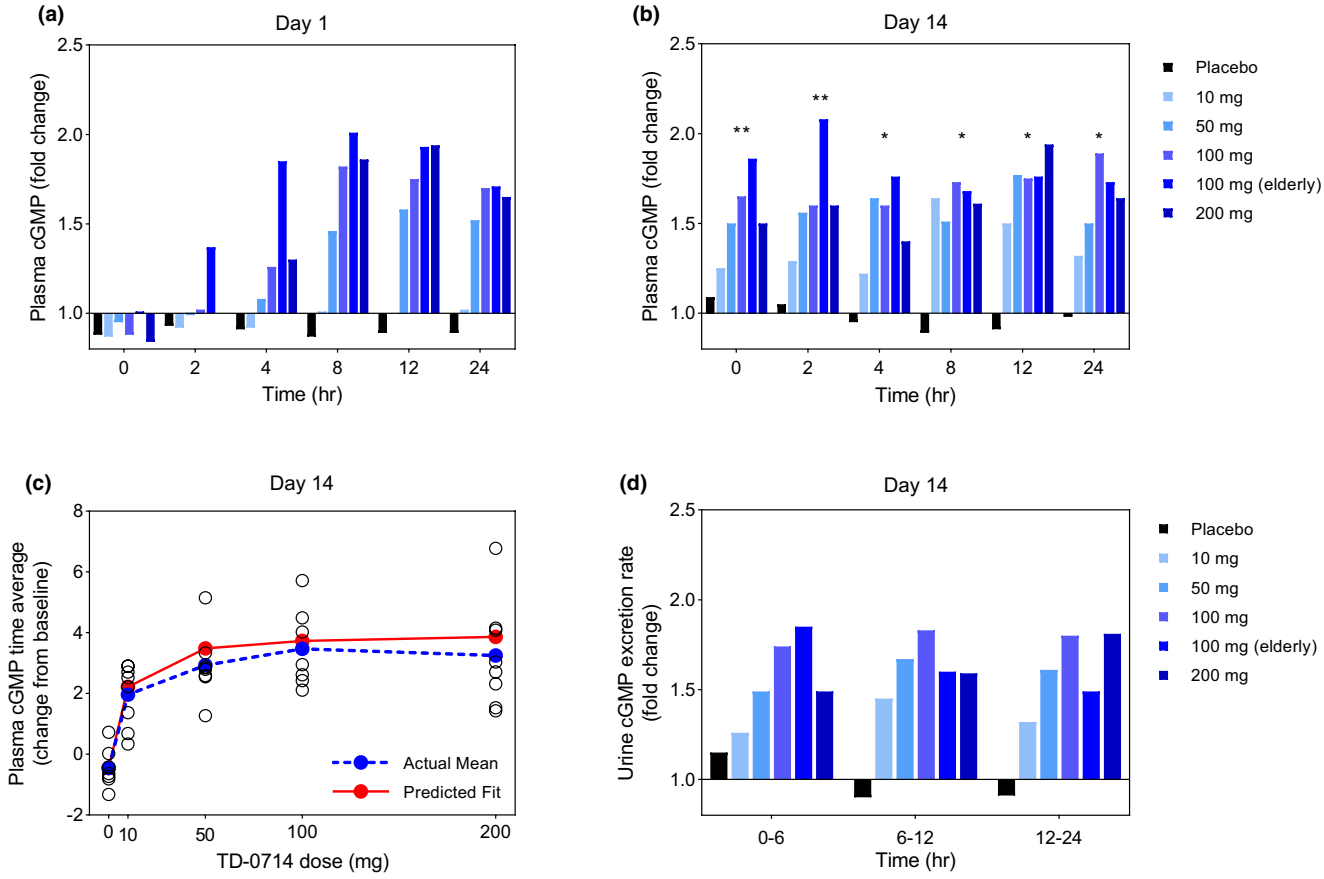

Figure 3 Plasma and urine pharmacodynamic data following dosing with TD-0714 on day 1 and day 14 of the multiple ascending dose study in healthy subjects. Geometric mean change from time-matched baseline in (**a**) plasma cyclic guanosine monophosphate (cGMP) on day 1, (**b**) plasma cGMP on day 14 (*indicates significance at $P < 0.05$ for all doses relative to placebo, **indicates significance at $P < 0.05$ for doses ≥ 50 mg relative to placebo), (**c**) maximum effect (E_{max}) model for change from baseline in plasma cGMP time average 24-hour period postdose was used to calculate an area under the effect curve ($AUEC_{0-24}$) on day 14, and (**d**) cGMP urine excretion rate over 24 hours.

