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Research Article

BIOEQUIVALENCE AND PHARMACOKINETIC EVALUATION OF TWO FORMULATIONS OF PARACETAMOL ER 650 MG: A SINGLE-DOSE RANDOMIZED TWO-PERIOD CROSSOVER COMPARISON IN HEALTHY INDIAN ADULT VOLUNTEERS

DHANESHWAR SHEP[1], ASHWINI OJHA[2], SWETA PATEL[3], RAJESHWARI RATHOD[4], MANISH NIVSARKAR[5], VIJAYA JAISWAL[6†] AND HARISH PADH [7]

[1]Sr. Manager, Medical Services, Troikaa Pharmaceuticals Ltd., Ahmedabad, [2]Scientist A, Department of Bio-analytical, B.V. Patel PERD Centre, Ahmedabad, [3]Research Assistant, Department of Biostatistics, B.V. Patel PERD Centre, Ahmedabad, [4]Scientist A, Department of Bio-analytical, B.V. Patel PERD Centre, Ahmedabad, [5]Joint Director, Department of Pharmacology and Toxicology, B.V. Patel PERD Centre, Ahmedabad, [6]Vice-President, Medical Services, Troikaa Pharmaceuticals Ltd, Commerce House-1, Satya Marg, Bodakdev, Ahmedabad – 54, Phone No- 91-79-26856242-45, Fax : 91-79-26856246. [7]Director, B. V. Patel PERD Centre & Project Director NIPER, Ahmedabad.

E-mail: medicalservices@troikaapharma.com

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ABSTRACT

Background: Paracetamol is one of the most popular and widely used drugs for the treatment of pain and fever. Due to its good tolerability profile, paracetamol is often the analgesic or antipyretic of choice, especially in patients in whom salicylates or other nonsteroidal anti-inflammatory drugs are contraindicated. OBJECTIVE: The aim of this study was to compare the bioavailability and tolerability of 2 oral formulations of paracetamol ER 650 mg. Methods: This single-dose, randomized, single-label, 2-period crossover study in healthy Indian adult volunteers was conducted at PERD Centre, Ahmedabad. Subjects received paracetamol ER 650 mg of either test or reference formulation with a washout period of 7 days. After study drug administration, serial blood samples were collected over a period of 24 hours. Plasma was analyzed for paracetamol concentration using a validated high-performance liquid chromatography method. Pharmacokinetic (PK) parameters C_{max}, T_{max}, $t_{1/2}$, AUC_{0-t}, AUC $_{0-\infty}$, and k_{el}, were determined for the 2 paracetamol formulations. The formulations were to be considered bioequivalent if the log-transformed ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence range of 80% to 125%. Results: A total of 18 subjects were enrolled (mean BMI 21.97, a mean age of 29.4 years, mean weight of 60.7 kg and a mean height of 166.6 cm). No significant differences were found based on analysis of variance, with mean values and 90% confidence intervals of test/reference ratios for these parameters as follows: C_{max}, 6.02 versus 6.17 µg/mL (89.9- 109.9); AUC_{0-t}, 30.54 versus 31.12 µg.hr/mL (92.31-104.1); and $AUC_{0-\infty}$, 31.36 versus 31.69 µg.hr/mL (91.58-106.6). CONCLUSION: In these healthy Indian volunteers, results from the PK analysis suggested that the test and reference formulations of paracetamol ER 650 mg tablets were bioequivalent, based on the regulatory definition.

Keywords: Paracetamol, Bioequivalence evaluation

INTRODUCTION

Paracetamol is one of the most popular and widely used drugs for the treatment of pain and fever.[1] Due to its good tolerability profile, paracetamol is often the analgesic or antipyretic of choice, especially in patients in whom salicylates or other nonsteroidal anti-inflammatory drugs are contraindicated.[2]

Paracetamol is well absorbed from the proximal small bowel and is not subject to significant first-pass metabolism in the liver, with oral bioavailability estimated at between 63–89% in adults.[3,4] Paracetamol is not significantly bound to plasma proteins, and has a volume of distribution of 0.7–1 L/kg. Maximal analgesic and antipyretic activity occurs 1–2 h after peak plasma levels, [3,5] and the time to achieve this varies with the route of administration. Peak plasma concentration (C_{max}) is achieved approximately at 45 min.[3] Metabolism of paracetamol occurs primarily in the liver, while elimination occurs almost entirely through the kidney. Following absorption of therapeutic doses, approximately 90% is metabolised by glucuronidation and sulphation to form non-toxic metabolites, which are excreted in the urine.

