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Bioequivalence Assessment of Nabumetone Tablets in Healthy Korean Volunteers

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Abstract – This study was performed to evaluate the bioequivalency between the Osmetone™ Tablet (Myeongmoon Pharm. Co., Ltd.) as a test formulation and the Relafen™ Tablet (Handok Pharm. Co., Ltd.) as a reference formulation. Twenty-four healthy male volunteers were administered the formulations by the randomized Latin square crossover design, and the plasma samples were determined by a high performance liquid chromatography (HPLC) with Ultra-Violet (UV) detector. AUC_t, C_{max} and T_{max} were obtained from the time-plasma concentration curves, and log-transformed AUC_t and C_{max} and log-untransformed T_{max} values for two formulations were compared by statistical tests and analysis of variation. AUC_t was determined to be 897.8±431.1 ug.hr/ml for the reference formulation and 902.3±408.4 ug.hr/ml for the test formulation. The mean values of C_{max} for the reference and test formulations were 24.2±8.9 and 24.0±9.5 ug/ml, respectively. The AUC_t and C_{max} ratios of the reference Relafen™ Tablet to the test Osmetone™ Tablet were +5.01% and -0.83%, respectively, showing that the mean differences were satisfied the acceptance criteria within 20%. The results from analysis of variance for log-transformed AUC_t and C_{max} indicated that sequence effects between groups were not exerted and 90% confidence limits of the mean differences for AUC_t and C_{max} were located in ranges from log 0.80 to log 1.25, satisfying the acceptance criteria of the KFDA bioequivalence. The Osmetone™ Tablet as the test formulation was considered to be bioequivalant to the Relafen™ Tablet used as its reference formulation, based on AUC_t and C_{max} values.

Keywords □ Nabumetone, Bioequivalence, Pharmacokinetics, HPLC

INTRODUCTION

Nabumetone is an antiinflammatory agents approved in 1991 for use in the United states. Clinical trials with nabumetone have indicated substanial efficacy in the treatment of rheumatoid arthritis and osteoarthritis, with a relatively low incidence of side effects and the dose typically is 1000 mg given once daily (Friedel *et al*., 1993). Nabumetone is absorbed rapidly and is converted in the liver to one or more active metabolites, principally 6-methoxy-2-naphthylacetic acid, a potent inhibitor of cyclooxygenase. This metabolie is inactivated by demethylation in the liver, is then conjugated before excretion, and is eliminated with a half-life of about 24 hours (Friedel *et al*., 1993). Side effects of treatment with nabumetone include lower bowel complaints, skin rash, headache, dizziness, heartburn, tinnitus and pruritus. The incidence of gastrointestinal ulceration appears to be lower with nabumetone than with other Non-steroidal antiinflammatory drugs (Patrignani *et al*., 1994; Scott and Palmer, 2000).

Various kind of methods for determining nabumetone in human plasma have been reported (de Jager *et al*., 2000; Kobylinska *et al*., 2003; Mikami *et al*., 2000; Nobilis *et al*., 2004; Nobilis *et al*., 2003; Qin *et al*., 1999; Ray and Day, 1984).

In the current work a sensitive and simple HPLC method is described for the quantitation of nabumetone in human plasma and the bioequivalence study was performed by using the HPLC in healthy human volunteers after oral administration of nabumetone.

MATERIALS AND METHODS

Nabumetone tablets as the test product (Osmetone™ tablet, a 500 mg tablet) was obtained from Myeongmoon Pharm. Co. (Seoul, Korea). The reference product (Relafen™ tablet, a 500 mg tablet) was obtained from Handok Pharm. Co. (Seoul, Korea). Ketoprofen used as the internal standard and citric acid

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were purchased from Sigma (St. Louis, MO, USA). 6-Methoxy-2-Naphthylacetic acid and 1-1-heptane sulfonic acid was obtained from Mp. Acetonitrile was purchased from Merck. All agents were of analytical grade.

