Indian J Med Res 154, September 2021, pp 509-519
DOI: 10.4103/ijmr.IJMR_1119_18

Quick Response Code:

A randomized, double-blind, parallel-group, single-dose comparative pharmacokinetic study of DRL_TZ, a candidate biosimilar of trastuzumab, with Herceptin® (EU) in healthy adult males

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Received June 15, 2018

***Background & objectives*: Trastuzumab (TZ) is a recombinant DNA-derived humanized monoclonal antibody approved for human epidermal growth factor receptor 2 positive early breast cancer, metastatic breast and gastric cancers. For the development of TZ biosimilars, establishing pharmacokinetic equivalence is required. The primary objective of this study was to compare the pharmacokinetics (PK) of Dr Reddy's Laboratories TZ (DRL_TZ) with that of EU-approved Reference Medicinal Product (RMP), Herceptin® in healthy adult male subjects.**

***Methods*: In this double-blind, parallel-group, phase I study (TZ-01-003), healthy male subjects aged 18-55 yr were randomized 1:1 to receive a single intravenous infusion of 6 mg/kg of TZ as DRL_TZ or RMP. Similarity for primary PK parameters was defined as the 90 per cent confidence intervals (CIs) for the geometric mean ratios (GMRs) falling within 75-133 per cent limits. Primary endpoints included area under the concentration–time curve - from time zero (pre-dose) to the last quantifiable concentration [$AUC_{(0-t)}$] and from time zero (pre-dose) extrapolated to infinity [$AUC_{(0-\infty)}$], and maximum observed serum concentration (C_{max}). Secondary objectives were to compare the safety and immunogenicity of DRL_TZ with that of the RMP.**

***Results*: Thirty two subjects were dosed (DRL_TZ, 16; RMP, 16). Primary PK parameters were found to be comparable with their 90 per cent CIs for the GMR falling within the usual more stringent limits of 80-125 per cent. The number of subjects reporting at least one TEAE in both the arms was similar. No serious adverse events were reported. Fifteen subjects, eight in DRL_TZ arm and seven in Herceptin® arm, tested positive for anti-drug antibodies (ADAs), none of the ADAs were neutralizing in nature.**

***Interpretation & conclusions*: In this study, DRL_TZ demonstrated PK equivalence with the RMP and had comparable safety and immunogenicity profiles in healthy adult male subjects.**

Key words Biosimilar - healthy volunteers - pharmacokinetics - trastuzumab

Trastuzumab (TZ) (Herceptin®, Genentech, San Francisco, CA, USA) is a recombinant, humanized IgG monoclonal antibody which specifically binds with high affinity to the extracellular domain of the human epidermal growth factor receptor 2 [HER2 or Neu or erbB2 (erythroblastic oncogene B)][1-3]. It was first approved in the United States (US) in 1998 followed by European Union (EU) in 2000. TZ is indicated for the treatment of HER2 overexpressing early as well as metastatic breast cancer and metastatic gastric or gastro-esophageal junction adenocarcinoma[4,5].

Trastuzumab, with its known efficacy and good tolerability, is one of the key drugs currently considered in the treatment regimen of HER2-positive breast cancer, both in early as well as in the advanced stage[6].

Trastuzumab, as the innovator reference medicinal product (RMP), Herceptin® has been available in the market for almost two decades, however, the access to the same has remained limited in many regions, especially the developing countries, on account of the high cost[7]. The development and introduction of a TZ biosimilar can provide a safe, effective and affordable option towards improving patient access to this important anti-cancer biologic therapy, while also contributing to the overall reduced healthcare costs leading to efficiencies in the healthcare system[7,8]. To this end, Dr. Reddy's Laboratories Ltd., Hyderabad, India, developed Dr Reddy's Laboratories TZ (DRL_TZ) as a potential biosimilar of Herceptin®.

Extensive physicochemical and analytical comparability and pre-clinical evaluations have shown DRL_TZ to be comparable to innovator TZ (Herceptin®)- both EU (RMP, EU-approved Herceptin®) & US Reference Product (data on file, Dr Reddy's Laboratories Ltd., Telangana, India). In this study, the pharmacokinetics (PK), safety and immunogenicity of a single intravenous 6 mg/kg dose of DRL_TZ was compared with that of the RMP in healthy adult male subjects.

Regulatory agencies require extensive and comprehensive physicochemical, analytical and pre-clinical comparability evaluations of developmental biosimilars alongside their reference products[9-11]. Relevant non-clinical studies should be performed before initiating clinical trials[11]. Comparative clinical pharmacology studies are considered essential to support a demonstration that there are no clinically meaningful differences between the proposed biosimilar and the reference product as these provide data that describe the degree of PK similarity between the two[12] and assess the feasibility, and the PD similarity[10,12]. EU and US guidances require comparative immunogenicity assessments depending on the class of the product[9,10]. As per EMA guidance, if there are no surrogate efficacy markers and once PK similarity is established, the sponsor is expected to demonstrate comparable clinical efficacy of the developmental biosimilar and the reference product in randomized, parallel-group comparative clinical trial(s), preferably double-blind. The patient population should be generally representative of the approved product indications and sensitive for detecting potential differences between the biosimilar and the reference product[10]. This study aimed to provide the PK similarity between DRL_TZ and RHP among healthy volunteers.

