ORIGINAL ARTICLE

Chronic dosing with mirtazapine does not produce sedation in rats

Alberto Salazar-Juárez, Susana Barbosa-Méndez, Paola Merino-Reyes, Maura Matus-Ortega, Jorge A. Hernández-Calderón, Benito Antón

Laboratorio de Neurobiología Molecular y Neuroquímica de Adicciones, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico City, Mexico.

Objective: Sedation/somnolence are major side effects of pharmacotherapies for depression, and negatively affect long-term treatment compliance in depressed patients. Use of mirtazapine (MIR), an atypical antidepressant approved for the treatment of moderate to severe depression with comorbid anxiety disorders, is associated with significant sedation/somnolence, especially in short-term therapy. Nonetheless, studies with human subjects suggest that MIR-induced sedation is transient, especially when high and repeated doses are used. The purpose of this study was to explore the effects of acute and chronic administration of different doses of MIR on sedation in the rat.
Methods: Assessment of sedation was carried out behaviorally using the rotarod, spontaneous loco-motor activity, and fixed-bar tests.
Results: A 15-mg/kg dose of MIR induced sedative effects for up to 60 minutes, whereas 30 mg/kg or more produced sedation within minutes and only in the first few days of administration.
Conclusion: These results suggest that 30 mg/kg is a safe, well-tolerated dose of MIR which generates only temporary sedative effects.

Keywords: Mirtazapine; sedation; depression; dosing schedules; pharmacotherapy; antidepressant

Introduction

Mirtazapine (MIR), a noradrenergic and specific serotonergic antidepressant, has a unique pharmacologic profile that differs from those of other currently available antidepressants.[1] Its therapeutic effects are the result of antagonist activity at pre- and postsynaptic α_2-adrenergic receptors and blockade of postsynaptic $5HT_{2A}$ and $5HT_3$ and histamine 1 receptors (H1R) in the central nervous system of mammals, including humans.[2] Nonetheless, recent evidence suggests that MIR can also act as an inverse agonist of the $5\text{-}HT_{2C}$ receptor and indirectly as an agonist of the $5\text{-}HT_{1A}$ receptor.[3,4]

A recent systematic review and meta-analysis of randomized controlled trials showed that MIR was the most effective among 12 new-generation antidepressants studied.[5-7]

Despite the efficacy of MIR, a crucial clinical problem is its tolerability, and the most commonly reported side effects are sedation and somnolence.[8,9] Several studies suggest that MIR-induced sedation can be mainly attributed to its potent blockade of H1Rs.[10,11] Nevertheless, some authors consider that sedative antidepressants like MIR may be a useful treatment option for some patients with agitation or insomnia.[12,13]

Clinical studies have shown that a lower initial dose of MIR (i.e., $\leqslant$15 mg/kg) provides potent histaminergic blockade that induces clear sedation and sleepiness,[14-16] whereas a higher initial MIR dose (i.e., $\geqslant$ 30 mg/kg) is associated with decreased sedative antihistaminergic activity due to increased noradrenergic transmission.[17,18] Other studies indicate that MIR-induced sedation decreases over time.[19]

Since clinical evidence suggests that chronic administration of high doses of MIR does not induce sedation, the present study aimed to evaluate the effects of acute and chronic administration of different doses of MIR on sedation in rats.

Assessment of sedation was carried out behaviorally using the rotarod, spontaneous locomotor activity, and fixed-bar tests, which are widely used to study the mechanisms of action of sedative drugs.[20]

Materials and methods

Animals

Male Wistar rats (baseline weight 250-280 g) were used. Rats were housed four to a cage in standard plastic rodent cages (57 cm x 35 cm x 20 cm) in a colony room maintained at 21±2 °C and 40-50% humidity, under a 12-h light/dark cycle (lights on at 7:00 a.m.). The animals had free access to water and rodent chow pellets, except during experimental sessions. All experiments were conducted during the light phase of the light/dark cycle (between 9:00 a.m. and 3:00 p.m.). All experimental procedures

were approved by the Institutional Care and Use of Laboratory Animals Committee and Bioethics Committee, in strict accordance with the Guidelines for the Care and Use of Laboratory Animals of the National Institutes of Health.