After approval of pre-planed proposal by Korea Food and Drug Administration (KFDA), male volunteers who submitted the agreement to attend to this project were medically examined and 24 volunteers were selected by a medical doctor in Hanyang Medical Center (Seoul, Korea), based on clinical examination including seropathological (hemoglobin, hematocrit, WBC, platelet), serochemical (blood urea nitrogen, creatinine, total protein, albumin, SGOT, SGPT, total bilirubin, cholesterol, glucose fasting, akaline phosphatase) and urological (specific gravity, color, pH, sugar, alumin, bilirubin, RBC, WBC) data. An exclusion criterion for selecting volunteers includes talking frequently any medicine such as hypertensive agent and vitamins or nutrient aids. They were accommodated at the clinical pharmacokinetic room at the Hanyang Medical Center one day before blood collection. They were fasted for at least 12 hr before administration of tablets. Lunch and dinner were allowed, respectively, 4 and 12 hr after drug intake. Physical and biological examinations were carried out before and after completion of the study.

A 22-gauge i.v.. catheter on arm vein was established on the arm vein of each volunteer and 7 ml of the blood were collected for blank. According to the prescription directed by a doctor, two tablets (a 500 mg tablet, 1000 mg nabumetone) were orally taken by each volunteer of the designated group at random design (12 volunteers a group) with 240 ml of water. One group received test tablets, and the other reference tablets. These two groups were taken the formulation by the randonmized Latin square crossover design after a 2-week washing out period. Blood was collected into EDTA-treated tubes (Vacutainer) at 0, 0.33, 0.66, 1, 2, 4, 6, 8, 10, 12, 24, 48, 72 and 96 hr after the oral administration. The time interval of blood sampling between volunteers was 2 min to consider blood collection time. Blood was centrifuged to obtain plasma. The plasma was stored at -70°C until analyzed.

Nabumetone analysis was performed by HPLC with UV detector (Waters 486). Shiseido Nanospace SI-2 3023 autosampler was used and ds Chrom data module was supported by computer. The detector was set at 254 nm. Symmetry C_{18} (4.6× 120 mm, 5 μm, Shiseido, Japan) column was used. The mobile phase for nabumetone analysis consists of acetonitrile and citric acid (20 mM), with 1-1-heptane sulfonic acid (550 : 450 : 0.3 <v/v/v>, pH 3.0). The flow rate of the mobile phase was set to 1.0 ml/min. As the nabumetone is metabolized to 6-MNA (6-methoxy-2-naphthylacetic acid), we measured the concentration of 6-MNA.

The stock solution of 6-MNA was prepared by dissolving it in acetonitrile as 1000 μg/ml. Plasma sample were made. The concentrations of 6-MNA of plasma sample were 0.3, 1, 2.5, 5, 10, 25 and 50 μg/ml. Ketoprofen, internal standard, was dissolved in acetonitrile as 5 μg/ml. To 180 ul of the blank plasma, 20 ul of the 0.3, 1, 2.5, 5, 10, 25 and 50 ug/ml of 6-MNA solution was added, respectively. 400 μl Ketoprofen solution (5 ug/ml) was added. The tube was agitated for 1 min on a shaker. After centrifugation at 13000 rpm and 4°C for 10 min, the 100 ul supernatant was applied to the instrument. Calibration curves were made by ploting the concentrations of 6-MNA added at x-axis and peak area ratios of the 6-MNA to internal standard at y-axis. Intra- and inter-day precisions and accuracies were obtained from the five repeated experiment, respectively.

The frozen plasma samples were thawed at room temperature, vortex-mixed, and 200 μl of the sample wa assed to the eppendorf tubes. Ketoprofen (5 μg/ml in acetonitrile) was added to human plasma. The rest of the clean-up procedure was the same as described above. The nabumetone plasma concentrations in human volunteers were determined based on the calibration curves from peak area ratios of nabumetone to the internal standard.

Pharmacokinetic parameters were determined from the time-plasma concentrations of nabumetone. The highest concentration (C_{max}) and the time to reach the highest concentration (T_{max}) were read directly from time-plasma concentration curves of nabumetone. The area under the curve of time-plasma concentrations of nabumetone until the last sampling time ($AUC_{0\ to\ last}$) was determined by the equation of $AUC_{0\ to\ inf} = AUC_{0\ to\ last} + C_{last}/\beta$, where β, is the slope of the terminal phase of the time-log plasma concentration curve and C_{last} is the concentration at the last sampling time (Shargel and Yu, 1993).