Material & Methods

Study design: This randomized, double-blind, single-dose, parallel-group, phase I study was conducted at Nucleus Network (formally known as Centre for Clinical Studies), Burnet Institute Melbourne, in Australia, between March and August 2016. Of the patients visiting this centre, potential subjects, who provided a written informed consent were screened, and those who met the inclusion and exclusion criteria were enrolled in the study. On the next day, subjects received the study drug as per the randomization plan and were housed in the clinical centre, for seven subsequent days, during which blood samples for PK and anti-drug antibody (ADA) analysis were collected. Thereafter, they were followed up on an ambulatory basis for the next 70 days (Fig. 1).

This study was reviewed and approved by the Alfred Hospital Ethics Committee, and the Australian Regulatory Authority, Therapeutic Goods Administration, prior to initiation and was conducted in accordance with the ICH Harmonised Tripartite Guidelines for Good Clinical Practice[13], the ethical principles laid down in the Declaration of Helsinki, and applicable local regulations. The trial was prospectively registered on the Australian New Zealand Clinical Trial Registry (ACTRN12616000084482).

Inclusion criteria: Male volunteers (any volunteer walking in, based on advertisement) in general good health, aged 18-55 yr, with a body mass index (BMI) between 18-30 kg/m^2, and body weight within 50-100 kg, with screening results (vital signs, physical examination, clinical laboratory tests, 12-lead electrocardiogram (ECG), echocardiogram and thyroid function tests) within the normal or clinically

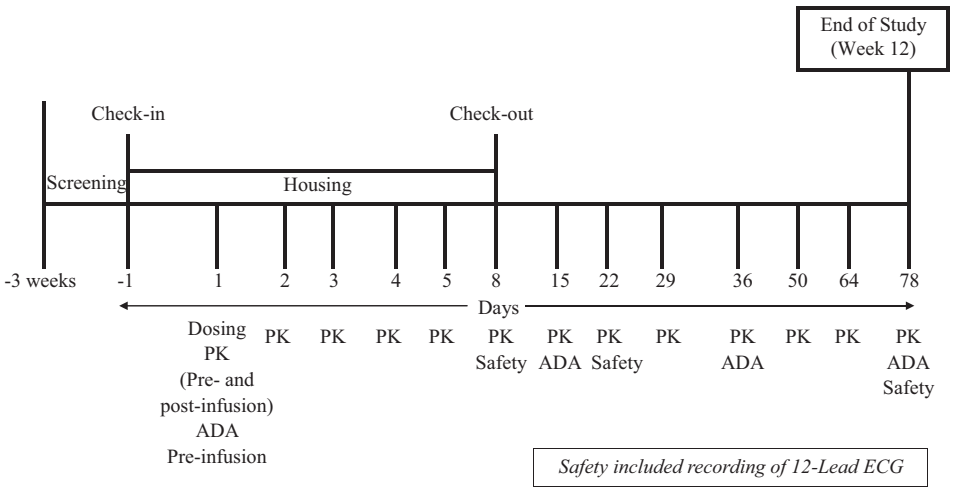

Fig. 1. Schematic representation of the study design.

acceptable range were eligible for enrolment into the study. Subjects had to be willing to use appropriate contraceptive measures for six months after the administration of the study drug.

Exclusion criteria: Key exclusion criteria were prior exposure to TZ or any of its excipients, or history of systemic disease or history of cancer, subjects with abnormal and clinically relevant findings, and/or history of cardiovascular diseases. The use of prescription or non-prescription drugs within 14 days (unless in the opinion of the Investigator, the medication did not interfere with the study procedures or compromise subject safety), hematopoietic growth factors, monoclonal antibodies or immunoglobulins within the last six months or five half-lives, whichever was longer, prohibited entry of subjects in the study. Subjects with a major surgery or trauma within the past one year of screening, difficulty in blood sampling or accessibility of veins, past or ongoing history of alcohol or drug abuse, and participation in a study with TZ or any other HER2 targeted antibody or any prior exposure to these drugs were excluded from the study.

Randomization and treatments: Subjects were randomized in a 1:1 ratio to receive a single dose of 6 mg/kg of DRL_TZ or RMP. Treatments were assigned according to a randomization schedule generated using a block randomization procedure. A variable block size of six, four and two was used. Both treatments were equally balanced within each block and among the blocks. A pre-dose health status recheck was performed. The study drug was administered as a single intravenous administration over 90 min using an electronic infusion pump.

