Pharmacokinetics of Talniflumate, a Prodrug of Niflumic Acid, Following Oral Administration to Man

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Plasma profile of niflumic acid following oral administration of talniflumate tablets (Somalgen) was compared to that of niflumic acid tablets in man. Plasma niflumic acid was assayed by HPLC method. Plasma niflumic acid profile from the talniflumate tablets was similar to that from the niflumic acid tablets resulting in no differences in AUC, C_{max}, t_{max} and MRT. It demonstrates that talniflumate is a prodrug of niflumic acid, and undergoes extensive first-pass biotransformation to niflumic acid. However, plasma niflumic acid concentration at 30 min after talniflumate dosing was significantly ($p<0.05$) higher than that of niflumic acid dosing. The more potent analgesic activity of talniflumate than niflumic acid might be related to this higher plasma drug concentration at the earlier phase. Considering that talniflumate is less irritant to gastrointestinal mucosa than niflumic acid, talniflumate seems to be advantageous over niflumic acid in therms of activity and side effects.

Key words: Niflumic acid, Talniflumate, Prodrug, Pharmacokinetics, HPLC, Oral absorption

INTRODUCTION

Niflumic acid, 2-(α, α, α-trifluoro-m-toluidino) nicotinic acid (Fig. 1), is a potent analgesic and anti-inflammatory drug widely prescribed in rheumatoid diseases (Lancranjan, 1981; Martindale, 1989). It is given in usual doses of 250 mg, three times daily by mouth, and shows rapid absorption followed by extensive metabolism, essentially hydroxylation or glucuroconjugation (Lan et al., 1973; Grossman and Besser, 1985). However, it has been reported both in experimental animals and in clinical use to demonstrate some side-effects such as gastrointestinal irritation (Martindale, 1989). In search for new non-steroidal antiinflammatory drugs with low ulcerogenic activity, a niflumic acid prodrug, talniflumate (Fig. 1), was synthesized by esterification of carboxyl group of niflumic acid with phthalidyl moiety (Los et al., 1981). Talniflumate exerts its activity in the body through conversion to niflumic acid by esterase. Talniflumate has been reported to show lower ulcerogenic activity and toxicity while exhibit greater anti-inflammatory activity than the niflumic acid (Los et al., 1981). Based on these clinical reports, talniflumate is now often

prescribed as tablets (Somalgen, Martindale, 1989) in place of niflumic acid tablets. Comparative studies on the pharmacokinetics of talniflumate and niflumic acid, however, are seldom available in the literatures. In this study, pharmacokinetics of niflumic acid in man following oral administration of talniflumate tablets and niflumic acid tablets was compared.

MATERIALS AND METHODS

Materials

Niflumic acid tablets (252 mg, plain tablets) and Somalgen tablets (film-coated, 370 mg as talniflumate which is equivalent to 252 mg niflumic acid) were obtained from Keun Hwa Pharmaceutical Company (Seoul, Korea).

Subjects

Five healthy male volunteers ranging in age of 23-25 years, in weight of 67-70 kg (68.2±0.43 kg), and in height of 167-180 cm (173.4±2.32 cm) were enrolled after granting written informed consent. Volunteers were evaluated for general good health on the basis of medical history and physical examination. Foods containing xanthines such as coffee, coke and chocolate, other drugs, alcohols, and fatty foods were not allowed for 2 days prior to and during the ex-

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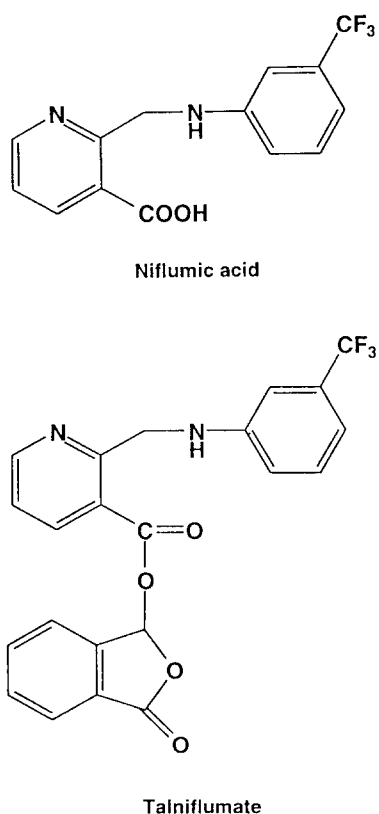
Niflumic acid

