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Bioequivalence Evaluation of Commercially Available Choline Magnesium Trisalicylate Tablets in Healthy Volunteers

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건강한 지원자를 대상으로 한 시판 Choline Magnesium Trisalicylate 정제의 생물학적 동등성 평가

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The bioequivalence of two commercial choline magnesium trisalicylate (CMT) tablets was evaluated in 10 normal male subjects (age 21-27 yr, mean 23 yr) following single oral administrations of two products. Test product was Trimax® tablet (Hyundai Pharm. Ind. Co., Ltd., Korea) and reference product was Trilisate® tablet (Purdue Frederick, U.S.A.). Both products contained 500 mg salicylate. In the study, ten volunteers were administered one tablet of Trimax® or Trisilate® with randomized two period cross-over study. The pharmacokinetic parameters of two products were statistically compared using Student's t-test and ANOVA. When Student's t-test was applied, mean area under the curves (AUC) of Trisilate® and Trimax® were 388.88± 74.99 μg·hr/m*l* and 390.63± 63.02 μg·hr/m*l*, respectively, which were not significantly different ($p>0.05$). The mean peak concentrations (C_{max}) and mean times to peak (T_{max}) of Trilisate® and Trimax® were 71.1± 12.2 and 72.9± 10.7 μg/m*l*, and 72± 33 and 57± 36 min, respectively, which were not significantly different ($p>0.05$). The mean terminal phase half-lives ($t_{1/2ter}$) of the two products were 2.57± 0.47 and 2.43± 0.40 hr, and also they were not significantly different ($p>0.05$). When ANOVA was applied, the parameters of the two products were not also significantly different each other. Based on the above results, it has been concluded that the bioavailability of Trimax® tablet was not significantly different from that of Trilisate® tablet.

Keywords—Bioavailability, Bioequivalence, Choline magnesium trisalicylate, Volunteers, Absorption, Pharmacokinetics

The salicylates have been considered to be a base therapy in the treatment of the arthritis.[1-3] Acetylsalicylic acid (ASA) has been used most commonly to obtain analgesic and anti-inflammatory effects. However ASA is associated with gastric irritation and hematologic dysfunction.[4,5] When using a salicylate such as ASA, the daily dose required to achieve analgesia or relieve inflammation is too high to avoid the risk of adverse effects. Among salicylate products, choline salicylate has less adverse effect on gastrointestinal tract, especially microbleeding.[6] Many clinical studies

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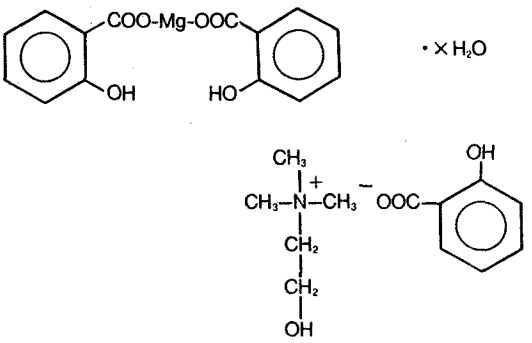

Figure 1—The structure of choline magnesium salicylate.

demonstrated the efficacy of choline salicylate, and pre-clinical bioavailability studies indicated rapidly-obtained serum salicylate levels[7-10] which were shown to be clinically important.[7-12] But, because of hygroscopic nature of choline salicylate, a solid dosage form had not been available yet in the market. The addition of magenesium salicylate to choline salicylate resulted in a product, choline magnesium trisalicylate (CMT) (Fig. 1), which could be formulated into a solid dosage form with desirable dissolution and availability to obtain therapeutic blood salicylate level. Each CMT tablet contains 293 mg of choline salicylate combined with 326 mg of magnesium salicylate to provide 500 mg salicylate content.

Previous investigations have described a close relationship between plasma salicylic acid concentrations and therapeutic and toxic effects of the drug, leading to the identification of an optimal range of therapeutic plasma levels.[13] However numerous clinical studies showed the bioinequivalence of available salicylate products, making it difficult to maintain optimal dosage on generic products.[14-16] To resolve this problem, the U.S. Food and Drug Administration (FDA) has implemented stringent bioequivalence requirements designed to ensure the adequate bioavailability of marketed products.[17]

Trimax® containing CMT has been introduced in 1987 in Korean market, but its bioequivalence to innovator's CMT product (Trilisate®) has not been studied. Therefore the objective of this controlled cross-over study was to compare the salicylate bioavailabilities of the two CMT products at equivalent single dose of 500 mg salicylate.