Study endpoints:

Pharmacokinetics (PK): The primary endpoints were, area under the concentration–time curve from time zero (pre-dose) to last quantifiable concentration [$AUC_{(0-t)}$], area under the concentration–time curve from time zero (pre-dose) extrapolated to infinity [$AUC_{(0-\infty)}$], and maximum observed serum concentration (C_{max}). Secondary endpoints included time to maximum concentration (t_{max}), terminal half-life ($t_{1/2}$), and systemic clearance (CL).

These are the usual endpoints determined using non-compartmental analysis for the evaluation of bioequivalence and PK biosimilarity[11,14].

Safety: Safety was assessed in terms of adverse events (AEs) as well as serious AEs (SAEs).

Immunogenicity: Immunogenicity was assessed in terms of the occurrence of ADAs and presence of neutralising antibodies (NAbs).

Pharmacokinetic assessments: Blood samples for PK assessments (3.5 ml each) were drawn pre-dose (*i.e*. 60 min pre-infusion), at the end of infusion, at 0.5, 1 and 6 h, as well as at 24, 48, 72, 96, 168 h after the end of infusion, while the subjects were housed (Fig. 1). For further ambulatory sampling, the subjects were asked to report on days 15, 22, 29, 36, 50, 64 and end of study (EOS) at day 78 following the end of infusion. These time points were selected to provide a

full coverage of the PK profile, with the later sampling time point covering four times the half-life (reported as about 18.3 days)[15].

A validated bioanalytical method using an enzyme-linked immunosorbent assay (ELISA) was used for the quantification of TZ in serum at Syngene International Ltd., Bengaluru. The recombinant human epidermal receptor-2 extracellular domain (rHER2-ECD; EMP Genentech, Germany) was coated on a 96-well plate and then blocked using a blocking buffer. The standards, quality controls and samples were treated for a minimum required dilution of 1:1000 fold in low cross buffer and added to the designated wells of the coated plate. The bound DRL_TZ and RMP were estimated by the addition of goat anti-human IgG (Fc specific; Jackson ImmunoResearch Laboratories, Inc., USA) coupled with horseradish peroxidase (HRP) followed by addition of a chromogenic substrate. The colour obtained after the addition of 1N sulphuric acid was measured on a microplate reader (SpectraMax® Plus 384, Molecular Devices LLC, USA) using validated SoftMax Pro GxP 5.4.1 software (Molecular Devices LLC, USA) at a wavelength of 450 nm with reference to 630 nm. The instrument response versus concentration relationship for standards was regressed according to a four parameter logistic regression model with mean optical density values as weighting factor of $1/Y^2$ using Laboratory Information Management System (Watson 7.3.0.01 Bioanalytical LIMS, Thermo Fisher Scientific, USA).

The lower limit of quantitation (LLOQ) was established at 500 ng/ml. Inter-assay precision and accuracy were established from QCs (Quality control) at five levels (LLOQ, low, medium, high, and upper limit of quantitation) in six different validation runs for DRL_TZ and RMP each. Incurred sample re-analysis was done to demonstrate reproducible quantitation of the drug in study samples.

For PK parameter calculations, in a subject profile, all samples before the first quantifiable sample (including pre-dose) were assigned a numerical value of zero. From the first to the last quantifiable sample, any sample below LLOQ was set to 0.0001. From the last quantifiable sample onwards, samples below LLOQ were set to missing.

Pharmacokinetic parameters were calculated by non-compartmental methods with Phoenix® WinNonlin® 6.4, (Certara USA, Inc., Princeton, New Jersey, USA), using actual sampling times elapsed from start of study drug administration. For the calculation of $AUC_{(0-t)}$, the linear-up/log-down trapezoidal rule was used. Standard non-compartmental methods were chosen for the study as for the evaluation of biosimilarity and bioequivalence[11,14].

Safety assessments: The safety and tolerability of both the treatments were assessed by means of vital signs, AEs, physical examination, ECG, echocardiogram and clinical laboratory (22.7 ml during screening and 12 ml for safety sample analysis) data. AEs were assessed for severity and relationship to the study drug and graded in accordance with the National Cancer Institute Common Terminology Criteria for AEs, version 4.03.

Immunogenicity assessment: Blood samples for immunogenicity analyses (8.5 ml each) were obtained within one hour prior to the study drug infusion and post dose on days 15, 36, and 78 (end-of-study visit) (Fig. 1). At all time points, the samples were tested and confirmed for the presence of anti-TZ antibodies. The confirmed ADA-positive samples of DRL_TZ and RMP in human serum samples were tested for titre estimation and neutralizing antibodies.

All study samples were screened in an affinity capture elution (ACE) based ELISA assay for ADA and the samples above screening cut-point were considered as potentially positive. The potentially positive samples and all confirmed positive samples were subjected to titre estimation and detection of NAb for further confirmation.