Table 3 Treatment emergent adverse events (TEAES) occurring in more than one subject in the SAD study

	Placebo					TD-0714			
	Fasted (*n* = 10)	Fed (*n* = 2)	50 mg (*n* = 8)	100 mg (*n* = 8)	200 mg Fast (*n* = 8)	200 mg Fed *n* = 8)	400 mg (*n* = 8)	600 mg (*n* = 8)	100 mg PO, 10 µg IV (*n* = 6)
At Least One TEAE (%)	3 (30.0)	0	3 (37.5)	2 (25.0)	2 (25.0)	2 (25.0)	1 (12.5)	4 (50.0)	4 (66.7)
Dermatitis contact	2 (20.0)	0	1 (12.5)	2 (25.0)	1 (12.5)	0	0	1 (12.5)	0
Dizziness	0	0	2 (25.0)	0	0	0	0	1 (12.5)	0
Diarrhea	0	0	0	0	0	1 (12.5)	0	1 (12.5)	1 (16.7)
Dizziness postural	0	0	0	0	0	0	1 (12.5)	0	1 (16.7)
Headache	1 (10.0)	0	1 (12.5)	0	0	1 (12.5)	0	0	0

PO, oral; IV, intravenous.
Data shown as number of individuals (%)

Plasma and urine cGMP concentrations

A significant increase in plasma and urine cGMP was observed for all doses (50–600 mg) in the SAD and MAD studies (**Figure 3a–d**, **Figure S3**). As expected, PD response on day 1 in the SAD study were consistent with the PD response on day 1 for the MAD study in which plasma cGMP levels increased up to a peak of ~ 2-fold at 8 hours and then gradually decreased at 12 hours and 24 hours (**Figure 3a**, **Figure S3**). Peak cGMP levels were observed at the earlier time points (2–4 hours) on day 1 at the higher dose levels (≥ 100 mg). Due to limited data at subtherapeutic doses in the SAD study, a larger dose range (10–200 mg) was tested for cGMP response in the MAD study and an exposure response analysis was performed based on MAD study results at steady-state (**Figure 3b,c**).

In the MAD study, dose levels of TD-0714 ≥ 50 mg significantly increased cGMP 1.5 to 2-fold over baseline throughout the entire 24-hour dosing period in plasma on day 14 (**Figure 3b**). The 10 mg dose of TD-0714 elicited a minimal cGMP response in plasma on day 1 but a greater response (up to 1.6-fold over baseline) on day 14 (**Figure 3a,b**).

An E_{max} model to evaluate the relationship between dose and plasma cGMP $AUEC_{0–24}$ response after single and multiple doses of TD-0714 was constructed (**Figure 3c**). After multiple doses on day 14, the effective dose for 50% of the population was estimated to be 6.7 mg (95% confidence interval = 1.17–22.7 mg). The cGMP response in the 100 mg elderly group (66–80 years) was similar to the cGMP response in the 100 mg dose level in the non-elderly group (19–54 years).

Urine cGMP levels significantly increased over baseline at all dose levels (10–200 mg) after multiple doses and remained elevated above baseline over 24 hours indicating a sustained PD response to TD-0714 treatment (**Figure 3d**). A maximal PD response was achieved based on urine cGMP for doses ≥ 50 mg.

Safety and tolerability

A list of reported drug-related AEs in two or more subjects in the SAD and MAD studies are presented in **Table 3** and **Table 4**. Overall, in both studies, TD-0714 was well-tolerated and the frequency of AEs was comparable across all cohorts, including elderly subjects. No deaths or serious AEs were reported. Mild AEs in more than one subject in the SAD study included diarrhea, dizziness, and postural dizziness. Mild AEs in more than one subject in the MAD study included dyspepsia, nausea, postural dizziness, dry throat, and rhinorrhea. All other drug-related AEs reported are presented in **Tables S4 and S5**. No AEs related to hypotension were observed in either the SAD or the MAD study up to a single dose of 600 mg and 2 weeks of daily dosing of up to 200 mg TD-0714. One instance of orthostatic hypotension was observed in the 400 mg single dose cohort in the SAD study.