Experimental

Chemicals and Instruments

The test product (Trimax®) and reference product (Trilisate®) of choline magnesium trisalicylate (CMT) were supplied from Hyundai Pharm. Ind. Co., Ltd. and methanol and acetonitrile were obtained from Merck Co. (Rahway, N.J., U.S.A.). Salicylic acid (SA) was purchased from Kanto Co. (Tokyo, Japan). The other chemicals were of analysis grade and used without futher purification.

HPLC system consisted of an injector (Rheodyne, Model 7125), a solvent delivery system (Hewlett-Packard, Model 1090A), a reversed-phase (RP-18) column (4.6 mm×20 cm, 10 μm particle size, Waters Assoc.), a programmable fluorescence detector (Biosystem, Model 980), an integrator (Hewlett-Packard, Model 3392), and a data system (Hewlett-Packard, Model 85B).

Subjects

Ten healthy male volunteers were between 21 and 27 years of age (mean 23 years). Their body weights were between 57 and 70 kg (mean 61 kg), and were within ± 10% of their optimum weights. All subjects had no significant abnormal physical findings or hematologic laboratory values at the pre-treatment evaluation. The subjects were excluded in the present study if they had received any form of salicylate on an intermittent or continuous basis during the previous two weeks, or if they had had any symptoms of significant illness in the four weeks preceeding the study, or if they had any known sensitivity to salicylates.

All subjects were fully informed of the nature and intent of the study, and written informed consents were obtained. Prior to initiation of the study, the protocol was reviewed and approved by the sub-drug committee for pharmacokinetic study of the Seoul National University Hospital. The demographic data, including name, age, height, weight and the results of blood chemistry test were shown in Table I.

Test and Reference Products of CMT

Table I—*Informations and the results of blood chemistry examinations of the volunteers*

Subject No.	Age (yr)	Height (cm)	Weight (kg)	Glucose (mg%)	BUN[a] (mg%)	CHOL[a] (mg%)	T.P.[a] (g%)	ALB[a] (g%)	BILI-T[a] (mg%)	ALP[a] (IU/L)	GOT[a] (IU/L)	GPT[a] (IU/L)	Creat[a] (mg%)
Normal		ranges[b]		70-110	10-26	120-270	6.8-8.0	3.3-5.2	0.2-1.2	30-115	0-25	0-29	0.7-1.4
1	23	168	58	106	10	162	8.4	5.4	0.4	77	16	8	0.8
2	26	175	59	95	11	151	7.4	4.9	0.6	86	12	8	0.6
3	24	176	61	95	8	144	7.7	5.1	0.7	88	13	4	0.8
4	27	165	58	84	11	172	7.3	5.0	0.4	75	17	15	1.2
5	25	172	57	101	17	192	8.3	5.3	0.3	102	15	12	1.0
6	22	174	57	101	15	149	8.4	5.4	0.7	71	15	10	0.9
7	23	174	57	95	11	163	7.7	5.0	0.9	73	11	6	1.2
8	26	171	69	91	13	136	7.3	5.2	1.2	107	10	4	0.9
9	21	172	64	106	10	150	8.1	5.2	0.6	113	20	11	0.9
10	21	172	70	105	12	149	7.9	5.2	1.1	122	12	5	1.1

[a]Abbreviations: BUN(blood urea nitrogen), CHOL(cholesterol), T.P.(total protein), ALB(albumin), BILI-T(bilirubin total), ALP(alkaline phosphatase), GOT(glutamic oxalacetic transaminase), GPT(glutamic pyruvic transaminase), CREAT (creatinine)

[b]Represents the normal range of each blood chemistry examination

The product was Trimax® tablet (manufactured by Hyundai Pharm. Ind. Co., Ltd., Lot No. 7306) containing 293 mg of choline salicylate combined with 362 mg of magnesium salicylate to provide 500 mg salicylate content, and reference product was Trilisate® tablet (manufacured by Purdue Frederick, U.S.A., Lot No. E8726) also containing same quantity of 500 mg salicylate content as Trimax®.