Statistical analyses: The sample size calculation targeted to provide a statistical power of 90 per cent to demonstrate equivalence with acceptance limits for the test/reference GMR of 75-133 per cent and assuming an actual test/reference GMR (Geometric Mean Ratio) of 100 per cent. For the calculation, a coefficient of variation of 20 per cent was assumed on the basis of a published randomized controlled PK trial conducted on healthy volunteers[16] which indicated that the coefficient of variation (CV) for TZ C_{max}, $AUC_{(0-\infty)}$ and $AUC_{(0-t)}$ ranged from 16 to 19 per cent, a slightly higher estimate was used for caution. A sample size of 12 subjects per arm would provide a statistical power of 90 per cent under these conditions. The sample size calculation was performed using the R package PowerTOST over R version 2.15.0 (R Foundation for Statistical Computing, Vienna, Austria) specifying the exact method which uses

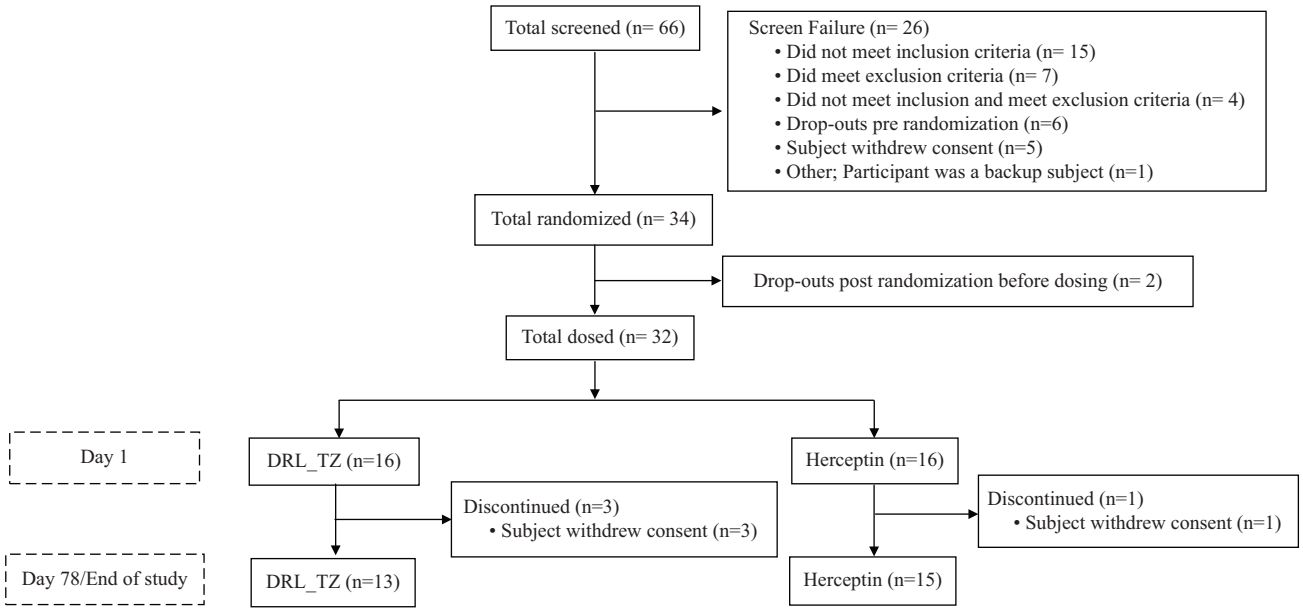

Fig. 2. Subject disposition DRL_TZ, Dr Reddy's Laboratories trastuzumab.

iterative power calculations based on the Owen's Q function[17].

The PK population included all randomized subjects who received the study drug, had primary PK parameters reliably calculated, and completed the study without major protocol deviations or factors that could significantly affect PK assessment. Subjects were included in PK analysis regardless of their ADA status. The safety population consisted of all subjects who were dosed and was analyzed according to actual treatment received. The immunogenicity population comprised all subjects for whom the pre-infusion and post-dose immunogenicity samples with valid results were available.

The primary PK parameters $AUC_{(0\text{-}t)}$, $AUC_{(0\text{-}\infty)}$, and C_{max} were compared among treatment arms using an analysis of variance (ANOVA) model with treatment as the only classification variable. The analysis was done after natural logarithm transformation of the parameters and the ANOVA results were used to construct 90 per cent confidence intervals (CIs) for the DRL_TZ/RMP GMR between both treatments following accepted standards for the comparison of biosimilarity and bioequivalence[10,11,14]. Statistical analysis was performed using SAS version 9.2 (SAS Institute, Inc., Cary, North Carolina, USA).

Results

A total of 34 subjects were randomized. Two subjects withdrew consent prior to dosing. Thirty two subjects, 16 in each arm, received the study drug (DRL_TZ, 16; RMP, 16). Subject disposition is shown in Fig. 2. Demographic and baseline characteristics of subjects were well balanced between both the arms (Table I). The mean (standard deviation) age and BMI of the subjects were 27.69 (5.98) yr and 23.78 (2.76) kg/m^2 respectively (Table I).

