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

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날트렉손은 μ-opioid 수용체에 특이적이고 선택적으로 길항작용을 나타내어 마약이나 마약성 진통제의 강한 의존성 치료에 쓰일 뿐만 아니라, 알코올 의존성 치료에도 쓰이는 약물이다. 본 연구는 날트렉손 제제인 레비아 정(50 mg tablet, 제일약품)을 대조약으로 하여 시험약인 명인 제약의 트락손 50 mg정의 생물학적 동등성 평가를 하기 위해 22명의 건강한 지원자를 모집하였다. 지원자를 두 군으로 나누어 1정씩 투여하였고 2×2 교차시험을 실시하였다. 날트렉손의 혈장 중의 농도를 정량하기 위하여 발리데이션된 LC/MS/MS를 사용하였다. 채혈 시간은 투약 전 및 투약 후 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 9, 12 시간에 걸쳐 시행하였다. 생물학적 동등성을 판정하기 위한 파라미터로 12시간까지의 혈장 중 농도 곡선 하 면적(AUC_{12hr})과 최고 혈중 농도(C_{max})를 사용하였다. AUC_{12hr} 의 평균은 43.45 ng·hr/ml (시험약) 과 43.31 ng·hr/ml (대조약) 으로 관찰 되었고, C_{max}의 경우 각각 12.01 ng/ml (시험약) 과 12.27 ng/ml (대조약) 으로 관찰 되었다. AUC_{12hr} 의 경우 로그변환한 평균치 차의 90%의 신뢰구간이 log0.95 – log1.07 이었고, C_{max}의 경우 log0.87 – log1.14로 계산되어 두 항목 모두 log0.8 – log1.25이어야 한다는 식품의약품 안전청과 FDA의 기준을 모두 만족시켰다. 이상의 결과를 종합하면 시험약 트락손 정 50 mg은 대조약 레비아 정 50 mg에 대하여 생물학적으로 동등한 것으로 판정되었다.

□ **Key words** – Naltrexone, bioequivalence, LC/MS/MS, Traxone, Levia®

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 serve as the characteristic of the extent of absorption, the peak concentration (C_{max}) and the time of its occurrence (T_{max}), reflect the rate of absorption, especially in fast-releasing drug formulations.[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_t (the area under the plasma concentration-time curve from 0 until the last sampling time, 12 hr) and C_{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 seletivity for the μ-opioid receptor at lower doses.[3] Naltrexone block the effects of opioids by competitive binding at opioid recepters. 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.[4]

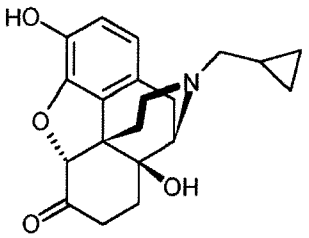

Fig. 1. Molecular structure of naltrexone

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 hepatin metabolism and the major metabolite of naltrexone is 6-β-naltrexol. The percentage of the administered dose excreted in urine as free naltrexone is about 1%.

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Reported half-life for naltrexone, after aral administration, is about 4hr.[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 elimination 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°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 electrospray ionization 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 μm particle size; Agilent technology, Wilmington, DE., USA). The column oven temperature was set at 30°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 aglilent 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 1min 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_{el}) was obtained from the least-square fitted terminal log-linear portion of the plasma concentration-time profile. The elimination half-life ($T_{1/2}$) was calculated as $0.693/k_{el}$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity (AUC_{inf}) was calculated as $AUC_{0-t} + (C_t/k_{el})$, where C_t is the last measurable concentration.[8]

Statistical analysis

For the purpose of bioequivalence analysis AUC_{0-t} 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 ($P\geq0.05$) 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 AUC_t and C_{max}. All statistical comparisons were made using EquivTest version 1.0 (Statical 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.