Paracetamol, like many other analgesics, has a short half-life around 2–3 hours which necessitates frequent dosing.[6] In UK the recommended regimen is 500–1000 mg every 4–6 hours. However, it would be advantageous if the duration of action were longer so that fewer daily doses could maintain therapeutic plasma levels. This would improve patient convenience and compliance and be of benefit to the patient at night-time.[6]

A generic version of the extended release formulation of paracetamol has been developed by Troikaa Pharmaceuticals Ltd, India which combines extended and immediate release paracetamol in a bi-layer tablet. This formulation (denoted as ER paracetamol) has been designed to be taken three times daily. Each bilayer tablet contains 650 mg paracetamol (325 as immediate release and 325 mg as extended release).

The aim of this study was to compare the bioavailability of the newly developed bilayer tablet formulation of paracetamol with innovator brand - Tylenol extended release caplets in healthy Indian adult male volunteers.

MATERIAL AND METHODS

The study was carried out at the B. V. Patel Pharmaceutical Education and Research Development centre, Ahmedabad. All the subjects provided written informed consent to participate in the study prior to enrolment and were free to withdraw at any time during the study. The study was approved by the institutional ethics committee and was conducted in accordance with good clinical practice and the declaration of Helsinki.

Study subjects

The study population consisted of 18, adult, male healthy subjects with mean BMI 21.97, a mean age of 29.4 years, mean weight of 60.7 kg and a mean height of 166.6 cm.

Design

The study was designed as Double blind, Balanced, Randomized, Two- Treatment, Two-Sequence, Two Period, Single Dose, Crossover Bioequivalence study with 7 days washout period.

The volunteers were administered one of the two study drugs after an overnight fast. The dose administration was performed as per the randomization schedule generated at B.V. Patel PERD Centre, Ahmedabad. Subjects received single oral dose of the test formulation (paracetamol ER 650 mg, Troikaa Pharmaceuticals Ltd. India) and reference formulation (paracetamol ER 650 mg, McNeil, USA).

Blood sampling

A total of 19 blood samples were collected during each period. Blood samples were collected through an indwelling cannula placed in the forearm vein using disposable syringe or with disposable syringes and needles. 4 mL of blood samples (including 0.2 mL discarded heparinised blood) were withdrawn at pre-dose and 0.25, 0.50, 0.75, 1.0, 1.25, 1.50, 2.0, 2.50, 3.0, 3.50, 4.0, 4.50, 5.0, 6.0, 8.0, 10.0, 14.0, 24.0 hrs following drug administration in each period. After centrifugation, plasma separated from blood samples was stored at –20 ± 5°C for interim storage and then at –70 ± 5°C until analysis.

Method of analysis

The analytical method used for determination of drug concentrations in *in-vivo* plasma samples was a validated high performance liquid chromatography method with ultravoilet detection at 242nm. 100µL plasma sample was extracted into ethyl acetate after addition of internal standard (4-amino acetophenone). The organic layer was evaporated under nitrogen and the sample reconstituted in mobile phase before injection on high performance liquid chromatograph (Jasco, Japan). The column used for separation from endogenous plasma components was HQ Sil HS C_{18}, 250 X 4.6mm, 5µm from Kya Technologies and the mobile phase was 20% acetonitrile and 80% 10mM ammonium acetate. Precision and accuracy were validated over the concentration range of 0.05 to 10µg/mL. The intra and inter day precision (%cv) of the method at low, medium and high concentrations were less than 15%. The method accuracy ranged from 91 to 112%.The lower limit of quantitation was 0.05µg/mL and the method recovery ranged from 72 to 81%.

Table 1: Summary of pharmacokinetic parameters of Paracetamol, following administration of the reference and test formulations

Products	Reference						Test					
Parameters	C_{max}	T_{max}	AUC_{0-t}	$AUC_{0-\infty}$	$t_{1/2}$	K_{el}	C_{max}	T_{max}	AUC_{0-t}	$AUC_{0-\infty}$	$t_{1/2}$	K_{el}
	(µg/mL)	(h)	(µg.h/mL)	(µg.h/mL)	(h)	(h^{-1})	(µg/mL)	(h)	(µg.h/mL)	(µg.h/mL)	(h)	(h^{-1})
MEAN	6.17	1.06	31.12	31.69	7.00	0.11	6.02	1.22	30.54	31.36	7.73	0.09
SD	1.47	0.72	8.02	8.12	2.10	0.03	1.56	0.80	7.57	7.70	1.64	0.03
SEM	0.35	0.17	1.89	1.91	0.50	0.01	0.37	0.19	1.78	1.81	0.39	0.01
% CV	23.76	67.75	25.76	25.63	30.02	29.95	25.98	65.40	24.78	24.55	21.22	26.49