Two subjects discontinued from the MAD study due to AEs; one event was considered by the investigator as unrelated to the study drug and one event related to

Table 4 TEAES occurring in more than one subject in the MAD study

	Placebo		TD-0714				
	Non-elderly (*n* = 8)	Elderly (*n* = 2)	10 mg (*n* = 8)	50 mg (*n* = 8)	100 mg (non-elderly) (*n* = 8)	100 mg (elderly) (*n* = 8)	200 mg (*n* = 8)
Subjects with treatment-related AEs (%)	2 (25.0)	1 (50.0)	1 (12.5)	0	1 (12.5)	4 (50.0)	4 (50.0)
Dyspepsia	0	0	0	0	2 (25.0)	0	2 (25.0)
Nausea	0	0	0	0	0	0	2 (25.0)
Dizziness postural	1 (12.5)	0	0	0	1 (12.5)	0	1 (12.5)
Dry throat	0	0	0	0	1 (12.5)	2 (25.0)	0
Rhinorrhea	1 (12.5)	0	0	0	1 (12.5)	2 (25.0)	0

AE, adverse event; MAD, multiple ascending dose; TEAEs, treatment emergent adverse events.
Data shown as number of individuals (%).

the study drug. The subject with drug-related AE discontinued TD-0714 due to a moderate increase in alanine aminotransferase and aspartate aminotransferase levels. This subject subsequently re-entered into the study and was re-challenged with TD-0714 at the 10-mg dose level (the subject's original dose level; additional extended screening assessments for safety were conducted and an updated informed consent was obtained in accordance to the amended protocol). No change in any of the liver function tests or other safety parameters was observed in this subject during the re-challenge period. The rationale for re-challenging this subject was to explore an assessment of causality with TD-0714 treatment. The initial increases in transaminases in this subject occurred at the lowest dose level in the study (10 mg) and no elevations in aminotransferase or aspartate aminotransferase were observed at doses up to 20-fold higher (200 mg) in other subjects exposed to TD-0714. No other likely causative factors were identified for the AE and the PK and metabolic profile (data not shown) of TD-0714 were unremarkable in the subject.

DISCUSSION

TD-0714 is a potent and highly selective hNEP inhibitor that is being developed as a nonrenally cleared drug for the treatment of CHF. The safety, tolerability, and PK-PD behavior of TD-0714 was characterized in SAD and MAD studies. TD-0714 was generally well-tolerated following single oral doses of 50–600 mg and multiple oral doses of 10–200 mg administered once daily for 2 weeks. No clinically significant changes in vital signs or electrocardiograms were observed in either study. The most common AEs in the SAD and MAD include dizziness and gastrointestinal-related events, including nausea, dyspepsia, and diarrhea. Frequencies of individual AEs were generally low with the gastrointestinal-related AEs occurring with higher frequency at higher doses. Notably, no AEs related to hypotension were observed in any of the subjects across both studies and no clinically significant decreases in systolic or diastolic blood pressure were observed in the study. The lack of effects on blood pressure in healthy volunteers is consistent with other NEP-inhibitors like racecadotril,[8] candoxatril,[9] omapatrilat,[10] and sacubitril/valsartan,[11] as normotensive subjects have low baseline renin angiotensin aldosterone system activation and compensatory regulatory mechanisms.

The plasma PK of TD-0714 was well-characterized with AUC_{0-24} and C_{max} values increasing linearly with single doses from 50–600 mg and multiple daily doses from 10–200 mg. Steady-state was achieved within 3–6 days and no accumulation of plasma TD-0714 was observed after 14 days of once-daily dosing. TD-0714 is well-absorbed after oral administration with a bioavailability of ~ 75%. Food had minimal impact on TD-0714 exposure and was not considered clinically meaningful.

Renal excretion was < 1% post single or multiple oral doses in healthy adults or healthy elderly subjects. This renal excretion profile is consistent with elimination via the hepatobiliary route, as observed in multiple nonclinical species.[6] Moreover, PK parameters of TD-0714 in elderly subjects at the 100 mg daily dose over 14 days were similar to non-elderly subjects at the same dose. Accordingly, the PK profile of TD-0714 is expected to be comparable across individuals independent of renal function or age. NEP inhibitors, such as sacubitril/valsartan, require downward dose adjustment in patients with heart failure with renal impairment as a result of increased exposure to sacubitrilat, the active metabolite of sacubitril.[12,13] TD-0714 may provide a more consistent dosing regimen in such patients without the need for dose adjustment in patients with heart failure with impaired renal function.