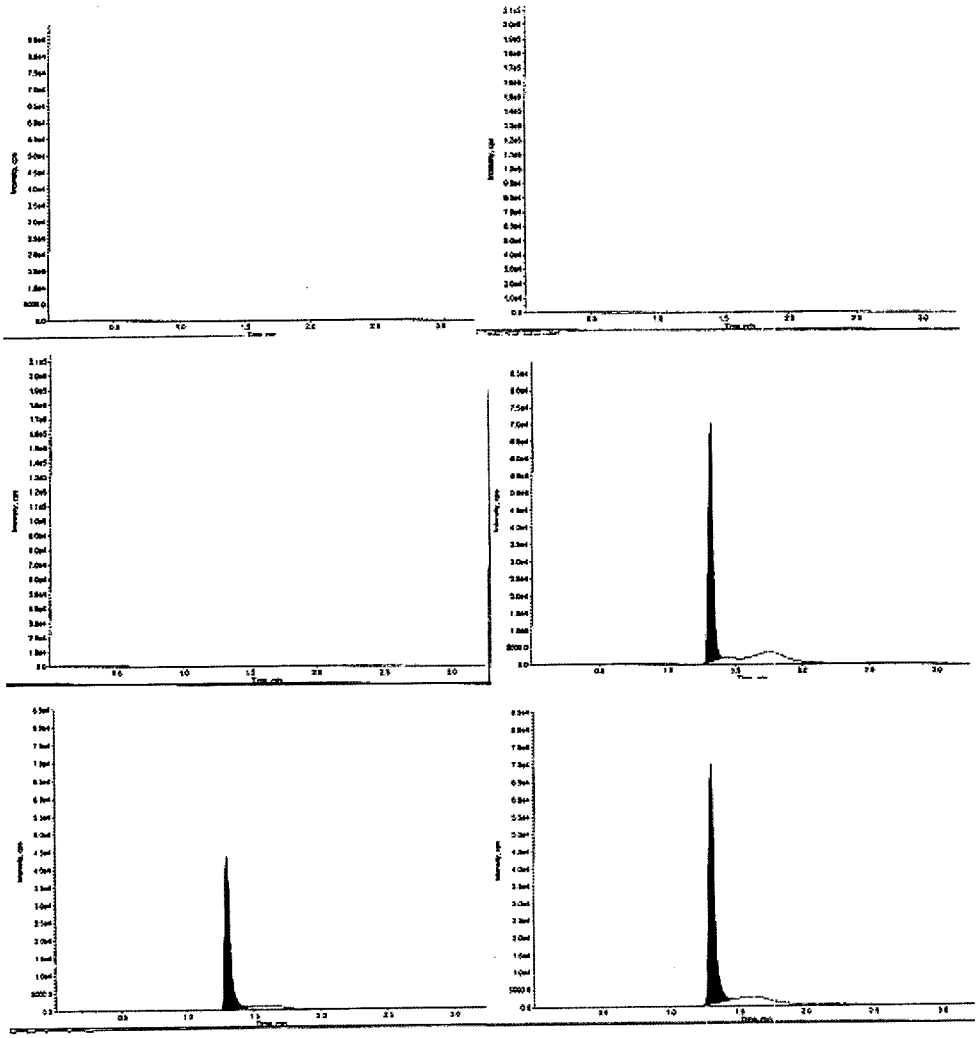

Fig. 2. Choromatogram of naltrexone. Upper chromatogram is double blank plasma uncontained naltrexone and internal standard, middle chromatogram is zero blank plasma contained only naltrexone. Lower chromatogram is plasma sample of subject 1 after 1 hour at a single naltrexone 50-mg oral dose.

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)

Pharmacokinetic parameter	Levia 50 mg (Reference)	Traxone 50 mg (Test)
AUC_t (ng·hr/ml)	43.31±10.72	43.45±9.91
AUC (ng·hr/ml)	67.68±15.56	70.15±22.26
C_{max} (ng/ml)	12.27±0.57	12.01±3.92
T_{max} (hr)	1.05±0.47	0.90±0.50
k_{el} (hr^{-1})	0.12±0.04	0.10±0.05
Cl_{total}/F(L/hr)	768.10±137.68	817.16±370.17

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

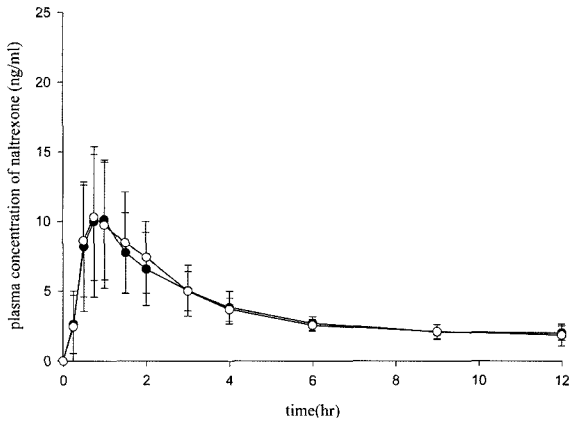

Fig. 3. Time course of the mean plasma concentration in healthy subjects after a single naltrexone 50-mg oral dose. Each point represents the mean + standard deviation. (n= 22, ●; Levia® (naltrexone 50 mg reference tablet), ○; Traxone (naltrexone 50 mg test tablet))

sequence (SEQ) for AUC_t and C_{max}, indicating substantial inter-subject variation in the pharmacokinetics of naltrexone from the two formulation (table 2). No significant period effect in AUC_t or C_{max} was detected in this study.

The detailed statistical and bioequivalence analyses of naltrexone for AUC_t and C_{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

Table 2. Analysis of varience test (α=0.05) for AUC_t (log-transformed) and C_{max} (log-transformed) for the sumatriptan tablets

ANOVA	Log-transformed AUC_t (F-value)	Log-transformed C_{max} (F-value)
Group or Sequence	0.049 (4.351)	0.291 (4.351)
Subjects/Group	9.171 (2.124)	3.515 (2.124)
Period	0.470 (4.351)	1.124 (4.351)
Drug	0.060 (4.351)	0.005 (4.351)

Table 3. The 90% confidence intervals and results of geometric means on the target pharmacokinetic parameters of naltrexone

	Geometric means			90% C.I.
	Test (T)	Reference (R)	T/R	
C_{max}	11.450	11.393	1.005	0.886-1.140
AUC_t	42.382	42.040	1.008	0.952-1.068

for AUC_t ans C_{max} were 1.008 and 1.005 (test/reference), respectively, and the parameteric 90% confidence intervals for AUC_t and C_{max} were 0.8862-1.1398

Conclusion

The Statistical comparision of AUC_t and C_{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_t and C_{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.

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