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Bioequivalence Study of Nicotine 4 Mg Lozenges in Indian Healthy Adult Human Male Smoker Subjects

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Abstract

Nicotine Lozenges are used to aid smokers wishing to quit or reduce prior to quitting. The aim of this study was to determine the bioequivalence of a test and reference formulation of Nicotine 4 mg Lozenge. This single dose, randomized, 2-period, 2-sequence, laboratory-blinded, crossover design study was conducted in 28 Indian healthy adult human male smoker subjects under fasting conditions with a washout period of 7 days. Study formulations were administered after a 10 hrs overnight fast. Blood samples for pharmacokinetic profiling were taken post-dose up to 16 hrs. Safety was evaluated through the assessment of adverse events, and laboratory tests. Plasma concentrations of Nicotine were determined with a validated LC-MS/MS method. Bioequivalence between the products was determined by calculating 90% confidence interval (90% CI) for the ratio of C_{max} and AUC_{0-t} values for the test and reference products, using logarithmic transformed data. The 90% CI of Nicotine were 109.85-123.32 and 101.48-115.41 for C_{max} and AUC_{0-t} respectively. Since the 90% CI for C_{max} and AUC_{0-t} were within the 80-125% interval, it was concluded that the two formulations of Nicotine 4 mg Lozenge are bioequivalent in their rate and extent of absorption.

Keywords: Nicotine 4mg lozenge; Smoking cessation; Nicotine replacement therapy; Bioequivalence

Abbreviations: AUC_{0-t}: Area under the Plasma Concentration versus Time Curve from Time 0 to Time t; AUC_{0-inf}: Area under the Plasma Concentration versus Time Curve from Time 0 Extrapolated to Infinity; C_{max}: Maximum Plasma Concentration; CI: Confidence Interval; CV: Coefficient of Variation; °C: Degree Centigrade; cm: Centimeter; ≥: Greater than or Equal to; hr(s): Hour(s); K_e: Elimination Rate Constant; kg(s): Kilogram(s); LC-MS/MS: Liquid Chromatography–Mass Spectroscopy/ Mass Spectroscopy; ≤ less than or equal to; LOQ: Lower Limit of Quantification; min(s): Minute(s); mm: Millimeter; m: Meter; mM: Millimol; µl: Microliter; ng/mL: Nanogram/ Milliliter; %: Percent; ppm: Parts Per Million; PK: Pharmacokinetic; rpm: Rotations Per Minute; SAS: Statistical Analysis Software; T_{max}: Time to reach C_{max}; $t_{1/2}$: Elimination Half-Life; yr(s): Year(s)

Introduction

Smoking accounts for more deaths and diseases worldwide than any other modifiable risk factors [1,2]. Literature suggests that approximately three quarters of smokers want to quit; however, smoking is highly addictive and smoking cessation is difficult with frequent relapses common amongst those who try to quit [1]. There is ongoing research on the effectiveness of various smoking cessation interventions. Nicotine replacement therapy (NRT), bupropion, varenicline and cytisine medications have been shown to improve the likelihood of quitting, with varenicline showing the greatest benefit. However, these products have relatively low consumer appeal and product satisfaction [3,4]. Evidence suggests that psychosocial smoking cessation interventions such as behavioral counselling, telephone support and self-help interventions are effective; behavioral interventions combined with pharmacotherapy increase the success rate of quitting [5,6].

Nicotine replacement therapy (NRT) helps smokers quit by providing nicotine at levels usually lower than those obtained through smoking and without the toxins contained in tobacco smoke. NRT can reduce the craving for nicotine and the nicotine withdrawal symptoms which might otherwise jeopardize the smoking cessation efforts [7].

Currently several dosage forms are available for Nicotine replacement therapy apart from lozenges such as- patches, nasal sprays, inhalers, sublingual tablets, chewing gums, and electronic cigarettes. Nicotine lozenges are the clinician's preferred form of nicotine replacement for users of chewing tobacco and snuff. Nicotine 4 mg Lozenges are suitable for adult (including the elderly) smokers who have their first cigarette of the day within 30 minutes of waking up [8].

