J. Pharm. Pharmacol. 1992, 44: 750–754
Received October 3, 1991

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Single- and Multiple-dose Pharmacokinetics of Nefiracetam, a New Nootropic Agent, in Healthy Volunteers

Y. FUJIMAKI, K. SUDO, H. HAKUSUI, H. TACHIZAWA AND M. MURASAKI*

*Drug Metabolism and Analytical Chemistry Research Center, Developmental Research Laboratories, Daiichi Pharmaceutical Co. Ltd, Kitakasai 1-16-13, Edogawa-ku, Tokyo 134, Japan, and *Department of Psychiatry, Kitasato University, School of Medicine, 863-1 Asamizodai, Sagamihara, Kanagawa 228, Japan*

Abstract—The pharmacokinetic profile of nefiracetam (*N*-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl)acetamide), a new nootropic agent, was studied in healthy Japanese male volunteers. Nefiracetam was administered orally at doses of 10–200 mg in the single-dose studies, and at doses of 200 mg three times a day for seven days in the multiple-dose study. An HPLC method was used to determine the concentrations of nefiracetam in serum, urine and faecal samples. Linear kinetic behaviour was obtained after single oral administration. Serum concentrations of nefiracetam reached maximum values (C_{max}) within 2 h for all dosage groups, and declined monophasically after C_{max} with half-lives of 3–5 h. The area under the concentration–time curve (AUC_∞) and C_{max} were linearly related to the dose. The apparent clearance (CL) values were 94·4–140·3 mL min^{-1}. Urinary excretion of nefiracetam was independent of the administered dose, and less than 10% of the dose was recovered in urine as the unchanged form within 24 h after administration. Renal clearance (CL_R) did not change significantly as dose increased from 10 to 1200 mg. Faecal excretion of nefiracetam was less than 0·1% of the dose up to 24 h after a 300 mg oral dose. Food intake delayed the absorption of nefiracetam but did not significantly modify its pharmacokinetics. No clinically significant accumulation of nefiracetam in the body was observed during and after multiple doses.

Nefiracetam (*N*-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl)-acetamide), a cyclic derivative of γ-aminobutyric acid (GABA), is now undergoing clinical trials as a novel nootropic agent. In pharmacological studies, nefiracetam was found to have anti-amnesic and antihypoxic effects and to enhance acquisition of learning (Nabeshima et al 1987, 1990; Kameyama et al 1989; Sakurai et al 1990; Tanaka et al 1990). Preclinical studies on the metabolic disposition of nefiracetam have been conducted in several experimental animals using [^{14}C]nefiracetam (Sudo et al 1988). Nefiracetam was well absorbed and distributed rapidly and widely to the tissues, and more than 80% of administered radioactivity was excreted into the urine within 24 h after administration in all species.

There are few pharmacokinetic and dose-proportionality studies of nefiracetam in man. An HPLC method for determination of the drug in human serum and urine has recently been published (Fujimaki et al 1988). The present study was undertaken to investigate the pharmacokinetic characteristics of nefiracetam after several single doses and multiple doses in healthy human subjects to assess the effects of dose on drug disposition following oral adminstration.

Materials and Methods

Chemicals

Nefiracetam *N*-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl)acetamide was supplied by the Production Technology Research Laboratories, Daiichi Pharmaceutical Co. Ltd (Tokyo, Japan), while *N*-(2,4,6-trimethylphenyl)-2-(2-oxo-1-pyrrolidinyl)acetamide (internal standard) was synthesized in our centre. Other reagents were all commercially available and of reagent grade.

Correspondence: Y. Fujimaki, Drug Metabolism and Analytical Chemistry Research Center, Daiichi Pharmaceutical Co. Ltd., Kitakasai 1-16-13, Edogawa-ku, Tokyo 134, Japan.

Dosage form

Doses were prepared in 10 or 100 mg tablets. The 10 mg (lot No. 1499-PJI-1) and 100 mg (lot No. 1499-PJI-2) tablets were manufactured by the Pharmaceutical Formulation Research Center, Daiichi Pharmaceutical Co. Ltd (Tokyo, Japan).

Subjects

Forty two Japanese male volunteers, 26–56 years old, weighing 48·8–100·0 kg, participated in the study. The nature and purpose of the study was explained to each volunteer before obtaining their consent to participate. All subjects passed medical examinations and routine clinical laboratory biochemistry and haematology tests, and were hospitalized in the Clinical Research Unit of Kitasato University during the course of the study. The subjects were asked not to take any medication for 2 weeks before the study and also to abstain from alcohol 24 h before dosing until 20 h after the collection of the last blood sample.