C_{max}: Maximum measured plasma concentration; T_{max}: Time of maximum measured plasma concentration; AUC_{0-t}: The area under the plasma concentration versus time curve from time zero to the last measurable concentration; $AUC_{0-\infty}$: The area under the plasma concentration verses time curve from zero to infinity; $t_{1/2}$: Time required for the plasma drug concentration to decrease by one half; K_{el}: Apparent first order elimination or terminal rate constant; SEM: Standard error of mean; %CV: Coefficient of variation; Test: Troikaa Pharmaceuticals Ltd. India, Reference: McNeil, US .

Table 2 : Point estimate and 90% confidence intervals for the ratio of the products averages of Test and reference formulations

Parameter	Point estimate test: reference	Lower confidence limit	Upper confidence limit
C_{max}	0.98	89.9	109.9
AUC_{0-t}	0.981	92.31	104.1
$AUC_{0-\infty}$	0.990	91.58	106.6

C_{max}: Maximum measured plasma concentration; AUC_{0-t}: The area under the plasma concentration versus time curve from time zero to the last measurable concentration; $AUC_{0-\infty}$: The area under the plasma concentration verses time curve from zero to infinity

Pharmacokinetic and statistical analyses

Maximal plasma concentration (Cmax) and time to reach the peak concentration (T_{max}) were obtained directly by the visual inspection of each subject's plasma concentration-time profile. The slope of the terminal log-linear portion of the concentration-time profile was determined by least-squares regression analysis and used as the elimination rate constant (K_{el}). The elimination half-life was obtained from the formula, $t_{1/2} = ln(2)/ K_{el}$ The AUC_{0-t} from time zero to the last quantifiable point (Ct) was calculated using the trapezoidal rule and the extrapolated AUC from Ct to infinity ($AUC_{0-\infty}$) was to be determined as Ct/ K_{el}. The area under the plasma concentration-time from 0 to infinity ($AUC_{0-\infty}$) was calculated as the sum of the AUC_{0-t} plus the ratio of the last measurable concentration to the elimination rate constant.

To test the bioequivalence of the test and reference formulations, analysis of variance (ANOVA) for the crossover design was conducted on log-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$. The formulations were to be considered bioequivalent if the log transformed ratios (test/reference) of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were within the predetermined bioequivalence range of 80% to 125% and if P was **>**0.05 for the 90% confidence intervals.[7]

Safety and tolerability

General clinical safety was assessed *via* physical examinations and vital signs conducted at screening and at the end of the study. Clinical laboratory tests and ECGs were also conducted at screening, before dosing within each treatment period, and at the end of the study. Adverse events were assessed for severity and relationship to treatment through out the study.

RESULTS

Pharmacokinetic analysis

The mean serum concentration–time curves of 2 ER formulations of paracetamol products each administered as a single 650 mg oral dose to 18 healthy Indian male volunteers are shown in the figure 1. The primary PK parameters for both formulations are listed in Table 1. The mean (SD) C_{max} values of the test and reference formulations were 6.02 (1.56) and 6.17 (1.47) µg/mL, respectively. The mean (SD) T_{max} values were 1.22 (0.8) and 1.06 (0.72) hours. Results for the extent of absorption, as determined from mean (SD) AUC_{0-t} and $AUC_{0-\infty}$ values, were 30.54 (7.57) and 31.36 (7.70) µg/mL/h respectively, after administration of the test formulation; and 31.12 (8.02) and 31.69 (8.12) µg/mL/h after administration of the reference formulation. The mean (SD) $t_{1/2}$ was 7.73 (1.64) hours for the test formulation and 7.0 (2.10) hours for the reference formulation. On ANOVA, no period, formulation or sequence effects were observed for any PK property. The 90% confidence intervals of the ratios (test vs reference) for the natural log (ln)-transformed C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ are shown in Table 2 and summary statistics are shown in Table 3. The 90% confidence intervals for the ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 89.9 to 109.9, 92.31 to 104.1 and 91.58 to 106.6 respectively, meeting the predetermined criteria for bioequivalence.