Drug Administration and Blood Sampling (treatment protocol)

Ten subjects were assigned in a random manner into Group 1 (n=5) and Group 2 (n=5). On each study day, after an overnight fast, a heparin lock was inserted into arm vein of each subject and control blood was withdrawn before drug administration. One tablet of either Trimax® or Trilisate® was administered to subjects along with 150 m*l* of water at 8:30 in the morning. In Period I, Group 1 and 2 received Trimax® and Trilisate® tablets, respectively. No food was allowed for 4 hr, and then meals of uniform composition were provided at 12:30 in day and at 18:30. After Period I, drug wash-out period for one week was given to all subjects. In Period II, the procedure was repeated in cross-over fashion. During each test period, all subjects were fully ambulatory but not allowed to engage in excessive or unusual exercise.

5 m*l* of blood samples were withdrawn at 0.25, 0.5, 1, 2, 4, 6, 10, 12 and 24 hr after the oral administrations of drugs with a syringe through an indwelling cannular or venipuncture, and then discharged into centrifuge tubes containing heparin as an anticoagulant. The plasma samples were separated immediately and frozen −70℃ until the analysis of salicylic acid (SA). For blood sampling, heparin injectable solution (25,000IU/5 m*l*, Choong Wae Pharm. Co.), disposable syringes (1 or 10 m*l*) and three way cock were used.

All subjects were questioned regarding the occurrence of adverse reactions after the drug administration and each blood sampling, or whenever necessary.

Assay of SA in Plasma using HPLC

SA in plasma was quantitated with a slightly modified HPLC method.[18] A volume of 200 μ*l* plasma was transferred to a 1.5 m*l* microcentrifuge tube (Eppendorf® tube). After the addition of 500 μ*l* of acetonitrile, the tubes were vortexed for 10 seconds and centrifuged at 10,000 rpm for 1 min using a microcentrifuge (Beckman). The supernatant was separated from the precipitate, and 100 μ*l* of the supernatant was injected directly to HPLC.

The concentrations of samples were calculated from the calibration curve over the range of 2~100 μg/ml of SA in plasma.

The mobile phase was a mixture of methanol: water:acetic acid (400:580:20 v/v/v%), and flow rate was 2.0 ml/min. The excitation and emission wavelengths of the fluorescence detector were 338 and 420 nm, respectively.

Pharmacokinetic Analysis

The model-independent pharmacokinetic parameters following single oral administrations of two CMT products were calculated from plasma SA concentration-time curve of the each subject. Peak concentration (C_{max}) and time to peak (t_{max}) were obtained from measured concentrations at various times, and area under the plasma concentration-time curve (AUC) was calculated using the trapezoidal method (Eq.1). Also half-life of terminal phase ($t_{1/2ter}$) was calculated using the Eq.2.

$$AUC_{0\to\infty} = AUC]_0^T + \frac{Cp(T)}{\lambda} \quad (1)$$

$$t_{1/2ter} = \frac{0.693}{\lambda} \quad (2)$$

Here, T and Cp(T) are the last sampling time and concentration, respectively, and λ is the slope of terminal phase.

Statistical Analysis

Statistical analysis of pharmacokinetic parameters, such as AUC, C_{max}, t_{max} and $t_{1/2ter}$ between two CMT products were performed using analysis of variance (ANOVA) (cross-over designed 2×2 Latin square method)[19] and Student's t-test for the comparison of matched data pairs. A probability value of 0.05 was used as criterion of statistical sinificance.

Results and Discussion

Chromatograms of SA in plasma

Typical chromatograms of a blank plasma, plasma spiked with SA (40 μg/ml) and postdose plasma obtained from volunteer 1, demonstrate the specificity with this method by absence of interfering peaks (Fig. 2). The calibration curve between

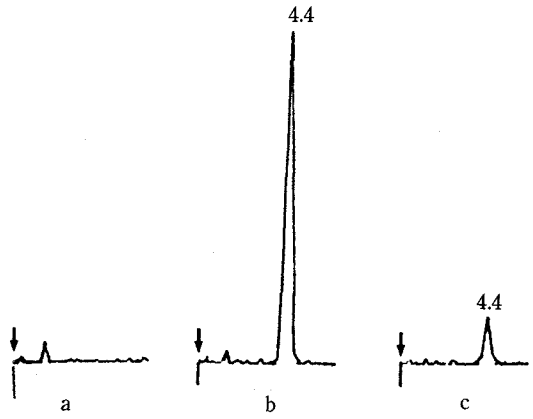

Figure 2—The chromatograms of plasma salicylic acid. The retention time of salicylic acid was 4.40 min. (a) blank plasma, (b) plasma siked with salicylic acid (40 μg/ml), (c) plasma obtained from a volunteer.