Study design

The study, which was approved by the ethics committees of the Higashi Hospital of Kitasato University and Daiichi Pharmaceutical Co. Ltd, was carried out in two parts: single- and multiple-dose studies.

Single dose study. A total of 39 subjects participated. They were divided into 8 groups, according to a balanced randomized design. Each subject of 7 groups ingested either a 10, 30, 100, 200, 300, 600 or 1200 mg dose of nefiracetam with water (100 mL), 30 min after a standardized light breakfast. The remaining groups received a 100 mg dose after an

overnight fast. Blood samples were obtained from a forearm vein at 0 (predose), 0·5, 1, 2, 3, 4, 6, 8, 12 and 24 h after administration. For the low oral doses of 10 and 30 mg, blood sampling at 3 and 6 h after administration was not done. All blood samples were collected in glass tubes. After centrifugation for 15 min, the serum was separated and frozen at −20°C until analysis. Urine samples were collected for the following periods: 0–2, 2–4, 4–8, 8–12 and 12–24 h after administration, and stored at −20°C until analysis. Faecal samples were collected at 24 h after administration in the single 300 mg dose study and frozen until analysis.

Multiple dose study. Six subjects received a 200 mg dose of nefiracetam 30 min after meals, three times a day for 7 days. Blood samples were obtained at 0 (predose), 0·5, 1, 2, 5, 6, 7, 10, 11, 12, 24, 26, 31, 36, 48, 49, 50, 53, 54, 55, 58, 59, 60, 72, 74, 79, 84, 96, 98, 103, 108, 120, 122, 127, 132, 144, 145, 146, 149, 150, 151, 154, 155, 156 and 168 h after the first dose of the multiple administration, and the serum was separated and stored at −20°C until analysis. Urine samples were also collected for the following periods after the first dose: 0–5, 5–10, 10–24, 24–29, 29–34, 34–48, 48–53, 53–58, 58–72, 72–77, 77–82, 82–96, 96–101, 101–106, 106–120, 120–125, 125–130, 130–144, 144–149, 149–154, 154–168 h. The volumes of these urine samples were measured and portions were frozen at −20°C until analysis.

Analytical procedures

Serum and urinary concentrations. After the addition of *N*-(2,4,6-trimethyl-phenyl)-2-(2-oxo-1-pyrrolidinyl)acetamide as the internal standard, samples were extracted with chloroform and analysed by HPLC as previously described (Fujimaki et al 1988). Standard curves obtained were linear over the concentration range studied.

Faecal concentrations. Water (200–750 mL) was added to the faeces which were then shaken for 30 min. A portion of the mixture (2% of the total weight) was diluted 2-fold with water and shaken for a further 30 min. After centrifugation at 3000 rev min^{-1} for 10 min, the supernatant was collected in a glass tube, and a sample was processed through the same analytical procedure as for the serum samples.

Pharmacokinetic analysis

Serum concentrations of nefiracetam from the single dose study were plotted vs time, and the resulting curves were fitted using a one-compartment open model with first-order absorption:

$$C_t = (K_a \cdot D/Vd(K_a - K_e)) \cdot (e^{-K_e(t\ to)} - e^{-K_a(t-to)})$$

where C_t = serum concentration of nefiracetam at time t (μg mL^{-1}), D = dose (mg), Vd = apparent volume of distribution (L), K_a = absorption rate constant (h^{-1}), K_e = elimination rate constant (h^{-1}), t = time after administration (h) and t_o = lag-time for absorption (h).

The pharmacokinetic parameters were calculated using a computer by a modification of the program devised by Lowenthal & Vitsky (1967). The maximum serum concentration (C_{max}) and the time to reach the maximum concentration (t_{max}) were determined directly from the raw data. The area under the curve of serum concentration vs time (AUC_{o-t}) was calculated from the observed values by the trapezoidal method and extrapolated to infinity (AUC_∞). The half-life ($t_{\frac{1}{2}}$) for nefiracetam was calculated by the following equation:

$$t_{\frac{1}{2}} = \ln 2/K_e$$

The apparent clearance (CL) and renal clearance (CL_R) were calculated using the following equations:

$$CL = D/AUC_\infty$$

$$CL_R = [X]/AUC_{o-t}$$

where [X] is cumulative urinary excretion of nefiracetam from 0 to 24 h after administration.

