Evaluation of the Bioequivalence of Two Brands of Naltrexone 50 mg Tablet in Healthy Volunteers

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Bioequivalence of two formulations of the same drug comprises equivalence with respect to the rate and extent of their absorption. While the area under concentration time curve (AUC) generally serves as the characteristic of the extent of absorption, the peak concentration (C\textsubscript{max}) and the time of its occurrence (T\textsubscript{max}) reflect the rate of absorption, especially in fast-releasing drug formulations.\textsuperscript{1,2)} The present study was conducted to evaluate the bioequivalence of two brands of naltrexone 50 mg tablets in fasting, 22 healthy human volunteers. Typical bioavailability, including AUC\textsubscript{t} (the area under the plasma concentration-time curve from 0 until the last sampling time, 12 hr) and C\textsubscript{max} (the maximum plasma concentration) parameters were compared.

Naltrexone, 17-(cyclopropylmethyl)-4,5-epoxy-3,14-dihydroxymorphinan-6-one, as shown in Fig. 1, has long been available as an orally available antagonist at opioid receptors, with a relative selectivity for the \(\mu\)-opioid receptor at lower doses.\textsuperscript{3)} Naltrexone block the effects of opioids by competitive binding at opioid receptors. Also, naltrexone is effective medication for treatment of alcohol dependence but the mechanism of action of naltrexone in alcoholism is not understood but involvement of the endogenous opioid system is suggested.\textsuperscript{4)}

Following oral administration, naltrexone undergoes rapid and nearly complete absorption with approximately 96% of the dose absorbed from gastrointestinal tract. Naltrexone is primarily eliminated from the body by hepatic metabolism and the major metabolite of naltrexone is 6-\(\beta\)-naltrexol. The percentage of the administered dose excreted in urine as free naltrexone is about 1%.
Reported half-life for naltrexone, after aral administration, is about 4 hr.\(^5\)

The purpose of this study was to determine the pharmacokinetic parameters of two brands of naltrexone 50 mg capsules and then to compare these parameters statistically to evaluate the bioequivalence between the brands. Traxone 50mg (Myung In Pharm. Co., Ltd., Seoul, Korea) was used as test product while Levia\(^®\) 50 mg (Jeil Pharm. Co., Ltd., Seoul, Korea) was used as reference product in 22 healthy volunteers.

### Materials and Methods

#### Test and reference products

The test product, Traxone 50 mg (50 mg of naltrexone hydroxide, lot no. 353501, Myung In Pharm. Co., Ltd.) and the reference product, Levia\(^®\) 50mg (50 mg naltrexone hydroxide, lots no. RVE301, Jeil Pharm. Co., Ltd.) were supplied by tablets.

#### Subjects and methods

The 50 mg naltrexone bioequivalence study involved 22 healthy volunteers with the age from 19 to 28 years (23.09±2.18 years), in weight from 55 to 96 kg (71.00±9.15 kg), and height from 167 to 182 cm (174.77±4.25). All the volunteers were enrolled after passing a clinical examination, including a physical examination and laboratory tests (blood analysis: hemoglobin, hematocrit, WBC platelets, WBC differential, blood urea nitrogen, total bilirubin, cholesterol, total protein, albumin, alkaline phosphate, glucose fasting, ALT, and AST, and urine analysis: specific gravity, color, pH, sugar, albumin, bilirubin, RBC, WBC, and casts). Any with potential hypersensitivity to this type of medication, a history of the hepatic, renal, or cardiovascular disease, or chronic alcohol consumption or other medications was excluded. This criteria was applied to eliminating the source of variation which can influence the pharmacokinetics of the drug. All the volunteers were restricted not to take using other drugs from at least one week before the study and until the completion of the study. They also refrained from alcoholic beverages and xanthine-containing foods and beverages 48 hr before the study, until the last sampling time.\(^6\)

This study was based on a single-dose, randomized, two-treatment, two-period crossover design and was approved by a local ethics committee. All the volunteers signed a written informed consent, in accordance with the Korea Guidelines for Bioequivalence Tests (KGBT 1998). In the morning of period ², after an overnight fasting (10 hr) volunteers were given single dose of either formulation (reference or test) of naltrexone 50mg with 240 ml of water. No food was allowed after 4 hr after dose administration. Water intake was allowed after 2 hr of dose; water, lunch and dinner were given to all volunteers according to the time schedule. They were not permitted to lie down or sleep for the first 4 hr after the dose. Approximately 10ml of blood samples for naltrexone assay were drawn into heparinized tubes through indwelling cannula before(0 hr) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 9, and 12 hr after drug administration. The blood samples were centrifuged at 3000 rpm for 15 min; plasma was separated and kept frozen at -70\(^\circ\)C until the LC/MS/MS analysis. After a washout period of 6 days the study was repeated in the same manner to complete the crossover design.