Pharmacokinetics (PK): The time versus serum drug–concentration profile curves for both DRL_TZ and RMP exhibited a similar pattern over the entire profiling interval (Fig. 3). Concentrations remained quantifiable in all the subjects until at least week six after dosing. In all subjects included in the final PK analysis, $AUC_{(0\text{-}t)}$ accounted for at least 80 per cent of $AUC_{(0\text{-}\infty)}$.

The peak serum concentrations were similar and were achieved at about the same time after the end of infusion (median: 2.02 h for DRL_TZ; 2.00 h for the RMP, Table II). No infusion interruptions or delays were reported during the study.

The extent of drug exposure post drug administration, as assessed by $AUC_{(0\text{-}t)}$ and $AUC_{(0\text{-}\infty)}$, was also comparable between the arms. The terminal phase for both DRL_TZ and RMP showed a similar pattern with comparable $t_{1/2}$. PK parameters were comparable for both of the products (Table II) with geometric mean values for C_{max} of 137.11 and 136.40 µg/ml for DRL_TZ and RMP, respectively. Geometric Mean $AUC_{(0\text{-}t)}$ values were 33538.10 and 35580.65 µg*h/ml for DRL_TZ and

Table I. Demographics and baseline characteristics

Demographics and baseline characteristics	DRL_TZ (n=16), n (%)	RMP (n=16), n (%)	Total (n=32), n (%)
Gender			
Male	16 (100.0)	16 (100.0)	32 (100.0)
Race			
American Indian or Alaska native	1 (6.3)	0	1 (3.1)
Asian	2 (12.5)	2 (12.5)	4 (12.5)
Black	1 (6.3)	0	1 (3.1)
White	9 (56.3)	11 (68.8)	20 (62.5)
Other	3 (18.8)	3 (18.8)	6 (18.8)
Ethnicity			
Hispanic or Latino	3 (18.8)	1 (6.3)	4 (12.5)
Not Hispanic or Latino	13 (81.3)	15 (93.8)	28 (87.5)
Age (yr), mean (SD)	27.48 (5.63)	27.89 (6.49)	27.69 (5.98)
BMI (kg/m^2), mean (SD)	23.47 (2.85)	24.09 (2.71)	23.78 (2.76)

DRL_TZ, Dr. Reddy's laboratories trastuzumab; RMP, reference medicinal product (EU-approved Herceptin®); SD, standard deviation; BMI, body mass index; EU, European Union

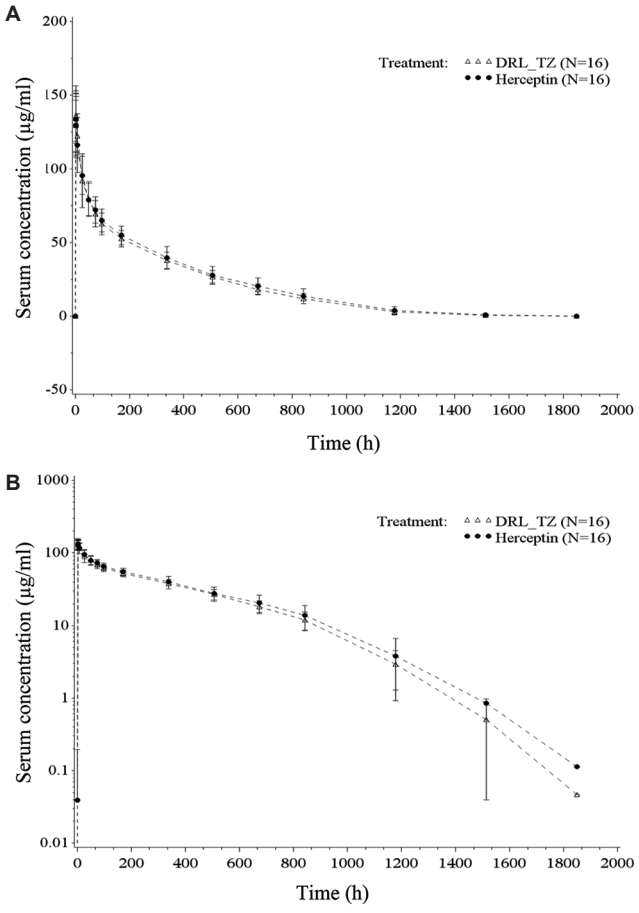

Fig. 3. Mean (±SD) serum concentration-time profiles for all treatments on (**A**) linear and (**B**) semi-logarithmic scales.

RMP, respectively. Geometric Mean $AUC_{(0-\infty)}$ values were 34053.42 and 35932.10 µg*h/ml for DRL_TZ and RMP, respectively. For $t_{1/2}$, the geometric mean values were 179.10 h and 184.70 h for DRL_TZ and RMP, respectively.