All values were presented as the mean ± s.e.

Linear regression analysis of pharmacokinetic parameters (C_{max} and AUC_∞) and the doses was performed to evaluate the dose-proportionality of nefiracetam in doses ranging from 10 to 1200 mg.

Statistical analysis

Analysis of variance was used to compare pharmacokinetic parameters at different doses, and assessment of the homogeneity of variances was by Bartlett's test. Comparison between groups was by Scheffe's test. When variances were not homogenous, statistical comparison was assessed by the "H" Kruskal–Wallis test. Comparison of pharmacokinetic parameters in the fasting and non-fasting states was by Student's *t*-test.

Values were considered significant at $P < 0.05$.

Results

Single-dose study

The mean serum concentration–time curves following single oral doses of nefiracetam (10–1200 mg) after a meal are shown in Fig. 1. Mean pharmacokinetic parameters obtained from inspection of individual data are summarized in Table 1. Serum concentrations were detectable 30 min after administration of tablets, and reached maximum values (C_{max}) between 1 and 2 h after administration. C_{max} values for doses of 10, 30, 100, 200, 300, 600 and 1200 mg increased in a dose-proportional manner (Table 1). Nefiracetam concentrations declined after the peak, with half-lives of 3–5 h, independent of the administered dose. Mean AUC_∞ values varied from 1·78 to 167·63 μg mL^{-1}, depending on the dose administered. The correlation between doses and mean C_{max} or AUC_∞ was linear over the dose range of 10–200 mg (C_{max}, r = 0·9993; AUC_∞, r = 0·9985).

Values for K_a, K_e, Vd and CL_o are given in Table 1. In each case, no differences between doses were noted. As shown in Fig. 2, approximately 5–8% of the administered dose was recovered as intact drug in urine within 24 h after dosing (10–1200 mg). CL_R values ranged from 6·11 to 10·95 mL min^{-1}, and the value obtained after the 1200 mg oral dose was significantly higher than corresponding values after the 10, 200 and 300 mg doses. However, values did not change according to administered dose.

Faecal excretion of nefiracetam after a single 300 mg dose was 0·056 ± 0·022% of the administered dose up to 24 h post-dosing.

Table 1. Pharmacokinetic parameters of nefiracetam after single oral doses (10–1200 mg) to healthy volunteers (mean ± s.e.).

Dose (mg)	K_a (h^{-1})	K_e (h^{-1})	t_0 (h)	t_{max} (h)	C_{max} ($\mu g\ mL^{-1}$)	$t_{\frac{1}{2}}$ (h)	AUC_∞ ($\mu g\ mL^{-1}$)	Vd ($L\ kg^{-1}$)	CL ($mL\ min^{-1}$)	CL_R ($mL\ min^{-1}$)
10 (n = 3)	2·31 ± 0·57	0·16 ± 0·03	0·11 ± 0·07	1·33 ± 0·33	0·23 ± 0·03	5·05 ± 0·87	1·78 ± 0·44	0·56 ± 0·06	109 ± 32	6·11 ± 1·24
30 (n = 3)	2·15 ± 0·19	0·16 ± 0·02	0·31 ± 0·16	1·67 ± 0·33	0·68 ± 0·09	4·43 ± 0·50	5·67 ± 1·04	0·57 ± 0·06	94 ± 17	6·78 ± 0·44
100 (n = 5)	2·04 ± 0·32	0·17 ± 0·03	0·30 ± 0·08	1·20 ± 0·20	1·97 ± 0·10	5·86 ± 1·85	19·5 ± 6·75	0·66 ± 0·05	113 ± 21	7·65 ± 0·61
200 (n = 5)	1·63 ± 0·43	0·29 ± 0·06	0·33 ± 0·08	1·60 ± 0·25	4·02 ± 0·22	3·33 ± 0·64	27·1 ± 5·46	0·58 ± 0·04	140 ± 22	6·81 ± 0·97
300 (n = 5)	1·59 ± 0·11	0·21 ± 0·02	0·17 ± 0·07	1·40 ± 0·25	5·77 ± 0·14	3·46 ± 0·16	38·5 ± 0·69	0·67 ± 0·02	130 ± 2·4	7·00 ± 0·62
600 (n = 5)	1·54 ± 0·23	0·19 ± 0·01	0·37 ± 0·05	1·80 ± 0·20	10·6 ± 0·50	3·85 ± 0·58	78·9 ± 7·64	0·65 ± 0·04	131 ± 11	8·64 ± 0·53
1200 (n = 5)	1·41 ± 0·37	0·23 ± 0·02	0·20 ± 0·09	1·40 ± 0·25	22·6 ± 2·04	3·77 ± 0·49	167 ± 40	0·52 ± 0·07	139 ± 21	10·9 ± 0·67*

* Significantly different with respect to 10, 200 and 300 mg doses ($P < 0·05$, analysis of variance followed by Scheffe's test).

