HUMAN PSYCHOPHARMACOLOGY
Hum Psychopharmacol Clin Exp 2003; **18**: 255–259.
Published online 3 March 2003 in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/hup.484

Milnacipran plasma levels and antidepressant response in Japanese major depressive patients

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The relationship between antidepressant effects and plasma levels of milnacipran was studied in 49 cases of major depression without psychotic features during 6 weeks of milnacipran treatment. The daily dose of milnacipran was 50 mg/day for the first week, and up to 100 mg/day thereafter. Depressive symptoms were evaluated by the Montgomery and Åsberg depression rating scale (MADRS) before treatment and at 1, 2, 4 and 6 weeks after the beginning of this study. Thirty-four patients (69.4%) were responders (defined as a 50% or greater decrease in the baseline MADRS score). Significant differences of MADRS scores were seen from 1 week after the beginning of this study ($p = 0.004$, unpaired t-test) between responders and nonresponders. The mean plasma milnacipran level of responders, 82.0 ± 29.4 ng/ml, was similar to that of non-responders, 78.6 ± 23.1 ng/ml; there was no significant difference between responders and nonresponders. Neither a significant linear nor a curvilinear relationship was obtained between the final MADRS score and the plasma levels of milnacipran. Although there was no significant relationship between the plasma levels of milnacipran and the antidepressant response, milnacipran should be considered an efficacious agent in the treatment of major depressive patients.
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KEY WORDS — milnacipran; plasma level; major depressive disorder; antidepressant response

INTRODUCTION

Milnacipran is a new specific serotonin and noradrenaline reuptake inhibitor (SNRI) that has been marketed recently in Japan. Its inhibitory potency is of approximately the same order as that of imipramine for serotonin and noradrenaline reuptake *in vitro* and *in vivo* (Mochizuki *et al.*, 2002). Meta-analysis of seven double-blind trials of equivalent design comparing milnacipran 100 mg/day with imipramine or clomipramine 150 mg/day showed that the response rate with milnacipran was similar to that with imipramine or clomipramine, but with a more benign side-effect profile (Kasper *et al.*, 1996). Tricyclic antidepressants (TCAs) such as imipramine interact directly with various receptors (e.g. cholinergic muscarinic receptors, alpha-adrenergic receptors, H1 histamine receptors) responsible for their major side effects (Richelson, 1996). Contrary to TCAs, milnacipran has little or no affinity for any postsynaptic receptor (Mochizuki *et al.*, 2002) and this is thought to be responsible for its favourable tolerance profile. Therefore, milnacipran should be considered as a promising agent for the treatment of major depressive patients.

Since Åsberg *et al.* (1971) reported the presence of a therapeutic window for plasma levels of nortriptyline, many studies have been conducted on the relationship between plasma levels and therapeutic response with TCAs. The clinical usefulness of plasma levels measurements of these drugs has been emphasized by

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Received 3 December 2002
Accepted 22 January 2003

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Glassman (1985). Regarding milnacipran, there have been no reports about the relationship of the antidepressant response and the plasma levels of milnacipran except for two preliminary reports dealing with a small number of patients (Retz *et al.*, 1995; Higuchi *et al.*, 2002). The two preliminary studies did not show a correlation between plasma levels of milnacipran and the antidepressant response in major depressive patients.

The purpose of this study was to clarify the relationship between plasma levels of milnacipran and the antidepressant response in more subjects compared with our previous report (Higuchi *et al.*, 2002).

MATERIALS AND METHODS

Subjects and treatment

Fifty-two patients meeting the DSM-IV diagnosis of major depressive disorder without psychotic features, whose score on the Montgomery and Åsberg depression rating scale (MADRS) (Montgomery and Åsberg, 1979) was more than 20 points, were included in this study. The patients were free from psychotropic drugs for at least 14 days before their entry into the present study. Their compliance of milnacipran was confirmed by detailed interviews in consultation with a doctor. Three cases were excluded from the analysis of this study because of their poor compliance. The mean age of 49 patients (34 females, 15 males) was 51.7 ± 12.3 years. 27 cases were outpatients and 22 cases were inpatients. The mean entry MADRS score was 28.5 ± 5.5. All patients provided informed consent. Milnacipran was administered divided in two equal doses (after dinner and at bedtime) for 6 weeks. The daily dose was 50 mg/day for the first week, and up to 100 mg/day thereafter. No other psychotropic drugs were given except occasional brotizolam 0.25 or 0.5 mg as a hypnotic.