Safety and tolerability

All 18 subjects completed the study and there were no premature withdrawals, replacements or death during the study. No serious adverse events were recorded, and there were no clinically significant changes in vital signs, clinical laboratory variables, ECG parameters or physical examination findings during the study. There were no adverse events reported during the study.

Table 3: Summary statistics of paracetamol in 18 adult, subjects under fasting conditions.

Parameters Summary statistics	Product	C_{max} (µg/mL)	AUC_{0-t} (µg.h/mL)	$AUC_{0-\infty}$ (µg.h/mL)
Geometric Mean	Test	5.47	28.68	29.46
	Reference	5.90	29.27	29.82
Least Square Mean (LSM)	Test	5.47	28.68	29.46
	Reference	5.90	29.27	29.82
LSM Ratio B/A %		92.7	98.0	98.8
90 % Confidence Interval : B/A				
Lower Limit		89.9	92.31	91.58
Upper Limit		109.9	104.1	106.6
p - value (ANOVA)				
Period		>0.05	>0.05	>0.05
Formulation		>0.05	>0.05	>0.05
Sequence		>0.05	>0.05	>0.05
Intra-subject Variability: CV(%)		17.45	10.5	28.1

A: Reference Product; B: Test Product; ANOVA: Analysis of variance; B/A: Bioavailability ratio Test (B) / Reference (A); %CV: Coefficient of variation.

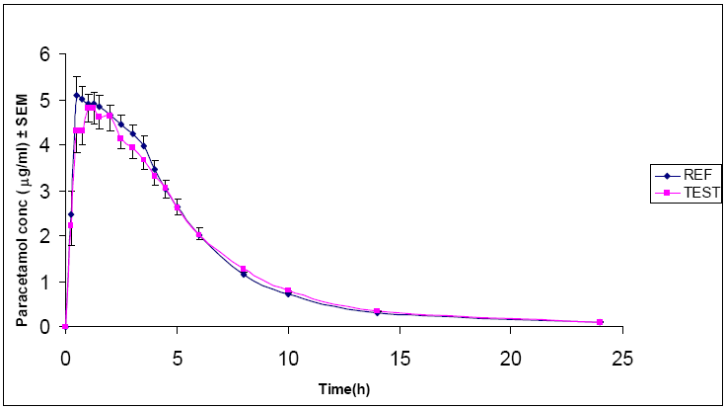

Fig. 1: linear plot of mean paracetamol concentration versus time in 18 male subjects under fasting conditions

DISCUSSION

This study examined the PK properties and bioequivalence of 2 formulations of paracetamol ER —a newly developed extended release bilayer tablet and an established branded tablet—in healthy Indian adult male volunteers. The 90% confidence intervals were completely contained within the predefined bioequivalence criteria of 80% to 125% for the primary end point of C_{max} and AUC. The study results revealed that the 2 formulations of paracetamol were similar in PK characteristics among these healthy Indian male volunteers. The 90% confidence intervals for the ratios of C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ were 89.9 to 109.9, 92.31 to 104.1 and 91.58 to 106.6 respectively, meeting the predetermined criteria for bioequivalence. The mean $t_{1/2}$ obtained in this study was 7.73 hours for the test formulation, which was comparable to that of the reference formulation at 7.0 hours. The mean C_{max} of the test was 6.02 µg/mL, which was comparable to that of the reference formulation 6.17 µg/mL.

The ER formulation of paracetamol and immediate release (IR) paracetamol have been reported to be clinically and statistically equivalent. Both formulations were similar in terms of both onset of analgesia and peak analgesic effect.[6]

The ER paracetamol taken three times daily was reported to be statistically and therapeutically non inferior to IR paracetamol taken four times daily in patients with knee pain due to osteoarthritis. The ER paracetamol may thus, be more convenient for patients with chronic pain and has the potential to enhance compliance and therefore pain relief.[8] It may be of benefit to the patient at night-time.[6]

Paracetamol is very well tolerated. Systematic reviews have found the rate of adverse events following its administration is not significantly different to that following administration of placebo,[3,9,10] while hypersensitivity reactions are rare.[3,11]

Although the major concern with paracetamol administration relates to the potential for hepatotoxicity, this is extremely rare following therapeutic dosing.[3,12] In the present study both formulations were well tolerated and no adverse events were reported during the study.

CONCLUSION

In this study in healthy Indian adult male volunteers, a single 650 mg dose of the extend release bilayer formulation (test) of paracetamol met the regulatory criteria for bioequivalence to a single 650 mg dose of the established tablet formulation (reference) based on the rate and extent of absorption. Both formulations were well tolerated.

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