Talniflumate

Fig. 1. Chemical structures of niflumic acid ([2-(α, α, α-trifluoro-m-toluidino)nicotinic acid, m.w.=282.2]) and talniflumate (2-{[3-(trifluoromethyl)phenyl]amino}-3-pyridinecarboxylic acid 1,3-dihydro-3-oxo-1-isobenzofuranyl ester, m.w.=414.3)

periment.

Study design

All the subjects were fasted for 12 hr prior to drug administration and 4 hr after. They received a single dose of two niflumic acid tablets (504 mg) between 8 and 9 o'clock a.m of the day of administration. After washout period of a week, they received a single dose of two Somalgen tablets (504 mg as niflumic acid) in the same manner. The tablets were administered with 200 ml of tap water. Subjects were not allowed to remain in a supine position or to sleep during the experiment. Blood samples (10 ml) were collected by venipunture from a forearm into heparinized (25-unit) vacuum tubes through an indwelling butterfly needle (21 G Scalp-vein set) predose and at 30, 60, 90, 120, 180, 210, 240, 360, 480, 600 and 1440 min (24 hr) after dosing. The plasma was separated by centrifuging blood specimens at 2700 g for 20 min, and frozen at -20°C until the analysis.

HPLC assay of niflumic acid and talniflumate in plasma

Plasma niflumic acid following oral administration of niflumic acid tablets and talniflumate tablets was quantified according to the method of Avgerinos and Malamataris (1990) with a slight modification. Briefly, a stock solution of niflumic acid was prepared by dissolving niflumic acid in methanol and serially diluted to obtain the standard solutions of 0.1-50 μg/ml, and 100 μl of the standard solutions were evaporated to dryness under the nitrogen stream in 5 ml conical tubes. To these tubes, 0.5 ml of human blank plasma were added and vortexed for 1 min. To these standard (0.5 ml) and plasma samples (0.5 ml), 1 ml of methanol was mixed and the tubes were centrifuged at 1500 g for 10 min to deproteinize the samples. After centrifugation, the 100 μl aliquot of the supernatant was directly injected onto the HPLC column.

The HPLC system consisted of a Shimadzu liquid chromatography system, pump (LC-9A), a UV-spectrophotometric detector (SPD-6AV) and an integrator (C-R6A). Separations were performed on a μ-Bondapak C18 column (3.9×300 mm, 10 μm particle sizes, Waters) under ambient temperature. The mobile phase used in the separation was a mixture of acetonitrile (HPLC grade) and 0.1 M sodium acetate with the volume ratio of 40 : 60 (v/v), and adjusted to pH 6.4 with glacial acetic acid and filtered through a 45 μm MF membrane filter. The solvents were degassed before use and delivered at a flow rate of 1.0 ml/min. Detection was performed at the wavelength of 279 nm. Plasma concentration of niflumic acid was calculated from the standard curves of peak hight of niflumic acid.

Plasma talniflumate following oral administration of talniflumate tablets was assayed as follows. A stock solution of talniflumate was prepared by dissolving talniflumate in acetonitrile and serially diluted to obtain the standard solutions of 0.1-100 μg/ml, and 100 μl of the standrd solutions were evaporated to dryness under the nitrogen stream in 5 ml conical tubes. To these tubes, 0.5 ml of human blank plasma samples (0.5 ml), 1 ml of acetonitrile was added to deproteinize the samples and the tubes were centrifuged at 1500 g for 10 min. After centrifugation, 100 μl of the supernatant was injected onto the HPLC column. The HPLC system and column were identical to those for niflumic acid assay, except the mobile phase which was a mixture of methanol, water and glacial acetic acid with the volume ratio of 80 : 19.7 : 0.3 (v/v). Detection was performed at the wavelength of 285 nm. Plasma concentration of talniflumate was determined from the stanard curves of peak hight of talniflumate.