Data collection

Depressive symptoms were evaluated by the MADRS before treatment and at 1, 2, 4 and 6 weeks after the beginning of this study. A clinical response was defined as a 50% or greater decrease in the baseline MADRS score at the end of this study. A clinical remission was defined as a final MADRS score of 7 or less. Four weeks after the administration of milnacipran, blood samples were collected approximately 12 h after the bedtime dose and were analysed using high-performance liquid chromatography (HPLC) with a fluorescent detector (Asahi Kasei Corporation, Tokyo, Japan).

Quantification of plasma milnacipran level

Milnacipran and p-methyl-milnacipran (IS) were purchased from Asahi-kasei (Tokyo, Japan) and HPLC-grade acetonitrile and all other chemicals were purchased from Wako (Kyoto, Japan).

HPLC was performed with an 851-AS auto sampler with two PU-880 pumps and an 802-SC system controller (Jasco, Tokyo, Japan). Separation was carried out at 40°C with an analytical column, YMC-Pack ODS-AQ (150×4.6 mm i.d., 5 μm particle size). The following solvents were used: (A) 0.05 M potassium dihydrogen phosphate, (B) acetonitrile. The potassium dihydrogen phosphate was filtered through a type HA filter (0.45 μm, Millipore, MA, USA), and degassed just prior to use. Gradient elution was employed according to the following linear programme: time 0, 20% solvent B; 10 min, 30% solvent B; 20 min, 30% solvent B; 25 min, 20% solvent B. The flow rate was 1.0 ml/min. For postcolumn derivatization, a PU-880 pump (Jasco, Tokyo, Japan) connected to a junction and reaction coil was used. The postcolumn solution consisted of 0.04 M boric acid buffer (pH 10.0) containing 0.8 g of o-phutalaldehyde, 2 ml of 2-mercaptoethanol, 4 ml of 5% Briji-35 per litre. The flow rate of the postcolumn solution was kept at 0.5 ml/min and the reaction coil was kept in a box at 48°C.

The fluorescence intensities were measured with excitation at 340 nm and emission at 440 nm, using a RF-530 fluorescence spectrophotometer (Shimadzu, Kyoto, Japan). The detector signal was recorded, and the peak area was quantified with a Chromatopac C-R4A integrator (Shimadzu, Kyoto, Japan).

About 3 ml of whole blood was collected in a vacutainer tube containing heparin. The tube was immediately centrifuged at 1000 x**g** for 5 min at 4°C and stored at −20°C until use. The plasma sample (0.5 ml) was mixed with 0.5 ml distilled water and 0.05 ml of IS solution (1500 ng/ml). The mixture was applied to a Bond Elut C2 solid-phase extraction column (Varian Inc., CA, USA), which had been preconditioned by 1 ml of water and 1 ml of methanol. After loading the mixed sample on the extraction column, it was washed by 1 ml of water and 1 ml of acetonitrile, and the retained sample components were eluted with 1 ml of 0.01 M potassium dihydrogen phosphate–methanol (20:80, v/v). The eluted samples were evaporated to dryness using a centrifugal evaporator (Yamato, Tokyo, Japan) at 50°C.

The residue was resuspended in 250 μl of 0.05 M potassium dihydrogen phosphate–acetonitrile (80:20, v/v), and filtered through an Ultrafree filter (Millipore, MA, USA) at 1000 x**g** for 5 min. An aliquot of 50 μl

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was subjected to HPLC analysis. Quantification was performed in duplicate and the mean was determined as a plasma level.

A stock standard solution of milnacipran and stock IS solution of p-methyl-milnacipran were prepared in water and stored at -20°C until use. The working standard solutions for calibration curves and IS solution were prepared daily by diluting drug-free human plasma.