#### Chromatographic conditions

The plasma naltrexone concentrations were quantified using liquid chromatography-mass spectrometry with a PE SCIEX API 2000 LC/MS/MS System (Applied Biosystems Sciex, Ontario, Canada) equipped with an electro spray ionization (ESI) interface used to generate positive ions [M+H]\(^+\). The HPLC system was an Agilent 1100 series (Wilmington, DE., USA). The separation was performed by using a reversed-phase Eclipse XDB-C\(_{18}\) column (2.1×100 mm internal diameter, 3.5 \(\mu\)m particle size; Agilent technology, Wilmington, DE., USA). The column oven temperature was set at 30\(^\circ\)C. The mobile phase consisted of 0.1% formic acid in acetonitrile and 0.1% formic acid in purified water (95: 5% [vol/vol]). The mobile phase was eluted using an agilent 1100 series pump G1312A (Agilent technology, Wilmington, DE., USA) at 0.2 ml/min.\(^7\)
A PE SCIEX API2000 triple-stage quadrupole mass spectrometer interfaced to a TurboIonSpray® source was used for mass analysis and detection. Ionization of analytes was carried out using the following settings of the electrospray ionization (ESI) in the positive ion mode: TurboIonSpray® temperature, 500°C; ion source voltage, 5500V; nebulizing gas flow (high-purity air), 1.04 L/min; curtain gas flow (nitrogen), 1.44 L/min; auxiliary gas flow, 4.0 L/min; collision gas (nitrogen) pressure, 5×10⁻⁵ torr; orifice voltage (declustering potential), 76 V; ring voltage (focusing potential), 320 V; entrance potential, 12 V; collision energy, 25 V; collision exit potential, 8.0 V. Quantitation was performed by multiple reaction monitoring (MRM) of the protonated precursor ion and the related product ion for naltrexone using the internal standard method with peak area ratios. The mass transition used for naltrexone and internal standard were m/z 342 ⇒ 324, 328 ⇒ 310, respectively (dwell time 150 ms). Quadrupoles Q1 and Q3 were set on unit resolution. The analytical data were processed by Analyst software (version 1.2).

Extraction of naltrexone from plasma

The naltrexone concentration in plasma was analyzed using a reported LC/MS/MS method, with slight modification.⁷ 1 ml of plasma was extracted with 5 ml of methyl tert-butyl ether containing internal standard (naloxone 250 ng/ml in methanol) for 10 minute. After mixing and centrifugation, the organic phase was transferred and evaporated to dryness under nitrogen stream at about 40°C and residue was reconstituted in 100 µl of 0.1% fomic acid in acetonitrile. After brief mixing for 1 min on a vortex mixer, 5 µl of the reaction mixture was injected onto the chromatographic column.

Pharmacokinetic analysis

The pharmacokinetic analysis was performed using non-compartmental methods and the non-compartmental parameters were derived using standard method. The maximum naltrexone concentration (C_max) was determined by the inspection of the individual drug plasma concentration-time profile. The elimination rate constant (K_e) was obtained from the least-square fitted terminal log-linear portion of the plasma concentration-time profile. The elimination half-life (T_e) was calculated as 0.693/K_e. The area under the curve to the last measurable concentration (AUCₜₜ) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity (AUCₜₜ) was calculated as AUCₜₜ + (Cᵣ/K_e), where Cᵣ is the last measurable concentration.⁸

Statistical analysis

For the purpose of bioequivalence analysis AUCₜₜ and C_max were considered as primary variables. Bioequivalence was assessed by analysis of variance between groups (ANOVA) for crossover design and calculating standard 90% confidence intervals of the ratio test/reference. The product were considered bioequivalent if the difference between two compared parameters was found statistically insignificant (P≥0.05) and 90% confidence intervals for these parameters fell within 80-125%, and the range of equivalence for the non-parameter analysis was set to 20% of the reference mean. ANOVA was performed using logarithmic transformed AUCₜₜ and C_max. All statistical comparisons were made using EquivTest version 1.0 (Statistical Solution Ltd., Sangus, MA, USA)⁶,⁸