A new generic formulation of Nicotine 4 mg Lozenge has been developed having the same composition as innovator brand, NIQUITIN® 4 mg Lozenge of Glaxosmithkline Consumer Healthcare, UK. A single dose of Nicotine 4 mg Lozenge has been evaluated in this study. The pharmacokinetics of Nicotine was evaluated in 28 healthy adult male smoker subjects. The aim of this study was to determine the bioequivalence and to compare the pharmacokinetics of two formulations (innovator vs. generic) of Nicotine 4 mg Lozenge.

Materials and Methods

Subjects

A total of 28 Asian Indian healthy adult human male smoker subjects who were part of Sitec healthy volunteer pool representing the general population were enrolled in this study. Male subjects (light smokers) between 18-45 years of age having body mass index ≥ 18.5 kg/

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m^2 and ≤ 30.00 kg/m^2, who smoke ≤ 10 cigarettes per day regularly since last three months and who had exhaled carbon monoxide (CO) levels ≥ 10 ppm at the time of screening were eligible for participation in the study. The demographics of all 28 recruited subjects are summarized in Table 1.

The subjects were screened within 21 days prior to study enrolment. The screening procedure included general history (like previous participation in clinical study/blood donation, alcohol and tobacco consumption); demographic data, including name, sex, race, age, body weight (kg), height (m); medical history, physical examination, vital signs measurement, a 12-lead electrocardiogram (ECG), hematology, biochemistry, urine analysis, testing for HIV I and II; hepatitis B and C.

Subjects were judged to be healthy based on acceptable physical examination, and clinical laboratory test results. The clinical investigator reviewed the screening data and performed the physical examinations.

Informed consent and ethical approval

The protocol and informed consent forms (ICFs) were reviewed and approved prior to study initiation by an independent ethics committee. All the subjects were informed about the nature, risks and purpose of the study in the language they understand. Adequate time was given to read and understand the ICF and a written informed consent was obtained from each one of them prior to study initiation. This clinical trial was conducted in accordance with the Declaration of Helsinki, good Clinical Practice guidelines and national regulatory requirements [9-12].

Study design

This study was an open label, randomized, two-treatment, two sequence, two-period, cross-over, single-dose comparative oral bioavailability study of Nicotine 4 mg Lozenge (Test) and Niquitin* 4 mg Lozenge (Reference) of Glaxosmithkline Consumer Healthcare, UK.

The subjects did not consume any food and beverages containing xanthine or alcohol (48 hrs before dosing and throughout the period of sample collection), grapefruit (7 days before dosing and throughout the study), or vitamins (throughout the confinement period). Medications (including herbal and over-the-counter products) were prohibited for the 14 days preceding the study and also during the study.

On check in day, at least 36 hrs prior to each dosing, all subjects were screened for cocaine, cannabinoids, benzodiazepines, Opioids, Amphetamines, barbiturates and alcohol. Oral cavity examination and exhaled CO levels were checked. A total of 28 subjects who satisfied all the criteria for inclusion were admitted to the study center in the evening before dosing (Day-1). Subjects' belongings were thoroughly checked and they were asked to remove all outer garments and take a shower (including hair wash). Subjects wore clothing provided by Sitec for the duration of confinement.

	Age (yrs)	Weight (kgs)	Height (m)	BMI (Kg/m²)
Number of Observations	28	28	28	28
Mean	26	67.1	1.69	23.5
Standard Deviation	5	8.9	0.06	2.7
Median	27	65.4	1.70	23.8
Minimum	19	51.5	1.57	18.9
Maximum	36	86.1	1.79	28.5

Table 1: The demographics of all 28 recruited subjects are summarized.

Then, they were assigned to each treatment sequence as per the randomization scheme. All study medications were kept in a pharmacy and temperature and humidity were monitored continuously. A SAS generated randomization code was used to ensure balanced permutation of the treatments.