Data are presented as mean±SD. K-BE test software (Seoul National University, Seoul, Korea) was used for data handling of bioavailability difference and 90% confidence limit for log-transformed C_{max} and AUC_t (Lee *et al*., 1993). In addition, ANOVA test was performed. The statistical analysis was performed for C_{max} and AUC_t after log-transformation: 90% confidence intervals and one-sided t-tests of Schuirmann were estimated and if 90% confidence limits of log-transformed C_{max} and AUC_t. ranged from log 0.8 to 1.25 at α=0.05, two products are concluded to be bioequivalent (Schuirmann, 1987;

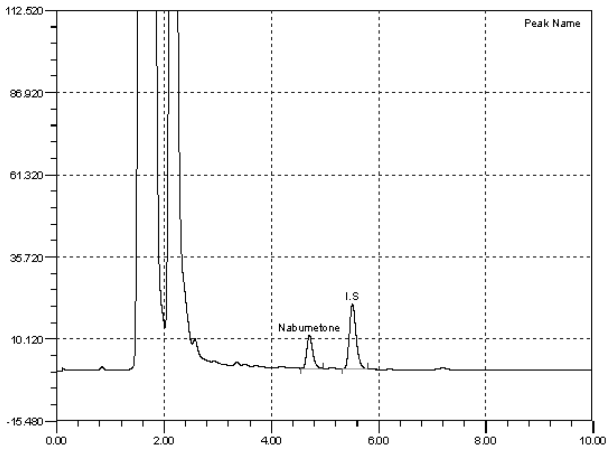

Fig. 1. Chromatograms obtained by HPLC with a UV detector from human plasma blank with the internal standard (I.S.) and nabumetone. Ketoprofen was used as the internal standard. Nabumetone was eluted at 4.5 min and ketoprofen at 5.5 min.

KFDA, 2002a; KFDA, 2002b).

RESULTS AND DISCUSSION

The plasma concentrations of nabumetone in healthy volunteers were determined and validated by HPLC with a UV detector. The HPLC chromatograms obtained from either its internal standard and nabumetone were showed in Fig. 1. Retention times of internal standard and nabumetone were about 5.5 and 4.5 min, respectively and no interfering peaks were observed at these times, showing good separation between peaks. Total run time for determing one sample was within 10 min. Precision and accuracy were presented in Table I. The lower limit of quantitation for nabumetone in human plasma was decided to be 0.3 ug/ml, at which the within- and between day precision was less than 20% and 15%, respectively, and 0.3 μg/ml, at which the within- and between day accuracy was less than 5% and 15%, respectively. The signal to noise ratios for nabumetone peaks were larger than 3. The linearity of nabumetone calibration curve was good (r^2=0.998) within the equation of y(ratio of peak area nabumetone to internal standard)=0.0824×concentration of nabumetone - 0.006 at concentrations ranging from 0.3 to 50 ug/ml. This data suggest that the method was suitable to determine the plasma concentrations of nabumetone and applicable to the pharmacokinetics and bioequivalence studies.

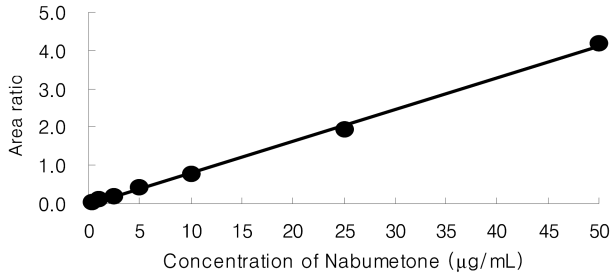

Fig. 2. The linearity of nabumetone calibration curve : y(ratio of peak area nabumetone to internal standard)=0.0824 x concentration of nabumetone - 0.006 (r^2=0.998).

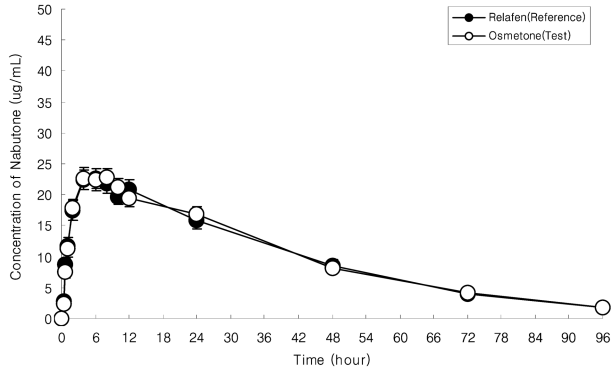

Fig. 3. The time-plasma concentration curves of nabumetone in human volunteers after oral administration of two different products of nabumetone to healthy volunteers over 96 hr at a dose of 1000 mg. Mean (±SD) values of plasma nabumetone concentrations of 24 volunteers for test or reference formulations were represented.