Results and Discussion

HPLC/MS/MS analysis

With the LC/MS/MS method, no interference was observed in human plasma. The retention times for naltrexone and the internal standard (naloxone) were approximately 1.30 min (Fig. 2). The quantification limit for naltrexone in human plasma was 2 ng/ml, based on a single-to-noise ratio of 5.0. The intra- and inter-day coefficients of variation were less than 11.520% and 9.762%, respectively, for the concentration range from 2 to 50 ng/ml.

Clinical observation

The tolerability of naltrexone 50 mg medication was acceptable. Clinically relevant or drug-related adverse effects were not observed in any of the 22 volunteers.
Pharmacokinetic characteristics
The mean concentration-time profiles for the two brands of naltrexone 50 mg tablets are shown in Fig. 3 and the pharmacokinetic parameters for both formulations are shown in Table 1. All calculated pharmacokinetic parameter values were in good agreement with the previously reported values. The mean terminal half-life of naltrexone of reference and test brands was 7.99±5.64 hr and 9.40±5.25 hr, respectively (mean terminal half-life of two products 8.70±5.43).

Table 1. Pharmacokinetic parameters of naltrexone for two brands (mean±S.D., n=22)

<table>
<thead>
<tr>
<th>Pharmacokinetic parameter</th>
<th>Levia 50 mg (Reference)</th>
<th>Traxone 50 mg (Test)</th>
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<tbody>
<tr>
<td>AUC (ng·hr/ml)</td>
<td>43.31±10.72</td>
<td>43.45±9.91</td>
</tr>
<tr>
<td>AUC (ng·hr/ml)</td>
<td>67.68±15.56</td>
<td>70.15±22.26</td>
</tr>
<tr>
<td>C_max (ng/ml)</td>
<td>12.27±0.57</td>
<td>12.01±3.92</td>
</tr>
<tr>
<td>T_max (hr)</td>
<td>1.05±0.47</td>
<td>0.90±0.50</td>
</tr>
<tr>
<td>k_el (hr⁻¹)</td>
<td>0.12±0.04</td>
<td>0.10±0.05</td>
</tr>
<tr>
<td>Ctot/F (L/hr)</td>
<td>768.10±137.68</td>
<td>817.16±370.17</td>
</tr>
</tbody>
</table>

Standard bioequivalence analysis
No significant sequence effect was found for any of the bioavailability parameters, indicating that the cross-over design was properly performed. Significant F-test values were found between subjects and the subjects' nested
sequence (SEQ) for AUC\textsubscript{t} and C\textsubscript{max}, indicating substantial inter-subject variation in the pharmacokinetics of naltrexone from the two formulation (table 2). No significant period effect in AUC\textsubscript{t} or C\textsubscript{max} was detected in this study.

The detailed statistical and bioequivalence analyses of naltrexone for AUC\textsubscript{t} and C\textsubscript{max} under the assumptions of multiplicative model are given in table 3. The geometric means of the parameters are given for the test and reference formulations of naltrexone, separately and as combined estimates. The parametric point estimates of the ratio of geometric mean of test and reference products for AUC\textsubscript{t} and C\textsubscript{max} were 1.008 and 1.005 (test/reference), respectively, and the parametric 90% confidence intervals for AUC\textsubscript{t} and C\textsubscript{max} were 0.8862-1.1398

**Conclusion**

The statistical comparison of AUC\textsubscript{t} and C\textsubscript{max} clearly indicated no significant difference in the two brands of naltrexone 50 mg tablet. 90% confidence intervals for the mean ratio (T/R) of AUC\textsubscript{t} and C\textsubscript{max} were entirely within the Korea Food and Drug Administration acceptance range. Based on the pharmacokinetic and statistical results of this study, we can conclude that Traxone 50 mg tablets (Myung In Pharm. Co., Ltd., Seoul, Korea) is bioequivalent to Levia 50 mg tablets (Jeil Pharm. Co., Ltd., Seoul, Korea), and that two products can be considered interchangeable in medical practice.

**Acknowledgment**

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