Statistical analysis

Linear regression, quadratic regression, unpaired *t*-test and Spearman rank correlation were used for statistical analysis where appropriate, and a *p*-value of 0.05 or less was regarded as significant.

RESULTS

Table 1 shows the clinical characteristics and plasma milnacipran levels of the patients in this study. Thirty-four patients (69.4%) were responders according to the definition mentioned above. The plasma milnacipran levels of 49 patients ranged between 38.9 and 156.8 ng/ml. The mean plasma milnacipran level of responders, 82.0 ± 29.4 ng/ml, was similar to that of non-responders, 78.6 ± 23.1 ng/ml; there was no significant difference between responders and nonresponders ($p = 0.70$, unpaired *t*-test). The mean plasma level of remitters was 84.2 ± 31.4 ng/ml, neither was there a significant difference between remitters ($n = 23$) and nonresponders ($p = 0.56$, unpaired *t*-test). The change in MADRS scores during treatment in responders and nonresponders is shown in Figure 1. Significant differences were seen from 1 week after the beginning of this study ($p = 0.004$, unpaired *t*-test) between responders and nonresponders. Neither a significant linear ($r = 0.16$, $p = 0.28$) nor a quadratic curvilinear relationship ($r = 0.19$, $p = 0.42$) was obtained between the final MADRS score and the plasma levels of milnacipran (Figure 2). Neither was it obtained between the difference in MADRS from the baseline to final scores and the plasma levels of milnacipran (linear: $r = 0.09$, $p = 0.54$, quadratic curvilinear: $r = 0.16$, $p = 0.55$) (Figure 3). The plasma levels of milnacipran and the time to sustained response showed no significant correlation ($\rho = -0.19$, $n = 34$, $p = 0.28$, Spearman rank correlation) (Figure 4).

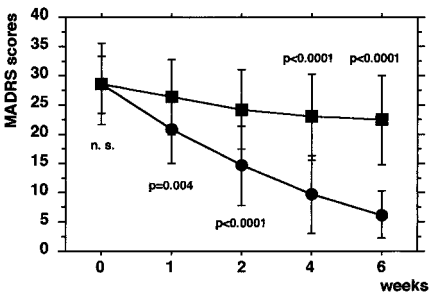

Figure 1. Change in MADRS scores during tratment in ● responders ($n = 34$) and ■ nonresponders ($n = 15$). Each point represents the mean ± SD scores

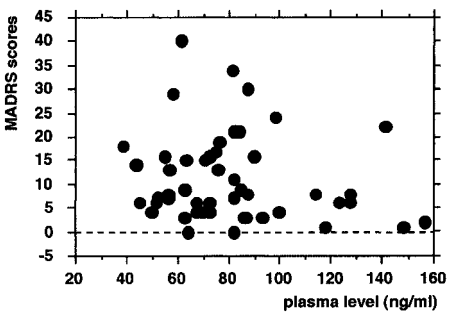

Figure 2. Plasma levels of milnacipran and the end-point MADRS scores in each patient

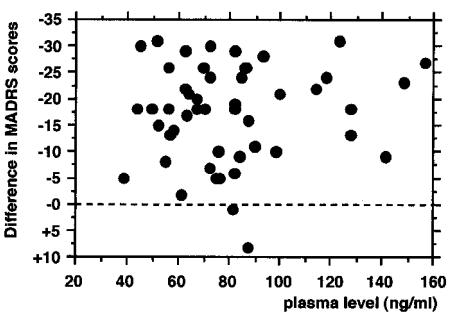

Figure 3. Plasma levels of milnacipran and the difference in MADRS from baseline to final scores in each patient

Table 1. Clinical characteristics of the patients in this study

	Responders	Non-responders	
Number	34	15	
Sex (male/female)	12/22	3/12	
Age (yr) (±SD)	50.7 ± 12.7	54.0 ± 11.5	n.s. ($p = 0.40$)
No. of previous episodes (±SD)	0.59 ± 1.50	0.27 ± 0.59	n.s. ($p = 0.43$)
Melancholia (+/−)	9/16	5/10	
Plasma milnacipran levels (ng/ml) (±SD)	82.0 ± 29.4	78.6 ± 23.1	n.s. ($p = 0.70$)