The analytical recovery, precision and accuracy of the above methods were assessed at 5 and 50 μg/ml for both compounds. The peak hights of three extracted plasma samples and three direct injections of

the same amount of drug were determines and analytical recovery was calculated from peak hight$_{extracted}$ $_{drug}$/mean peak hight$_{direct\ injection}\times 100\%$. The intra- and inter-day precision of the assay was determined by the analysis of three quality control samples. The accuracy of the assay was assessed by comparing the results of the precision study to known concentration.

Pharmacokinetic evalution of niflumic acid and talniflumate

Noncompartmental pharmacokinetic characteristics were derived by standard methods with plasma niflumic acid and talniflumate concentrations above each assay limit of quantification (0.1 µg/ml). The maximum plasma concentration C_{max} and the time of its occurrence t_{max} were compiled from the concentration-time data. The area under the plasama drug concentration-time curve (AUC) was calculated by the linear trapezoidal rule to the last blood concentration $Ct_{(z)}$ above the limit of quantification and extrapolated to infinity by the addition of the term $C't_{(z)}/\lambda'_z$, where $C't_{(z)}$ and λ'_z are the predicted concentration at time t_z and the terminal elimination rate constant determined by nonlinear regression analysis. The extrapolations contributed on average 2.1% (range, 0.6 to 5.4%) to the total AUC, indicating that of our sampling time was adequate in the estimation AUC. The mean residence time MRT of niflumic acid and talniflumate in the plasma was calculated from Eq. 1 using AUC and the area under the first moment of the plasma drug concentration-time curve, AUMC. The AUMC was calculated by the linear trapezoidal rule from time zero to t_z and extrapolated to infinity by a standard method.

$$MRT=AUMC/AUC \qquad\qquad Eq.\ 1$$

Significant difference of the pharmacokinetic characteristics between niflumic acid tablets and talniflumate tablets was examined by the paired t-test at the level of 5%.

RESULTS AND DISCUSSION

Fig. 2 shows typical chromatograms for the extracts of blank plasma and a plasma sample taken after oral administration of talniflumate tablets. No interfering endogeneous peaks were observed in the blank plasma. Niflumic acid and talniflumate had retention times of 6.0 and 8.0 min, respectively. The assay was linear over the range of 0.1 to 50 µg/ml for niflumic acid, and 0.1 to 100 µg/ml for talniflumate with a lower limit of quantitation of 0.01 µg/ml for both drugs. The intra- and interday coefficients of variation were less than 10%, and the accuracy of the assay was 95%. The analytical recovery of niflumic acid
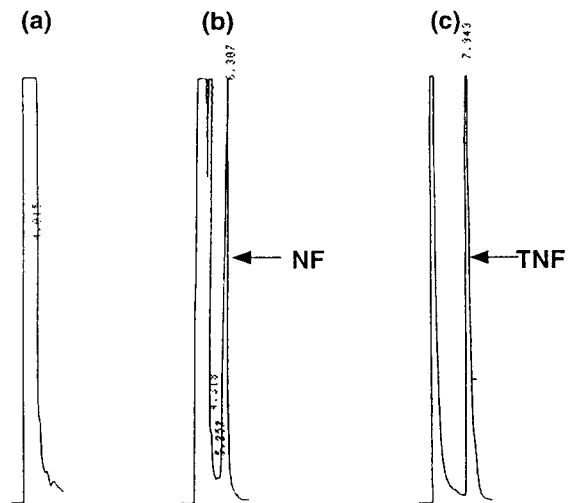

Fig. 2. HPLC chromatograms of blank plasma (A) under plasma niflumic aicd (NF) assay condition, plasma spiked with NF and talniflumate (TNF) under plasma NF assay condition (B), and plasma sample taken after oral administration of TNF tablets (C) under plasma TNF assay condition

and talniflumate was greater than 95%. In this method, the use of an internal standard such as indomethacin (Avgerions and Malataris, 1990) could be excluded since no extraction process was involved before HPLC injection. Niflumic acid and talniflumate were quatified by different analytical procedures. In the HPLC assay for niflumate, more lipopholic talniflumate peak was not apparent in a sample where both compounds were added (Fig. 2-B) and thus did not interefere with the assay. This observation was probably because of a longer retention of the lipophilic drug in the column and subsequent washing during equilibration period between sample analysis. In the HPLC assay for talniflumate, niflumate was not apparent in a plasma sample obtained from talniflumate administered patient (Fig. 2-C). The more hydrophilic metabolite may be eluted with the solvent front and not interfere with the assay