The 90 per cent CIs for the GMRs of the PK primary endpoints ($AUC_{(0-\infty)}$, $AUC_{(0-t)}$ and C_{max}) were within the usual acceptance margins (80-125%) for PK equivalence (Table III). For C_{max} the GMR (90% CI) values were 100.52 per cent (92.66-109.04). For $AUC_{(0-t)}$ the GMR (90% CI) values were 94.26 per cent (83.39-106.54). For $AUC_{(0-\infty)}$, the GMR (90% CI) values were 94.77 per cent (85.64-104.88).

The geometric mean (95% CI) measured serum TZ concentration at three weeks after dosing (which corresponds to the trough concentration in the frequently used every three weeks dosing schedule of TZ) was 26.39 (23.86-29.18) µg/ml for DRL_TZ and 27.10 (23.99-30.61) µg/ml for RMP.

Safety: In each of the treatment arms, a similar number of subjects (15 out of 16 treated or 93.8%) reported at least one treatment-emergent AE (TEAE). A numerically higher number of TEAEs (51 vs. 37) were reported in the DRL_TZ arm (Table IV).The most frequently reported TEAEs considered related to study drugs (DRL_TZ or RMP) were pyrexia (21.9%), headache (18.8%), fatigue (12.5%) and chills (12.5%).

Table II. Pharmacokinetic parameters for both the treatments

Treatment	Statistic	C_{max} (µg/ml)	$AUC_{(0-t)}$ (µg*h/ml)	$AUC_{(0-\infty)}$ (µg*h/ml)	T_{max} (h)	$t_{1/2}$ (h)	CL (ml/h)
DRL_TZ	n	16	11	14	16	14	14
	Mean	138.06	33890.54	34305.04	---	181.40	12.45
	SD	16.32	5049.39	4275.54	---	30.35	1.75
	Median	141.00	34077.86	34500.14	2.02	176.39	12.02
	Minimum	106.00	24569.53	25978.90	1.52	140.99	10.17
	Maximum	159.00	43263.56	43388.86	7.55	236.78	17.23
	Geometric mean	137.11	33538.10	34053.42	---	179.10	12.34
	GCV (%)	12.42	15.43	12.74	---	16.65	13.14
	95% LCL	128.36	30254.68	31648.50	---	162.79	11.45
	95% UCL	146.45	37177.87	36641.09	---	197.04	13.31
RMP	n	16	15	16	16	16	16
	Mean	137.81	36220.87	36526.80	---	190.13	12.94
	SD	21.04	6979.72	6781.78	---	46.79	2.35
	Median	131.50	35147.64	35012.98	2.00	185.69	12.86
	Minimum	111.00	25199.01	26082.58	1.50	114.64	9.05
	Maximum	178.00	49378.98	49881.80	2.55	275.30	17.03
	Geometric mean	136.40	35580.65	35932.10	---	184.70	12.73
	GCV (%)	14.73	19.94	19.01	---	25.47	18.72
	95% LCL	126.15	31895.74	32500.44	---	161.60	11.53
	95% UCL	147.47	39691.27	39726.09	---	211.10	14.06

$AUC_{(0-t)}$, area under the concentration-time curve from time zero (pre-dose) to the time of the last quantifiable concentration, estimated using linear up/log down trapezoidal rule; $AUC_{(0-\infty)}$, area under the concentration-time curve from time zero (pre-dose) extrapolated to infinity, calculated by linear up/log down trapezoidal rule and extrapolated to infinity by addition of $AUC_{(0-t)}$ to the last quantifiable concentration divided by the terminal rate constant, $AUC_{(0-t)} + C_{last}/\lambda z$. C_{max}, maximum observed concentration over the entire sampling interval; T_{max}, time to maximum observed concentration; λz, apparent terminal rate constant; $t_{1/2}$, terminal half-life; CL, total body clearance; GCV, geometric coefficient of variation. GCV%=(GSD−1)×100; where GSD geometric standard deviation obtained by back-transforming SD of log transformed data; 95% LCL, lower limit of 95% confidence interval for geometric mean; 95% UCL, upper limit of 95% confidence interval for geometric mean

Table III. Statistical comparison of key pharmacokinetic (PK) parameters

Study Parameters	n		Geometric mean		Geometric mean ratio(%)		90% CI (lower-upper)
	DRL_TZ	RMP	DRL_TZ	RMP	CV%	DRL_TZ/RMP	
Transformation							
PK parameters							
Mixed model-LN transformed data							
C_{max}	16	16	137.11	136.40	13.62	100.52	92.66-109.04
$AUC_{(0-t)}$	11	15	33538.10	35580.65	18.19	94.26	83.39-106.54
$AUC_{(0-\infty)}$	14	16	34053.42	35932.10	16.38	94.77	85.64-104.88

All the TEAEs were grade 1-2 in severity. Infusion reactions were experienced by five and two subjects in DRL_TZ and RMP arms, respectively. Of the four subjects (3 in DRL_TZ arm and 1 in RMP arm) who were withdrawn, three subjects (2 in DRL_TZ arm and 1 in RMP arm) reported at least one AE. Majority of the infusion reactions were of grade 1 (n=12; grade 1=8, grade 2=4). None of the infusion reactions triggered