Dose-dependent PD responses, as measured by increases in cGMP in plasma and urine, were observed at all doses in the SAD and MAD studies. Plasma cGMP levels were 1.5-fold to 2-fold greater than baseline throughout the 24-hour period at steady-state, and doses ≥ 50 mg were sufficient to elicit a maximal PD response at steady-state indicating NEP inhibition over the entire dosing interval. At the lowest 10 mg dose of TD-0714, a PD response was not observed on day 1, but a measurable submaximal response was observed at steady-state indicating accumulation of PD effect over time without a corresponding accumulation of plasma TD-0714.

The PD profile in healthy subjects is consistent with the PD results observed in the rat PK-PD study. Plasma TD-0714 concentrations over 12–24 hours at doses ≥ 50 mg in healthy volunteers were greater than the EC_{50} value estimated in rat plasma. However, there exists a lag between the PD response and plasma PK concentrations in both healthy volunteers and rats. The PD response reaches its peak 2–4 hours after the drug reaches C_{max} and remains elevated for multiple hours at plasma concentrations of TD-0714 less than the EC_{50} value.

The disconnect between the plasma PK profile of TD-0714 (C_{max} to C_{trough} ratio = 100) and the sustained PD response suggests the need for an effect compartment. We hypothesize that the high plasma protein and tissue binding property of the drug (f_u < 1%, human and rat plasma, internal data) may be responsible for the slower clearance of TD-0714 from the effect compartment, thus enabling a PD effect with long half-life and accumulation at steady-state. Alternatively, the PK-PD disconnect may be due to the slow dissociation of TD-0714 from hNEP ($t_{1/2}$ = 144 minutes). Investigation of the mechanisms driving the sustained PD response at steady-state is currently ongoing.

Modulators of the cGMP pathway have shown significant promise for therapeutic applications in cardiorenal diseases.[14] A recent meta-analysis of soluble guanylate cyclase (sGC) stimulators by Zheng *et al*. highlighted the potential for drugs like riociguat and vericiguat to improve the quality of life in subjects with heart failure,[14] whereas NEP inhibitors, like sacubitril/valsartan and omapatrilat, have demonstrated statistically significant improvements in patient outcomes during their respective phase III studies.[5,15] Once-daily administration of TD-0714 resulted in a robust and significant increase (1.5-fold to 2-fold from baseline) in cGMP that was sustained for 24 hours. The magnitude of cGMP activation for TD-0714 was higher relative to that observed for sGC activators, such as

praliciguat,[16] cinaciguat,[17] and riociguat.[18] Furthermore, the duration of cGMP activation for TD-0714 was longer as compared with NEP inhibitors, such as sacubitril[11,19] and omapatrilat.[10] Therefore, TD-0714 may have the potential for greater efficacy and higher patient compliance given the once-daily dosing regimen as compared with other modulators of the cGMP pathway. Additionally, the PD response at the 50 mg dose level is higher at steady-state as compared with day 1, allowing for a self-titrating effect. Thus, TD-0714 can potentially be developed at a single target dose level without the need for dose titration.

TD-0714 has been developed as a stand-alone NEP-inhibitor rather than as fixed dose combination with an angiotensin II receptor blocker like Entresto. Thus, it can be flexibly combined with existing standard of care treatments depending upon the individual indication (e.g., candesartan for HFrEF, irbesartan for diabetic nephropathy) and optimized to achieve an ideal benefit-risk profile.[20,21] Due to the AEs associated with on-target pharmacological effect leading to hypotension for sGC activators, like riociguat[22] and angioedema for NEP inhibitors like sacubitril,[23] these drugs are required to be titrated from a lower starting dose up to the target dose. Although the favorable benefit-risk profile of these drugs at the target dose has been adequately demonstrated, the benefit-risk profile at lower doses is less well understood, thus increasing the risk for lack of efficacy in patients who are unable to tolerate the target dose level. Owing to a sustained PD response during the entire dosing interval at steady-state for doses ≥ 50 mg, TD-0714 has the potential for a wide therapeutic margin allowing for development at a single dose level without the need for dose titration.

The PK-PD and tolerability profile of TD-0714 supports further investigation for once-daily administration in patients with cardiorenal diseases.

Supporting Information. Supplementary information accompanies this paper on the *Clinical and Translational Science* website (www.cts-journal.com).

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