DISCUSSION

Milnacipran is thought to be suitable for this study because its metabolites are not pharmacologically

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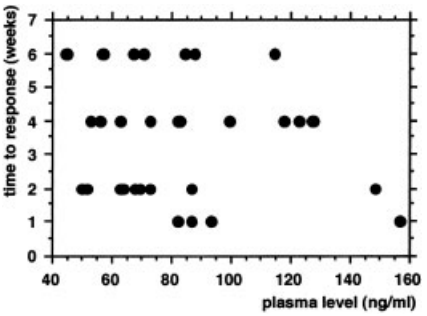

Figure 4. Plasma levels of milnacipran and time to sustained response in each patient

active at clinically relevant doses (Puozzo *et al.*, 2002). However, in the present study, no significant correlation was found between the plasma levels of milnacipran and the antidepressant response. Several studies have shown a significant positive correlation between the plasma levels of TCAs and the antidepressant response. Åsberg *et al.* (1971) reported that amelioration of depressive symptoms was most pronounced in the intermediate plasma level range (50–139 ng/ml) of nortriptyline and was slight both at lower and at higher plasma levels. Glassman *et al.* (1977) have demonstrated a sigmoid relationship between the plasma level and the clinical outcome for imipramine, such that when the level of imipramine and its desmethylated metabolite exceeds 200 ng/ml, the number of patients responding is significantly higher compared with patients with plasma levels below that amount.

There was an important difference between these previous studies and ours. Our study was conducted mainly on non-melancholic patients, while these previous studies were conducted on severely depressed inpatients meeting the criteria of endogenous depression. For example, a positive correlation between the blood levels of amitriptyline or nortriptyline and the antidepressant responses was not found in other previous studies dealing with non-endogenous depressive patients (Coppen *et al.*, 1978; Mendlewicz *et al.*, 1980). For many years TCAs have been the standard treatment of endogenous depression. Compared with other treatment alternatives, TCAs are advantageous, being both effective and acceptable. Therefore, it may be that a clear relationship between plasma levels of TCAs and antidepressant response is easily obtained in endogenous depressive patients. There was another reason for this negative correlation between plasma levels of milnacipran and antidepressant responses. It is well known that compliance tends to be less when patients are feeling better. Only a single rating time point was used for plasma levels of milnacipran in this study, and this might be a cause of variability of plasma levels and loss of correlation.

In our study, only 15 patients with melancholia were included, so it is impossible to analyse the relationship between plasma levels of milnacipran and antidepressant responses in patients with melancholia. A clinical study of major depressive patients with melancholia treated with milnacipran is under way. In the future, it may be possible to reveal a relationship between the plasma levels of milnacipran and the antidepressant response in major depressive patients with melancholia.

The most important findings in the present study were that significant differences of MADRS scores were seen from 1 week after the beginning of this study between responders and nonresponders. Furthermore, the response rate of milnacipran (69.4%) was higher than that in Caucasian major depressive patients (63%) reported by Kasper *et al.* (1996). The rapid response to milnacipran in the treatment of Japanese major depressive patients was confirmed in this study following our previous study (Higuchi *et al.*, 2002). However, this study is an open study without a placebo group, so that any conclusion concerning the rapid response to milnacipran should be made with caution. Other Japanese investigations also showed a rapid response to milnacipran for major depressive patients in a double blind comparative study vs imipramine (Matsubara *et al.*, 1995). On the other hand, a meta-analysis of the major comparative studies has not confirmed any difference in the onset of action of milnacipran compared with TCAs or SSRIs (Montgomery *et al.*, 1996). Considering these findings, further investigations of the onset of action of milacipran are needed.

In conclusion, there was no significant relationship between the plasma levels of milnacipran and the antidepressant response in Japanese major depressive patients. The response rate to milnacipran was considerably high (69.4%) and equal to that of TCAs in the previous studies. The rapid response to milnacipran in the treatment of Japanese major depressive patients is worthy of note. Milnacipran should be considered a promising agent for the treatment of major depressive patients in Japan.

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