Table IV. Summary of treatment emergent adverse events (TEAEs, occurring in any arm >0%) by system organ class and preferred term for each treatment

TEAEs system organ class preferred term	DRL_TZ (n=16), n (%), E	RMP (n=16), n (%), E
TEAE	15 (93.8), 51	15 (93.8), 37
General disorders and administration site conditions	11 (68.8), 16	5 (31.3), 7
Pyrexia	6 (37.5), 6	1 (6.3), 1
Fatigue	4 (25.0), 4	1 (6.3), 1
Chills	2 (12.5), 2	2 (12.5), 2
Influenza-like illness	1 (6.3), 1	1 (6.3), 1
Infusion site erythema	1 (6.3), 1	1 (6.3), 1
Feeling cold	1 (6.3), 1	0
Feeling hot	1 (6.3), 1	0
Infusion site pain	0	1 (6.3), 1
Infections and infestations	8 (50.0), 9	9 (56.3), 10
Upper respiratory tract infection	6 (37.5), 7	8 (50.0), 9
Gastroenteritis	1 (6.3), 1	1 (6.3), 1
Oral herpes	1 (6.3), 1	0
Nervous system disorders	9 (56.3), 9	3 (18.8), 4
Headache	8 (50.0), 8	3 (18.8), 3
Dizziness	1 (6.3), 1	1 (6.3), 1
Gastrointestinal disorders	4 (25.0), 6	3 (18.8), 4
Nausea	2 (12.5), 2	1 (6.3), 1
Diarrhoea	1 (6.3), 1	1 (6.3), 1
Abdominal discomfort	1 (6.3), 1	0
Aphthous ulcer	0	1 (6.3), 1
Bowel movement irregularity	1 (6.3), 1	0
Dyspepsia	0	1 (6.3), 1
Vomiting	1 (6.3), 1	0
Musculoskeletal and connective tissue disorders	4 (25.0), 4	4 (25.0), 5
Myalgia	2 (12.5), 2	1 (6.3), 1
Arthralgia	1 (6.3), 1	1 (6.3), 1
Back pain	0	2 (12.5), 2
Musculoskeletal chest pain	1 (6.3), 1	0
Pain in extremity	0	1 (6.3), 1
Skin and subcutaneous tissue disorders	3 (18.8), 3	2 (12.5), 2
Rash	2 (12.5), 2	1 (6.3), 1
Acne	0	1 (6.3), 1
Atopic dermatitis	1 (6.3), 1	0
Injury, poisoning and procedural complications	0	2 (12.5), 2
Infusion related reaction	0	1 (6.3), 1
Musculoskeletal injury	0	1 (6.3), 1
Respiratory, thoracic and mediastinal disorders	1 (6.3), 1	1 (6.3), 1
Cough	1 (6.3), 1	0
Oropharyngeal pain	0	1 (6.3), 1

Contd...

TEAEs system organ class preferred term	DRL_TZ (n=16), n (%), E	RMP (n=16) n (%), E
Cardiac disorders	0	1 (6.3), 1
Palpitations	0	1 (6.3), 1
Code not available	1 (6.3), 1	0
Surgical removal of birth mark (left flank)	1 (6.3), 1	0
Ear and labyrinth disorders	1 (6.3), 1	0
Ear congestion	1 (6.3), 1	0
Investigations	0	1 (6.3), 1
Transaminases increased	0	1 (6.3), 1
Renal and urinary disorders	1 (6.3), 1	0
Nephrolithiasis	1 (6.3), 1	0

dose interruption or subject withdrawal. All the TEAEs resolved with majority of them recovering within a week. The ECG and echocardiogram of all subjects were normal throughout the study with no abnormal left ventricular ejection fraction values. There were no SAEs reported during the study.

Immunogenicity: Fifteen subjects, eight in DRL_TZ arm and seven in RMP arm, showed ADA positive results by confirmatory assay at least once after dosing. The ADAs were observed transiently in these subjects with 14 out of 15 subjects becoming ADA negative by EOS. None of the ADA-positive subjects showed neutralizing antibodies.

Discussion

This phase I study was intended to compare the PK, safety and immunogenicity of DRL_TZ and RMP. A comparable PK profile supports the conclusion that there are no clinically meaningful differences between a proposed biosimilar and its reference product[18]. Hence, it is important for establishing the PK equivalence of a proposed biosimilar with a reference product in a study evaluating a population, route of administration, and dose that are adequately sensitive for detection of PK differences[18]. In this context, a study performed in normal healthy subjects has several advantages over a similar comparative patient study[12]. It allows for a long enough sampling to properly elucidate the terminal phase of the profile, as in this study. Patients on treatment with TZ need to be dosed at weekly or at three weekly intervals[5,6], which, given the observed half-life values (16.4 days[19] or 18.3 days with the three weekly regimen[15] and 6.2 days with the once-weekly regimen[20], are too short for a proper elucidation, whereas the present study had a 77 day sampling schedule post-administration (approximately four times 16.4 days[19] to 18.3[15] days half-life) which has been previously to be sufficient for full PK profiling. In addition, patient populations have potential confounding factors such as underlying disease burden, concomitant illnesses/co-morbidities, and concomitant medications[12].