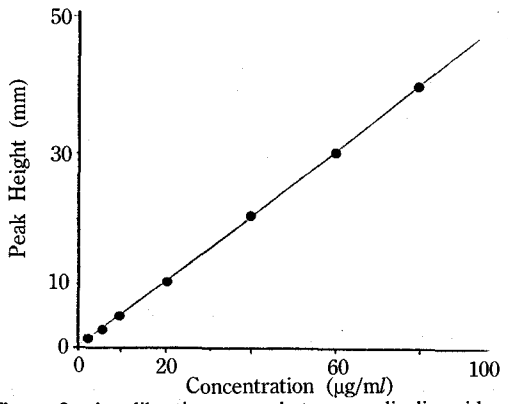

Figure 3—A calibration curve between salicylic acid concentrations in spiked plasma and peak heights.

SA concentrations (X) and peak heights (Y) were linear over the ranges of 2-100 μg/ml, Y=0.0016+0.5216X (r=0.9998) (Fig. 3). The retention time of SA was 4.40 min. The intra-assay coefficients of variation (CV) were ranged from 4.2% and 9.7% (n=6), and inter-assay CVs were ranged from 6.5 % to 15.2% (n=6). Limit of detection with this assay method was 1.5 μg/ml of SA using 200 μl plasma. Although the metabolites of SA were not considered, it was found that applications of this assay method is very simple and convenient in pharmacokinetic study of salicylate products.

Monitorings of Side Effects of CMT during Study

Table II—*Comparisons of the Plasma SA Concentrations of Two CMT Products at Each Sampling Time*

Time (hr)	Plasma Concentration (μg/ml)	
	Trilisate®	Trimax®
0.25	33.9± 13.6	51.5± 12.3
0.5	56.5± 16.9	66.0± 11.2
1	65.4± 14.9	68.4± 11.0
2	56.8± 7.5	58.9± 7.4
4	39.9± 7.8	39.2± 5.6
6	28.3± 6.4	27.9± 7.8
10	10.4± 4.5	9.4± 4.0
12	5.7± 2.7	5.0± 2.5

*The concentrations of two products at each time were not significantly different (p>0.05).

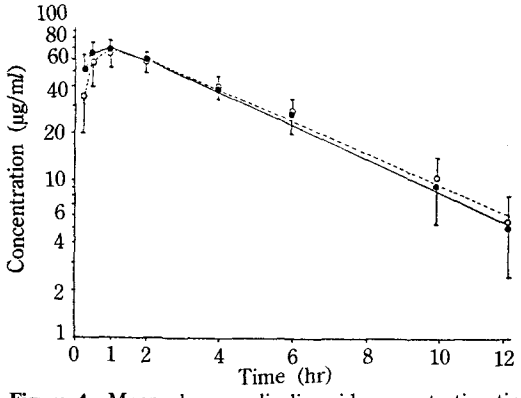

Figure 4—Mean plasma salicylic acid concentration-time curves of volunteers after the oral administrations of Trilisate® (-○-) and Trimax® (-●-). Each point represents mean concentration (± S.E.) of 10 normal volunteers.

Side effects of CMT during the study were monitored by a physician, and subject 5 claimed moderate gastric pains at 2 hr in period I and II, but recovered at 3 hr after drug administrations. No subjects except subject 5 claimed any disturbances with CMT.

Plasma Concentrations of SA after Single Oral Doses of CMT Products

The mean plasma concentrations at various times obtained from 10 subjects after single oral administrations of Trilisate® and Trimax® tablets were shown in Table II, and also were illustrated in Fig. 4 showing similar concentration-time curves with small differences of initial plasma concentrations, but which were not significantly different (Table II, p>0.05). The concentrations at 24 hr were not detected, since the limit of detection was 1.5 μg/ml. On the logarithm paper (Fig. 4), SA concentration-time curves of two products showed straight lines after peak times, and it was noted that the dose-dependency was not appeared under the single oral dose of one tablet of CMT products (500 mg salicylate content).