Fig. 3 shows mean plasma niflumic acid concentration curves versus time following oral administration of niflumic acid and talniflumate tablets at niflumic acid dose of 504 mg. Mean plasma talniflumate profile following oral administration of talniflumate tablets at the same dose was also shown in the figure. Mean plasma niflumic acid level following oral administration of the niflumic acid tablets showed its peak at 138 min and declined slowly with an elimination half-life of 184 min. Plasma niflumic acid profile from the talniflumate tablets was similar to that from the niflumic acid tablets, consistent with the hypothesis that talniflumate is absorbed at a similar rate to that of niflumic acid and b iotransformed rapidly to niflumic acid in the body. Actually, plasma talniflumate level following oral administration of tal-
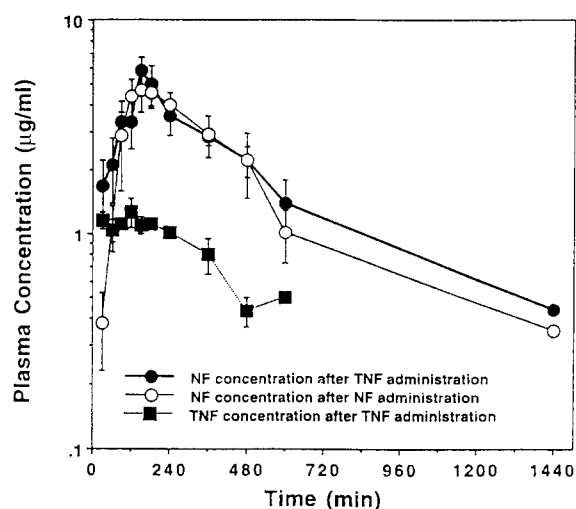
Fig. 3. Plasma concentration-time curves of niflumic acid (NF) following oral administration of NF tablet (504 mg NF), and NF and talniflumate (TNF) following oral administration of TNF tablets (740 mg TNF equivalent to 504 mg NF) to man. Each point of NF represents the mean±SE of five subjects, while that of TNF represents the mean±SE of three subjects who showed detectable plasma TNF level.

Table I. Mean pharmacokinetic parameters[a] of niflumic acid after single oral administration of niflumic acid tablets and talniflumate (Somalgen) tablets to man at a dose of 504 mg niflumic acid (two tablets)

	Following Oral Administration of	
	Niflumic acid	Talniflumate
AUC, $\mu g \cdot min/ml$	2030	2212
	(307)	(384)
C_{max}, $\mu g/ml$	7.1	5.7
	(0.6)	(0.3)
t_{max}, min	138.1	168.6
	(7.3)	(18.0)
MRT, min	373.0	425.4
	(73.4)	(47.9)
$t_{1/2}$, min	184.2	229.4
	(40.9)	(24.5)

[a]Parameter values are expressed as mean (SE); n=5.

niflumate tablets was below the detection limit (0.1 $\mu g/ml$) in two subjects among five subjects while their plasma niflumic acid level was considerably high. Plasma talniflumate concentrations in Fig. 3 are, therefore, expressed as mean±SE of three subjects who showed detectable plasma talniflumate concentrations. Pharmacokinetic parameters generated by area-moment analysis are presented in Table I. There were no statistically significant differences (p<0. 05) in any of the parameters of niflumic acid between the two drugs. However, plasma concentration of niflumic acid at 30 min, the first sampled time point, differed between the two tablets: $C_{30\,min}$ from the talniflumate tablets (1.67±0.54 $\mu g/ml$, n=5) was significantly (p<0.05) higher than that from the niflumic acid tablets (0.38±0.15 $\mu g/ml$, n=5). More rapid appearance of a drug in the plasma is likely to be desirable for analgesics since therapeutic concentration in the plasma may be achieved faster. In this sense, talniflumate appears to be advantageous over niflumic acid.