Fig. 3 shows mean serum concentration–time profiles after 100 mg doses of the drug in the fasting and non-fasting states. Pharmacokinetic characteristics are shown in Table 2. Pharmacokinetic data differed slightly between conditions. The t_0 for the absorption process was practically negligible in the fasting (less than 5 min) compared with the non-fasting group (0·30 ± 0·17 h), and t_{max} was shorter in the fasting (0·8 h) than in the non-fasting group (1·2 h). The $t_{\frac{1}{2}}$ and AUC_∞ values were also smaller in the fasting group. Except for t_0, however, the differences in these parameters between the fasting and non-fasting states were not statistically significant. The renal clearance values ($CL_R = [X]/AUC_{0-t}$) in the non-fasting state were higher than those in the fasting state ($P < 0·05$), but neither AUC_{0-t} values nor renal excretion of nefiracetam ([X]) differed between the states.

Multiple-dose study

Mean serum concentrations of nefiracetam were measured during and after multiple oral dosing with 200 mg nefiracetam three times a day for 7 days are shown in Fig. 4. Serum level just before and 2 h after every administration increased gradually on the first day. Serum profiles on days 3 and 7 showed a similar tendency to increase gradually during the day. Although concentrations of 1·45 ± 0·58 $\mu g\ mL^{-1}$ on day 3 and 1·99 ± 0·80 $\mu g\ mL^{-1}$ on day 7 were not statistically significantly different, peak concentrations of 4·1–5·1 $\mu g\ mL^{-1}$ at 2 h after each administration on days 3 and 7 were

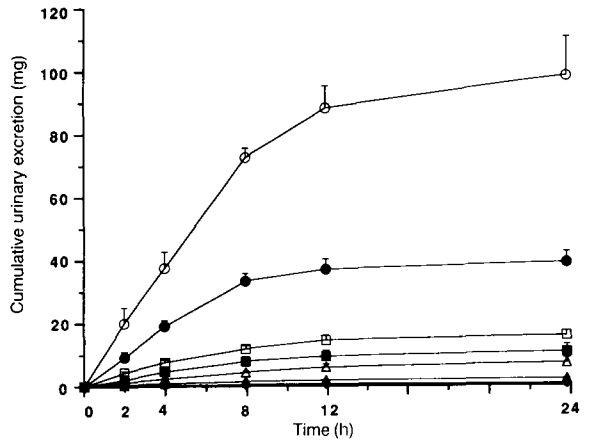

FIG. 2. Cumulative urinary excretion of nefiracetam after single oral administration of 10 (◇), 30 (▲), 100 (△), 200 (■), 300 (□), 600 (●) and 1200 (○) mg doses after a meal (mean ± s.e.).

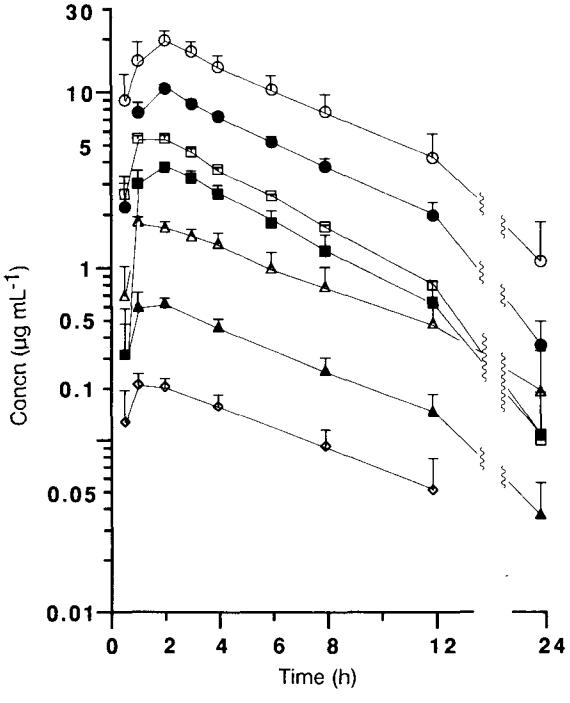

FIG. 1. Mean serum concentration-time profiles of nefiracetam after single oral administration of 10 (◇), 30 (▲), 100 (△), 200 (■), 300 (□), 600 (●) and 1200 (○) mg doses after a meal (mean ± s.e.).