Drug administration

All the subjects received doses of Nicotine 4 mg Lozenge on the dosing day. Training sessions on the appropriate technique of dose administration were conducted prior to each dosing period. Drug administration was standardized as follows: lozenges were moved from side to side in the mouth every 4 seconds until completely dissolved. The movement of the lozenge was marked by a timer with an audible signal (metronome), and swallowing was timed with a verbal command given every 30 seconds. The dissolution times for the lozenge were recorded.

Study subjects were required to abstain from smoking for at least 36 hr prior to dosing and were required to maintain abstinence until blood sampling was completed. Study subjects were confined to the study facility from at least 36 hrs prior to dosing until at least 24 hrs after dosing. Continued abstinence was monitored throughout the sample collection period with oral cavity examination and random carbon monoxide (CO) monitoring. Oral cavity examination and exhaled CO level were measured at before check-in (Day 1), 6 pre-dose readings as per randomization (Day 2), on dosing day prior to dosing (within 30 minutes) (Day 3) and 4 post-dose readings as per randomization (Day 3) for each study period. All subjects had exhaled carbon monoxide levels < 10 ppm in the morning prior to dosing. Each dosing period was separated by 7 days, and subjects were permitted to smoke during this interval.

During the trial, the subjects were to remain ambulatory or seated upright for the first 2 hrs after drug administration. During housing, post-dose meals were identical for both periods of the study. Lunch, snack and dinner were served at 4.0, 9.0 and 13.0 hrs, respectively, after dosing. Water was not permitted from 1 hr before dosing until 1 hr following dosing, but it was allowed at all other times.

Adverse events were monitored throughout the study, until resolution or lost to follow-up. Adverse events were described in terms of severity, seriousness, outcome, action, frequency and relationship to treatments. The principal investigator or sub-investigator was on-site, within the proximity of the subject confinement area for first 6 hrs after drug administration. Subjects were instructed to inform the study physician and/or nurses of any adverse events that occurred during the study.

Blood sampling

Blood samples (1 x 5 ml) for nicotine analysis were collected via an indwelling catheter (intra-venous) in vacuum collection tubes containing sodium heparin anticoagulant at hr 0.00 (pre-dose) within 15 minutes prior to dosing and at 0.08, 0.17, 0.33, 0.50, 0.67, 0.83, 1.00, 1.25, 1.50, 2.00, 3.00, 4.00, 6.00, 9.00, 12.00 and 16.00 hrs post dose. After blood collection, vacuum collection tubes were inverted gently several times to ensure the mixing of tube content and blood sample. Tubes containing blood samples were immediately placed in an iced water bath at approximate temperature of 8-12°C till they were centrifuged. The blood sample tubes were centrifuged to separate plasma as soon as possible at 3000 rpm for 10 minutes in a centrifuge set at a temperature of 8°C. Then plasma was stored below -70°C at the clinical unit of Sitec Labs Pvt Ltd and then transferred to the bioanalytical facility of Sitec

Labs Pvt Ltd under frozen condition and then samples were stored at –70°C or below until sample analysis. To avoid contamination of blood samples with nicotine, study staff was not allowed to smoke in the study surroundings. Study staff was also asked to refrain from smoking in the morning before commencing work on the study. They were only permitted to be in contact with the samples after having washed their hands with soap and water. They were also double-gloved and were wearing nose masks, hair nets and lab coats.

Analytical methods

Plasma concentrations of Nicotine were assessed by a method using high-performance liquid chromatography with mass spectrometry detection (LC-MS/MS). An aliquot 500 µl of human plasma containing the analyte and the internal standard was extracted using a liquid - liquid extraction technique. The internal standard for Nicotine assay was Nicotine D3. 20 µl of the internal standard working solution was added to 500 µl of plasma sample. After vortexing the tubes, 50 µl of 10 M Potassium hydroxide solution was added and the tubes were again vortexed. To this tube 5 mL of Diethyl ether was added and vortexed for 3 min with pulsation. Samples were centrifuged for 3 min at 4000 rpm and then kept in freezer at -70°C for freezing the aqueous layer. Subsequently the organic layer was transferred to a tube containing 100 µl of 0.1% formic acid and vortexed for 3 mins. After vortexing, tubes were centrifuged at 4000 rpm and transferred to freezer at -70°C. After freezing the aqueous layer the samples were withdrawn from freezer and organic layer was removed. To the aqueous layer 700 µl of reconstitution solution was added. The reconstitution solution comprised of 10 µl of triethylamine in 100 mL of mobile phase. This final extract was transferred to glass vial for analysis using LC-MS/MS.