Table I. Precision and accuracy in within-a day and between-days for the determination of nabumetone in the human plasma

Concentrations (μg/ml) of nabumetone	Precision(CV%)		Accuracy(%)	
	within-a day(n=5)	between-days (n=5)	within-a day (n=5)	between-days (n=5)
0.3(lower limit of quantitation for nabumetone)	2.4005	1.8488	102.78	114.49
1	0.7036	6.9793	113.94	110.56
2.5	0.4530	1.5210	102.42	103.09
5	0.3221	4.6847	100.45	104.77
10	0.1110	2.8029	95.89	97.48
25	0.1615	0.6465	100.91	98.27
50	0.1080	2.3561	99.96	100.55

Table II. Bioavailability parameters obtained after oral administration of reference (Relafen^{TM} Tablet) or test (Osmetone^{TM} Tablet) drug containing 500 mg nabumetone (as total 1000 mg) to human healthy volunteers

Subject	Bioavailability parameters					
	AUC (μg.hr/ml)		C_{max}		T_{max}	
	reference	test	reference	test	reference	test
A1	438.6	506.8	13.8	14.7	4	8
A2	637.4	476.4	20.3	13.2	6	12
A3	695.0	1073.3	17.2	25.2	6	24
A4	1354.1	963.5	26.5	23.5	24	8
A5	1154.6	867.0	36.1	26.4	2	2
A6	1572.0	992.3	32.7	20.1	6	10
A7	687.1	538.9	22.7	20.1	8	8
A8	1282.6	855.6	28.1	27.3	8	8
A9	1234.1	569.2	29.7	14.1	6	2
A10	443.3	1006.0	13.5	33.5	6	4
A11	1060.5	942.6	34.8	28.5	4	6
A12	1036.6	1647.2	21.1	36.8	12	4
B1	941.4	706.9	31.1	23.8	4	6
B2	814.4	774.8	32.2	19.2	6	6
B3	1229.6	1302.2	31.1	30.6	4	6
B4	1993.3	1938.6	37.8	46.7	12	6
B5	877.3	879.8	28.3	23.8	6	6
B6	903.5	1415.8	21.3	35.0	8	24
B7	897.6	902.1	24.1	24.1	6.4	7
B8	951.7	1220.6	30.0	33.3	6	4
B9	575.7	600.6	20.9	18.7	4	4
B10	790.2	930.7	21.3	26.2	2	4
B11	459.7	822.6	16.1	19.7	4	4
B12	415.4	624.6	14.7	16.7	4	12
MEAN	897.8	902.3	24.2	24.0	6.3	7.4
SD	431.1	408.4	8.9	9.5	4.7	5.9

Table III. Statistics for pharmacokinetic and bioequivalence parameters of nabumetone formulation

	Parameters	
	AUC_t	C_{max}
90% confidence limit[a)]	$0.90 \leq \delta \geq 1.16$	$0.86 \leq \delta \geq 1.13$

Log-transformed values of AUC_t and C_{max} were used for statistics of bioequivalence evaluation. [a)]$\alpha=0.05$.

From the time-plasma concentrations of nabumetone in healthy human after oral administration of two different products (Fig. 3), principal pharmacokinetic parameters were determined. The parameters for individual subjects are seen in Table II. The values of AUC_t for the reference and test products were 897.8±431.1 and 902.3±408.4 μg.hr/ml, respectively. The values of C_{max} for the reference and test products were 24.2±8.9 and 24.0±9.5 μg/ml, respectively. The values of T_{max} for the reference and test products were 6.3±4.7 and 7.40±5.9 hr, respectively.

The analysis of variance for AUC_t and C_{max} obtained from human volunteers was conducted and the results were presented in Table III. The mean differences of log-transformed AUC_t and C_{max} at 90% confidence limit were determined to be log 0.90~log 1.16 and log 0.86~log 1.13, in which these two parameters satisfied the condition of the bioequivalence criteria that should be ranged from log 0.80~log 1.25. Our data showed that most of these parameters satisfy the acceptance criteria of bioequivalence for two products.

The Osmetone^{TM} tablet as a test formulation was considered to be bioequivalent to the Relafen^{TM} tablet used as its reference formulation, based on pharmacokinetic data of nabumetone obtained from the time-plasma concentrations.

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