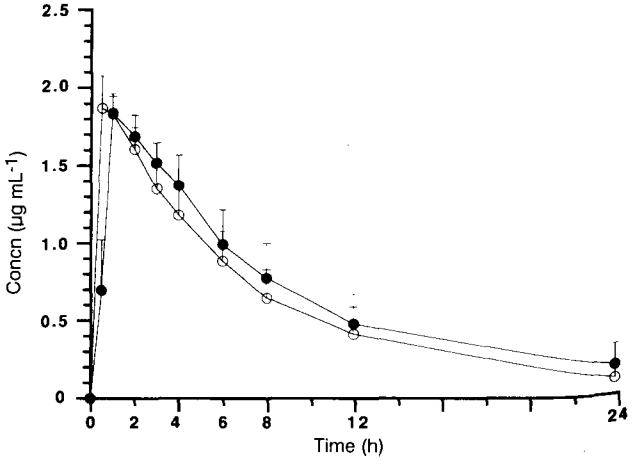

FIG. 3. Mean serum concentration-time profiles of nefiracetam after single oral administration of a 100 mg dose before (○) and after (●) a meal (mean ± s.e., n = 5).

Table 2. Pharmacokinetic parameters of nefiracetam after a single oral dose (100 mg), to healthy volunteers in the fasting and non-fasting states (mean ± s.e.).

Subject	K_a (h^{-1})	K_e (h^{-1})	t_0 (h)	t_{max} (h)	C_{max} ($\mu g\ mL^{-1}$)	$t_{\frac{1}{2}}$ (h)	AUC_∞ ($\mu g\ mL^{-1}$)	Vd ($L\ kg^{-1}$)	CL ($mL\ min^{-1}$)	CL_R ($mL\ min^{-1}$)
Fasting state										
I	3·22	0·19	0·00	0·5	2·27	4·04	13·97	0·58	119	7·07
II	2·03	0·08	0·00	1·0	2·26	9·65	34·62	0·53	48	3·58
III	4·15	0·25	0·00	0·5	2·44	2·69	9·93	0·65	168	7·99
IV	1·98	0·16	0·00	1·0	1·61	4·88	13·37	0·71	125	6·71
V	2·68	0·27	0·00	1·0	1·82	2·65	8·64	0·73	193	4·12
Mean	2·81	0·19	0·00	0·8	2·08	4·78	16·10	0·64	131	5·89
s.e.	0·41	0·03	0·00	0·1	0·16	1·29	4·74	0·04	25	0·86
Non-fasting state										
I	2·41	0·17	0·42	1·0	2·03	5·00	14·91	0·63	112	7·71
II	1·46	0·06	0·33	2·0	2·21	13·03	46·35	0·56	36	6·92
III	1·09	0·19	0·33	1·0	1·70	3·38	11·02	0·82	151	9·55
IV	2·61	0·16	0·42	1·0	1·80	5·01	14·51	0·70	115	8·17
V	2·61	0·26	0·00	1·0	2·13	2·82	10·94	0·59	152	5·89
Mean	2·04	0·17	0·30	1·2	1·97	5·85	19·59	0·66	113	7·65*
s.e.	0·32	0·03	0·08	0·2	0·10	1·85	6·75	0·05	21	0·61

*Significantly different from the value in the fasting state ($P < 0·05$).