Only male subjects were included considering possible risk for women of childbearing age and to avoid the possibility of developing anti-TZ antibodies as TZ is widely administered in women given its indication in breast cancer[21-24]. It is acknowledged that the differences between NHV (normal healthy) and patients in terms of PK may be present even if underlying processes remain the same, hence a limited evaluation of PK in patients while not essential might be desirable, in addition to proving PK similarity in NHV.

A parallel-group design (as used in this study) is usually considered appropriate for products that have a long half-life (values in this study ranging from 114.64 to 275.30 h; Table II) or for products where repeated exposures can lead to an increased immune response that can affect the PK and/or pharmacodynamic similarity assessments[12].

In this study, PK equivalence was demonstrated for DRL_TZ *vs*. RMP as the 90 per cent CIs for the GMR of $AUC_{(0-\infty)}$, $AUC_{(0-t)}$, and C_{max} were within the bioequivalence limits of 80 - 125 per cent[21]. The results show that the 6 mg/kg dose was appropriate for PK similarity evaluation[16]. Measured AUC [$AUC_{(0-t)}$] accounted for more than 80 per cent of total AUC [$AUC_{(0-\infty)}$] in all subjects analyzed for AUC (exclusions were due to missing samples).

Several comparable PK studies in healthy subjects evaluating candidate biosimilars have been published[16,21,24]. All these studies included only male

subjects and employed a parallel arm design. Several of the studies evaluated both the USA and the EU reference products[16,22,25,26], some included either the US or the EU reference product[23,24], only one study included a reference product from Japan[27]. The inclusion of both reference products is intended to scientifically bridge the EU and US reference products[28] and to provide the data justifying the use of a single reference product in further comparative clinical trials in patient populations[16]. In the present study, there was a single reference product as reference product bridging was not an objective of the study. Sampling for PK duration in other studies ranged from 56 to 84 days as compared to 77 days in this study. Overall, the results of these studies are comparable with those in the present study. If evaluating the results of the reference products and after linear normalization to a 6 mg/kg dose of the Waller *et al*[26] study results (tested dose 8 mg/kg) and considering only the 6 mg/kg group results from Wisman *et al*[28], arithmetic mean C_{max} values ranged from 140 to 174 µg/ml for the reference products as compared to 137.81 µg/ml in this study. Mean $AUC_{(0-t)}$ and $AUC_{(0-\infty)}$ ranged from 32690 to 39499 µg*h/ml and from 32729 to 41466 µg/ml respectively as compared to 36220.87 and 36526.80 µg*h/ml respectively in the present study. Finally, the arithmetic mean of $t_{1/2}$ ranged from 154 to 249 h as compared to 190.13 h in the present study (Table II).

DRL_TZ and RMP administered as a single 6 mg/kg intravenous dose were comparably well tolerated in the study population. The profile of drug-related events was similar to that observed in other TZ trials with frequently reported drug-related AEs being pyrexia, headache, chills and fatigue[16,27,29]. No new or unexpected safety events were observed. Given the sample size which was optimized for continuous scale PK parameters and not for the dichotomous incidence of AEs the slightly higher number was likely due to random fluctuation.

In this study, the occurrence of ADA seemed to be higher (15 out of 32 subjects) than that published in similar TZ healthy volunteer studies[16,27], though ADA rates among different studies are not directly comparable due to differences in bioanalytical methodology. ADA rate was similar between both the arms and none of them were neutralizing.

This study is however, not without some limitations. The study was conducted in a limited number of healthy subjects which was appropriate for its PK objectives as per the sample size calculations but provides limited evaluation of safety and tolerability. However, this was a secondary objective of the study, this being a single-dose administration of the investigational product. The development programme also included a clinical study in patients, providing a more direct and clinically relevant safety and tolerability assessment. The same applies to the evaluation of immunogenicity.

Regarding PK, while the initially estimated equivalence limit of 75 to 133 per cent could have been a potential limitation, this was not the case because the full 90 per cent CIs were found to be within the usual and more stringent 80 to125 per cent bioequivalence limits (due to the margin awarded by the use of a slightly higher expected variability as compared to the available literature values).

In conclusion, PK, safety and tolerability, and immunogenicity of DRL_TZ were comparable to the innovator EU-approved TZ in healthy male subjects. The results support the continued development of DRL_TZ as a candidate biosimilar to TZ.

***Financial support & sponsorship*:** This study was funded by Dr Reddy's Laboratories Ltd., Telangana, India.

***Conflicts of Interest*:** The first author (JDL) was the principal Investigator of this study and hence received a study grant. Authors (RND, RKT, SRNK & PKR) were employees of Dr Reddy's Laboratories Ltd.

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