The extracts were injected into the LC-MS/MS system equipped with MDS Sciex API-4000 mass spectrometer. Positive ions were monitored in the multiple reaction-monitoring (MRM) mode. The following ion transitions using analyst 1.4.2 were monitored 163.2/130.4 and 166.2/130.1 for Nicotine and internal standard respectively. Linearity for Nicotine was assessed by plotting area ratios versus standard concentrations and using a linear regression weighted $1/concentration^2$. Analytical range for Nicotine was 0.20-80 ng/mL. The column used for the analysis wss Inertsil HILIC 15 cm x 4.6 mm, 3 µ and the mobile phase composition was a mixture of acetonitrile, water and formic acid (90:10:0.75) and 10 mM Ammonium trifluroacetate. The retention time of Nicotine was 2.2 mins. Nicotine was chromatographically resolved from Anabasine which is a tobacco content and is detected in the same MRM ion channel as Nicotine. The blank plasma used for preparation of calibration standards and control samples was obtained from non-smoker subjects who were housed for three days and were provided control diet in order to reduce the Nicotine concentration in blood to acceptable level.

Method validation was performed according to the current international approach and the applicable regulations regarding bioanalytical method validation. The intra-batch and inter-batch accuracy and precision was evaluated at five different concentrations of control samples. The inter-batch accuracy ranged from 95.00 to 103.33% and the inter-batch precision ranged from 1.39 to 9.13%. The selectivity of the method was assessed by analyzing plasma samples from six normal and a haemolysed and lipemic source. Matrix effect was evaluated by performing post-extraction addition and post-column infusion experiments. Stabilities such as stock solution stability, short-term stability of analyte in plasma, freeze-thaw stability, post-preparative stability and long-term stability in plasma were assessed.

Pharmacokinetic analysis

The following pharmacokinetic (PK) parameters were calculated using validated PK software (WinNonlin version 5.3). The area under the curve from time zero to the last measurable concentration (AUC_{0-t}) using the linear trapezoidal rule, the area under the curve extrapolated to infinity (AUC_{0-t} + Clast /kel, where Clast is the last measurable plasma concentration), the maximum plasma concentration (C_{max}), and the time to maximum plasma concentration (t_{max}), the terminal rate constant of elimination (kel) and terminal elimination half-life ($t_{1/2}$). The ratio of AUC_{0-t} - $AUC_{0-\infty}$ ($AUC_{0-t}/AUC_{0-\infty}$) as well as the extrapolated area of the curve ($AUC_{0-\infty} = (AUC_{0-\infty} - AUC_{0-t})/ AUC_{0-\infty}$) were calculated as percentage.

Statistical analysis

A statistical analysis was performed using the SAS® GLM procedure (SAS® system for windows® release 9.2). Concentration values below the LOQ of the assay for nicotine (0.20 ng/ml) were set to zero. Analyses of variance (ANOVA) were performed on In-transformed AUC_{0-t}, and C_{max} parameters. The ANOVA model included sequence, subjects nested within sequence, period and drug formulation as factors according to regulatory guidance on Bioequivalence. Geometric least-square means (LSM) as well as ratio of LSM with corresponding 90% CI for the generic and innovator formulations were calculated. In addition, nonparametric methods were used to assess differences in median values of t_{max} between the two formulations and 90% CI were constructed.

Results

Safety

A total of 28 subjects were recruited in this study. There were 11 adverse events of mild and moderate severity. Overall, 8/28 (28.57%) subjects experienced an adverse event. Adverse events are summarized in Table 2. No deaths or serious adverse events (SAE) occurred during conduct of this study.