Table III—*Statistical Analysis of the Pharmacokinetic Parameters of Two CMT Products*

Products	AUC (μg·hr/ml)	C_{max} (μg/ml)	T_{max} (min)	$t_{1/2ter}$ (hr)
Trilisate®	388.88± 74.99	71.1± 12.2	72± 33	2.57± 0.47
Trimax®	390.63± 63.02	72.9± 10.7	57± 36	2.43± 0.40
t-value	0.145	0.339	0.958	1.245

*All phamacokinetic parameters were not significantly different (p>0.05).

Levy[20] has reviewed the metabolic behavior of salicylates in the body, and reported that increase in the dose of salicylate will increase the half-life of salicylate because of dose-dependent metabolism. Also Cohen *et al*[21] reported that the increases of terminal half-life at steady-state were shown from 7.6 hr to 18.3 hr when dosage regimen was changed from 1 gram, b.i.d. to 1.5 gram, b.i.d. medication of salicylate using CMT. In the present study, the dose of CMT was selected to 500 mg salicylate content (1 tablet) to avoid dose-dependency.

Comparisons of Pharmacokinetic Characteristics of CMT Products

Statistical analysis for each pharmacokinetic parameters (C_{max}, T_{max}, AUC and $t_{1/2ter}$) was performed using Student's t-test (Table III) and ANOVA (2×2 Latin Square method) (Table IV). As shown in Table III, the mean AUC of Trilisate® and Trimax® were 388.88± 74.99 and 390.63± 63.02 μg·hr/ml, respectively, and were not significantly different each other (p>0.05). Also, the mean $t_{1/2ter}$, C_{max} and T_{max} of Trilisate® and Trimax® were not significantly different each other. They weres 2.57 ± 0.47 and 2.43± 0.40 hr (p>0.05) for $t_{1/2ter}$, 71.1± 12.2 and 72.9± 10.7 μg/ml for C_{max} (p>0.05), and

Table IV—*ANOVA table of the pharmacokinetic parameters, AUC, C_{max} and T_{max} obtained from two CMT products (2×2 Latin square method)*

S.V.	D.F.	M.S. (F) AUC	C_{max}	T_{max}
B.S.	9	8867.3 (11.674)*	123.1 (2.569)	1305.0 (1.184)
G.S.	1	5535.0 (0.596)	7.5 (0.055)	3645.0 (3.601)
S.G.	8	9283.8 (12.223)*	137.6 (2.871)	1012.5 (0.918)
W.S.				
T.P.	1	465.7 (0.613)	893.7 (18.646)*	2205.0 (2.010)
Drug	1	15.5 (0.020)	16.4 (0.342)	1125.1 (1.021)
RE.	8	759	47.9	1102.5

*Significantly different ($p<0.05$).
#Abbreviation: S.V.(source of variance), D.F.(degree of freedom), M.S.(mean squares), F(variance ratio), B.S.(between subjects), G.S.(group or sequence), S.G.(subjects /group), W.S.(within subjects), T.P.(time period), RE.(residual)
#F-values: $F_{0.05}(1,8)=5.32$, $F_{0.05}(8,8)=3.44$, $F_{0.05}(9,8)=3.39$, $F_{0.10}(1,8)=3.46$

72± 33 and 57± 36 min for T_{max}($p>0.05$), respectively.

In standard procedures of rules for bioequivalence test, the ANOVA was recommended to compare data obtained from two products. And the results of ANOVA using Latin Square method were shown in Table IV. According to results of ANOVA, mean AUC, C_{max} and T_{max} of test product (Trimax®) were not significantly different from those of reference product (Trilisate®). The percent difference of average AUC was 1.3% which was less than 20%. In the case of AUC, all F-values of items were less than $F_{0.05}$(degree of freedom) except those of B.S. (between subjects) and S.G. (subjects/group). In the case of C_{max}, all F-values of items were less than $F_{0.05}$(degree of freedom) except that of T.P.(time period). Also all F-values of items were less than $F_{0.05}$(degree of freedom). Among the items of ANOVA test, G.S.(group or sequence) is the most important factor to evaluate the cross-over effect in the present study. And from the F-values of G.S., the results were well fit for cross-over design making it possible to objectively evaluate the bioeqivalence of two CMT two products.

Considering the results of the above Student's t-test and ANOVA test, it has been concluded that the bioavailability of Trimax® tablet is not significantly different from that of Trilisate® tablet, and that two products are bioequivalent.

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