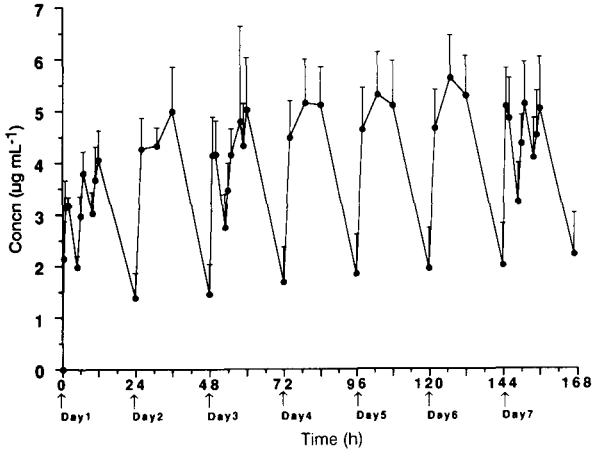

FIG. 4. Mean serum concentration-time profiles of nefiracetam during and after multiple oral dosing with 200 mg three times a day for 7 days (mean ± s.e.).

reasonably constant, indicating that steady state was reached within 3 days.

Urinary excretion was 5·53 ± 0·56% of the dose on day 1. Mean cumulative urinary excretion of nefiracetam was 7·35 ± 1·64% of the administered dose up to 14 h after the last administration on day 7.

Discussion

Serum concentration-time profiles for each subject were fitted to standard pharmacokinetic models; a one-compartment open model with first-order absorption provided the best fit. Nefiracetam was rapidly absorbed; serum concentrations were measurable at the first post-dose sampling time (0·5 h) and reached t_{max} within 2 h in the majority of subjects studied in the non-fasting state. Linear relations were observed between dose and C_{max} or AUC_∞, with correlation coefficients of over 0·99 for both parameters. The apparent volume of distribution (Vd) was virtually constant (0·52–0·67 L kg^{-1}) at each dose level studied, and the apparent clearance (CL) (94–140 mL min^{-1}) and the renal clearance (CL_R) (6·11–10·95 mL min^{-1}) did not change as the dose increased. These data indicate that nefiracetam has linear pharmacokinetics over a wide dose range, from 10 to 1200 mg in healthy subjects.

Clearance of the drug was essentially due to metabolism; renal excretion of the unchanged drug was very low (less than 10%).

Vd (0·52–0·67 L kg^{-1}) was almost identical with the total body water of 0·58 L kg^{-1} (Murata & Arita 1983), suggesting nefiracetam is widely distributed throughout the body. This was consistent with the findings of Sudo et al (1988) who showed that the drug was distributed in almost all the tissues in rats and monkeys.

When a drug is eliminated by hepatic metabolism and urinary excretion, systemic bioavailability (f) can be estimated by the following equation (Vaughan 1975):

$$f = F(Q + CL_R)/(Q + (F \cdot D)/AUC_\infty)$$

where D is the administered dose, Q is the average value for liver serum flow (940 mL min^{-1} (Gibaldi et al 1971)) and F is the fraction of the administered oral dose absorbed (this value was calculated from faecal excretion of the unchanged drug). In this study, f values were estimated to be 87·6–91·5% in the studied dose range. In monkeys, f values calculated from AUC values of the drug in intravenous and oral administration studies were 76·9 ± 6·9%. These data suggested that nefiracetam has relatively high systemic bioavailability and is rarely subject to substantial first-pass effects.

No obvious toxicity or significant changes in standard laboratory tests due to nefiracetam administration were noted at any dose (Murasaki et al 1988).

The effects of meals on the pharmacokinetics of nefiracetam were examined by comparing the pharmacokinetic parameters obtained with the single 100 mg dose in the fasting and non-fasting states. The mean CL_R value in the

non-fasting state is slightly greater than that in the fasting state, this difference being statistically significant. This difference might be caused in part by CL_R values obtained from subject II, whose urinary excretion ratio of nefiracetam in the non-fasting state (13·1%) was double compared with that in the fasting state (6·0%). As t_{max} and lag-time values (t_o) in the non-fasting state were slightly longer than those while fasting, food intake was assumed to delay the absorption of nefiracetam from the gastrointestinal tract. However, the pharmacokinetic profiles did not distinctly differ between states.

In the multiple-dose study, serum concentrations just before the first dose on days 3 and 7 showed similar values, and the urinary excretion ratio did not change throughout the experiment. These data indicate that nefiracetam does not accumulate during and after multiple dosing. Furthermore, no serious side-effects or abnormal laboratory parameters were reported during and after dosing (Murasaki et al 1988).

In conclusion, the present study in healthy subjects shows that the single-dose pharmacokinetics of nefiracetam are essentially independent of the oral dose, indicating the rates of absorption, distribution and elimination of the drug do not change within the studied dose range, and that the metabolic disposition of nefiracetam also does not vary during and after multiple dosing.

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