During vital signs examination, there were no clinically significant deviations observed from the baseline values and no clinically significant changes were noted in post-study clinical laboratory data. All subjects were found fit in post-study examination. There were

Adverse Event (Preferred Term)	Frequency (Percentage)	Relationship	Number Of Adverse Events	
			Test Product (T)	Reference Product (R)
Dizziness	10.71%	Related	2	1
Nausea	7.14%	Related	0	2
Vomiting	7.14%	Related	2	0
Hiccups	7.14%	Related	2	0
Headache	3.57%	Related	0	1
Toothache	3.57%	Not Related	0	1

Table 2: Adverse events are summarized.

no clinically significant changes observed in post-study ECGs when compared with pre-study ECGs.

Pharmacokinetics and statistics

A total of 28 subjects were recruited in this study, but only 26 subjects completed the study. One subject was discontinued due to an adverse event (vomiting). One subject dropped out in period-2 for personal reasons. The plasma samples of 27 subjects were analyzed for nicotine except the subject who was dropout in the study for period-2. Pre-dose concentration levels of nicotine of 5 subjects were greater than 5% of the C_{max} in Period-2. Therefore, data of 5 subjects was not considered for final pharmacokinetic and statistical analysis. Data of remaining 21 subjects was considered for pharmacokinetic and statistical analysis. For Nicotine concentrations which were quantifiable at pre-dose sampling (which were < 5% of C_{max}), all parameters were calculated after correction for individual pre-dose levels in the secondary analysis. Bioequivalence acceptance criteria concluded was based on without baseline-adjusted results only.

The blood samples were collected up to 16 hrs post dose. Mean plasma concentration profiles of nicotine under linear over the 16 hrs pharmacokinetic study are presented in Figure 1. Overall, mean plasma concentrations of nicotine peaked rapidly and then declined in a mono-exponential manner, with some plasma concentration values falling not below the LOQ of the assay at 16 hrs post dose. Therefore, 1-2 additional time points were required after 16 hrs post dose. Values below the LOQ were set to zero for pharmacokinetic analysis. A 36 hrs period of abstinence from smoking prior to dosing was not sufficient since pre-dose concentration levels of nicotine of 5 subjects were greater than 5% of the C_{max} in Period-2. Mean plasma concentrations of nicotine following oral administration of these formulations were almost superimposable during the early absorption, distribution and elimination phases of the products. Ratios of $AUC_{0-t}/AUC_{0-\infty}$ for all subjects were found to be more than 80%, indicating that blood samples collected adequately characterized the pharmacokinetic profile of the drug. In addition, 21 subjects provided > 99% power to detect a difference of at least 20% in C_{max}, and AUC_{0-t} between the two treatments.
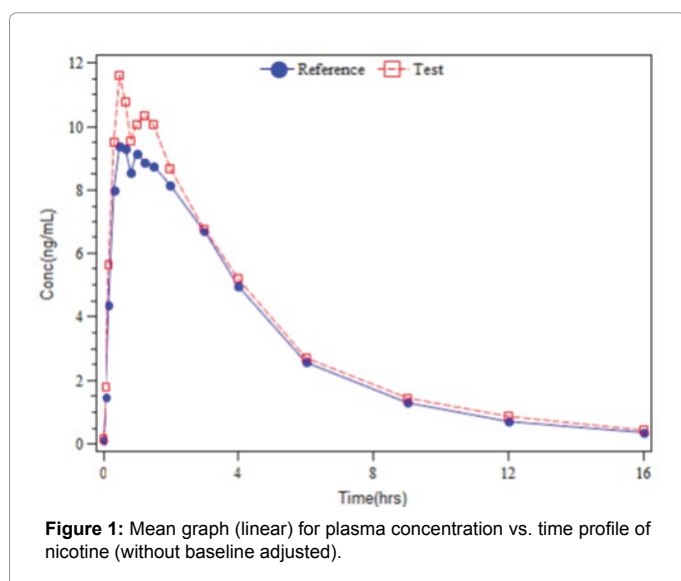
The statistical results of the primary pharmacokinetic parameters of nicotine are presented in Table 3. The geometric mean ratios, 90% CI, power and intra subject coefficient of variation of test and references for Ln transformed pharmacokinetic parameters C_{max}, and AUC_{0-t} for nicotine are presented in Table 4.