Partition coefficient of niflumic acid between n-octanol and pH 7.4 phosphate buffer was 82.3, but that of talniflumate was 524.2 (Kim, 1994). Thus, the higher $C_{30\,min}$ of niflumic acid from the talniflumate tablets may be a result of faster gastrointestinal (GI) absorption of talniflumate, a more lipophilic molecule than niflumic acid. Nevertheless, plasma levels of talniflumate were negligible in two subjects and significantly lower than plasma levels of its metabolite, niflumic acid, in three subjects indicating that talniflumate undergoes extensive first-pass metabolism to niflumic acid following GI absorption. Niflumic

acid seems to suffer further extensive metabolism in the body, essentially hydroxylation or glucuroconjugation (Lan et al., 1973); Only 4 mg of niflumic acid was recovered from the urine in 24 hr when talniflumate tablets were administered orally to man at a dose of 504 mg as niflumic acid (Kim, 1994). Renal clearance of niflumic acid varied as a function of plasma niflumic acid level: it was 9 ml/min/kg at plasma niflumic acid level of 0.5 $\mu g/ml$ far exceeding the reported glomerular filtration rate (GFR) of 4 ml/min/kg (Nightingale et al., 1979); it abruptly decreased down to less than 1 ml/min/kg, which is far lower than GFR, as the plasma niflumic acid level increased to 6 $\mu g/ml$ (Kim, 1994). It suggests that secretion and reabsorption, in addition to filtration, are involved in the urinary excretion process of niflumic acid. Therefore, saturation of the renal excretion process seems to be reponsible, at least in part, for the negligible urinary recovery of niflumic acid.

In this study, we compared the pharmacokinetics of niflumic acid in the plasma. But considering higher red blood cell distribution of niflumic acid in rat blood (86.5-88.5% for 5-50 $\mu g/ml$ blood concentration at 37°C, Kim, 1994), comparison of blood niflumic acid level may be necessary to understand fully the behavior of this compound in the body.

CONCLUSION

Talniflumate, a prodrug of niflumic acid, was immediately metabolized to niflumic acid following oral administration. Plasma niflumic acid profile from the orally administered talniflumate (Somalgen tablets) was similar to that from the orally administered niflumic acid except $C_{30\,min}$. The niflumic acid concentration in the plasma at 30 min from the talniflumate tablets was significantly (p<0.05) higher than that from the niflumic acid tablets. The ap-

pearance of a drug in the early phase plasma will be desirable for the analgesic drugs. Therefore, tan-iflumate seems to be advantageous over niflumic acid. In addition, less irritant characteristics of talniflumate to GI mucosa compared with niflumic acid (Los *et al.*, 1981) rather than its pharmacokinetics appers to be a major advantage of talniflumate over niflumic acid.

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REFERENCES CITED

Avgerinos, A. and Malamataris, S., High performance liquid chromatographic determination of niflumic acid in human plasma and urine. *J. Chromatogr.*, 533, 271-274 (1990).

Grossman, A. and Besser, G.M., Prolactinomas. *Br. Med. J.*, 290, 182-184 (1985).

Kim, H. J., Pharmacokinetics of talniflumate, a pro-drug of niflumic acid, following oral administration to human. MS theis, College of Pharmacy, Seoul National University, 1994.

Lan, S. J., Chando, T. J., Weliky, I. and Schreiber, E. C., Metabolism of niflumic acid-^{14}C: absorption, excretion and biotransformation by human and dog. *J. Pharmacol. Exp. Ther.*, 186, 323-330 (1973).

Lancranjan, I., The endocrine profile of bromocriptione: its application in endocrine diseases. *J. Neural. Transm.*, 51, 61-82 (1981).

Los, M., Boned, J. E. and Piccininali, C., Nuevos esteres de acidos anilinonicotinicos Y N-fenil-antranilicos sustituidos (New esters of substituted anilinonicotinic and phenylanthranilic acids). *Farmaco. Ed. Sci.*, 36, 372-385 (1981).

Martindale, *The Extra Pharmacopoeia.* The Pharmaceutical Press, London, 29th ed., 1989, p. 31.

Nightingale, C. H., French, M. A. and Quintiliani, R., Cephalosporin pharmacokinetics in beta-lactam antibiotics, ed. by Muranishi, S., Japan Scientific Societies Press, (1981). pp.259-298.