Discussion

This study demonstrates both generic and innovator formulations displayed similar rate and extent of bioavailability of nicotine. The median T_{max} for test and reference was found to be 0.67 hr and 1.00 hr respectively. Wilcoxon-Mann-Whitney two sample test for difference in median T_{max} was performed using SAS* 9.2. The difference between the median T_{max} of Test and Reference product is not statistically significant. The C_{max} was found to be consistent both for test and reference, indicating the attainment of similar body peak levels. The mean data are also comparable. For the AUC parameter, the results were found to be similar and there was not much difference in inter-subject variability. The $T_{1/2}$ values were also comparable and in the elimination phase there is no variation.

The statistical analysis was carried out for both untransformed and log transformed data. The data showed statistical equivalence for the important pharmacokinetic parameters i.e. C_{max}, and AUC_{0-t}. The 90% confidence intervals are well within the limits acceptable to any regulatory agency. A power of > 99% was achieved for the pharmacokinetic parameters. The intra subject CV was found to be 10.76% for C_{max}, and 11.97% for AUC_{0-t} for log transformed data.

The sample size of 28 subjects selected for this study was considered to be sufficient to provide adequate power to meet bioequivalence criteria. To avoid variability in the study, to minimize adverse events and to increase compliance healthy adult male human smoker subjects were selected. All the subjects were dosed between 07:00 to 09:10 in both the periods. The actual start and end time of dosing was recorded. The minimum and maximum dissolution time for the lozenges was 8 min and 32 min respectively.

During the clinical study there were no significant protocol/standard operating procedure (SOP) deviations and adverse events were mild to moderate in nature. The subjects tolerated the study medications well. During the study subject compliance to restriction of use of tobacco products was checked by oral cavity examination and exhaled CO level measurements, and all subjects were found compliant. During oral cavity examination no illicit use of tobacco products was found, and exhaled CO level measurements after check-in were < 10 ppm for all the subjects. The biological samples were successfully analyzed by LCMS/MS. The quality control data are found to be consistent and precise.

Conclusion

As a result, the generic formulation of nicotine 4 mg Lozenge should be equally effective and as safe as the innovator formulation of NiQuitin* 4 mg Lozenge of GlaxoSmithKline Consumer Healthcare, UK.

Acknowledgement

Disclosure

Figure 1: Mean graph (linear) for plasma concentration vs. time profile of nicotine (without baseline adjusted).

Pharmacokinetic Parameters	Mean ± SD	
	Test (T)	**Reference (R)**
N	21	21
C_{max} (ng/ml)	13.23 ± 3.68	11.13 ± 2.78
AUC_{0-t} (hr.ng /ml)	51.30 ± 16.12	46.83 ± 12.30
$AUC_{0-\infty}$ (hr.ng /ml)	55.23 ± 19.97	49.29 ± 13.55
*T_{max} (hr)	0.67 (0.33 – 1.50)	1.00 (0.33 – 2.00)
K_{el} (1/hr)	0.212 ± 0.082	0.224 ± 0.067
$T_{1/2}$ (hr)	3.85 ± 1.96	3.33 ± 0.91

*Median (range)

Table 3: The statistical results of primary pharmacokinetic parameters of nicotine (without baseline adjusted) are presented.

Pharmacokinetic Parameters	Geometric Mean		*(%)T/R	90% Confidence Interval	Power (%)	Intra Subject CV%
	Test	**Ref**				
N	21	21	-	-		
C_{max} (ng/ml)	12.6524	10.8705	116.39	109.85-123.32	100.00	10.76
AUC_{0-t} (hr.ng/ml)	49.6880	45.9137	108.22	101.48-115.41	99.98	11.97

*(%) T/R is ratio of Test Geometric Mean / Ref Geometric Mean

Table 4: The Geometric mean ratios, 90% CIs, power and intra subject coefficient of variation of test and reference for Ln transformed pharmacokinetic parameters C_{max} and AUC_{0-t} for nicotine (without baseline adjusted) are presented.

Conflict of Interest

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