Drugs

MIR (Remeron®; Schering-Plough-Organon, New Jersey, USA) dissolved in 0.9% NaCl was administered at doses of 15, 30, 60, and 90 mg/kg. Trazodone (TRZ) (100 mg/kg) and MIR solutions were kept at -20 °C before use and were freshly prepared. Saline (0.9% NaCl) was the control in all experiments. The treatments were administered intraperitoneally (i.p.) in a volume of 1 mL/kg. The optimal MIR or TRZ doses used in our experiments were based on previous studies,[21-23] which showed that lower MIR doses (< 15 mg/kg) produce hyperphagia, weight gain, and sedation, whereas higher doses (> 60 mg/kg) lead to hypoactivity, somnolence, and reduced exploratory activity, as well as altered behavioral responses.[21] In addition, we have reported elsewhere that daily administration of MIR did not alter spontaneous locomotor activity.[22]

Behavioral procedures: assessment of sedation levels

Spontaneous locomotor activity

Spontaneous locomotor activity in each animal was assessed in 50 × 50 × 30 cm transparent Plexiglas® activity chambers linked online to a compatible PC. Each activity chamber was surrounded by a 16 × 16 array of photocell beams located 3 cm from the floor surface to scan locomotor activity (OMNIALVA, Ciudad de México, Mexico). Interruptions of the photobeams were automatically quantified in OABiomed software version 1.1 for later analysis. Locomotor activity was defined as the interruption of consecutive photobeams (OMNIALVA).

Measurement of spontaneous locomotor activity was performed according to a standard protocol.[22] Briefly, animals were habituated to the activity chambers for three 30-minute sessions and were randomly assigned to different pharmacological treatment groups. Locomotor activity was recorded at 5, 10, 20, 30, 40, and 60 minutes after i.p. administration. The rats were returned to their home cages after each experimental session had been completed.

Fixed-bar test

The fixed-bar test was performed as described by Deacon.[24] A round wooden bar (2.5 cm in diameter and 50 cm long) was secured 60 cm above the cage floor. The rats were trained to stay on the fixed bar just before administration of the drug treatments. Only those rats that stayed on the bar for > 3 minutes were used for subsequent tests. Groups of rats (n=8) were administered test compounds, placed on the middle of the bar, and. their movements and time (in seconds) spent on the bar monitored via video. A maximum of 5 minutes per trial

was allowed. The rats were placed on the bar again 5, 10, 20, 40, and 60 minutes after treatment.

Rotarod test

The rotarod test was divided into two phases.[20] First, the rats were trained to stay on a rod (25 mm in diameter, 25 cm in height; OMNIALVA) rotating at 20 rpm for 5 minutes. Only those rats that were able to remain on the rod for > 3 minutes on at least two out of three consecutive trials were selected and used for subsequent tests.

During the second phase, TRZ (100 mg/kg), MIR (15, 30, 60, and 90 mg/kg), and vehicle (0.9% NaCl) were administered i.p. to each experimental group (n=10). Then, the animals were placed on four paws on the rotating rod and were observed for 3 minutes immediately after administration and at 5, 10, 20, 40, and 60 minutes after administration. The ability of the rats to remain on the rotarod for 3 minutes (time spent on the rotarod), latency, and number of falls were recorded for each rat at least in triplicate. After each test, the device was washed with soap and water, cleaned with 70% ethanol, and dried before the next rat was tested.

Experimental procedures

For the study, 96 male Wistar rats in two groups were used. Each experiment required 48 animals, which were further divided into six experimental groups (n=8). Each experimental group received a different pharmacological treatment.

Experiment 1: effect of acute MIR dosing on sedation

This experiment consisted of a single 1-day pharmacological phase to test sedation in six groups of rats (Figure 1A). One of the groups (saline solution [SAL]) received i.p. saline (0.9% NaCl), while a second group (TRZ) received i.p. TRZ (100 mg/kg). The other four groups were administered different doses (15, 30, 60, and 90 mg/kg) of i.p. MIR (MIR_{15mg}, MIR_{30mg}, MIR_{60mg}, and MIR_{90mg} respectively).

The rotarod, spontaneous locomotor activity, and fixed-bar tests were performed on each animal before (baseline) and 5, 10, 20, 30, 40, and 60 minutes after i.p. administration of MIR (15, 30, 60, or 90 mg/kg), TRZ (100 mg/kg), or vehicle (SAL).

Experiment 2: effect of chronic MIR dosing on sedation

Experiment 2 (Figure 2A) followed the same protocol used in experiment 1. The protocol was repeated for 5 consecutive days. The vehicle (SAL) and TRZ groups received i.p. SAL (0.9% NaCl) and TRZ (100 mg/kg), respectively. The MIR_{15mg}, MIR_{30mg}, MIR_{60mg}, and MIR_{90mg} groups received i.p. MIR (15, 30, 60, and 90 mg/kg respectively) daily.

The rotarod, spontaneous locomotor activity, and fixed-bar tests were performed on each animal before (baseline) and 5, 10, 20, 30, 40, and 60 minutes after i.p. administration of MIR (15, 30, 60, or 90 mg/kg), TRZ (100 mg/kg), or vehicle (SAL). Body weight was measured daily prior to administration of each treatment.
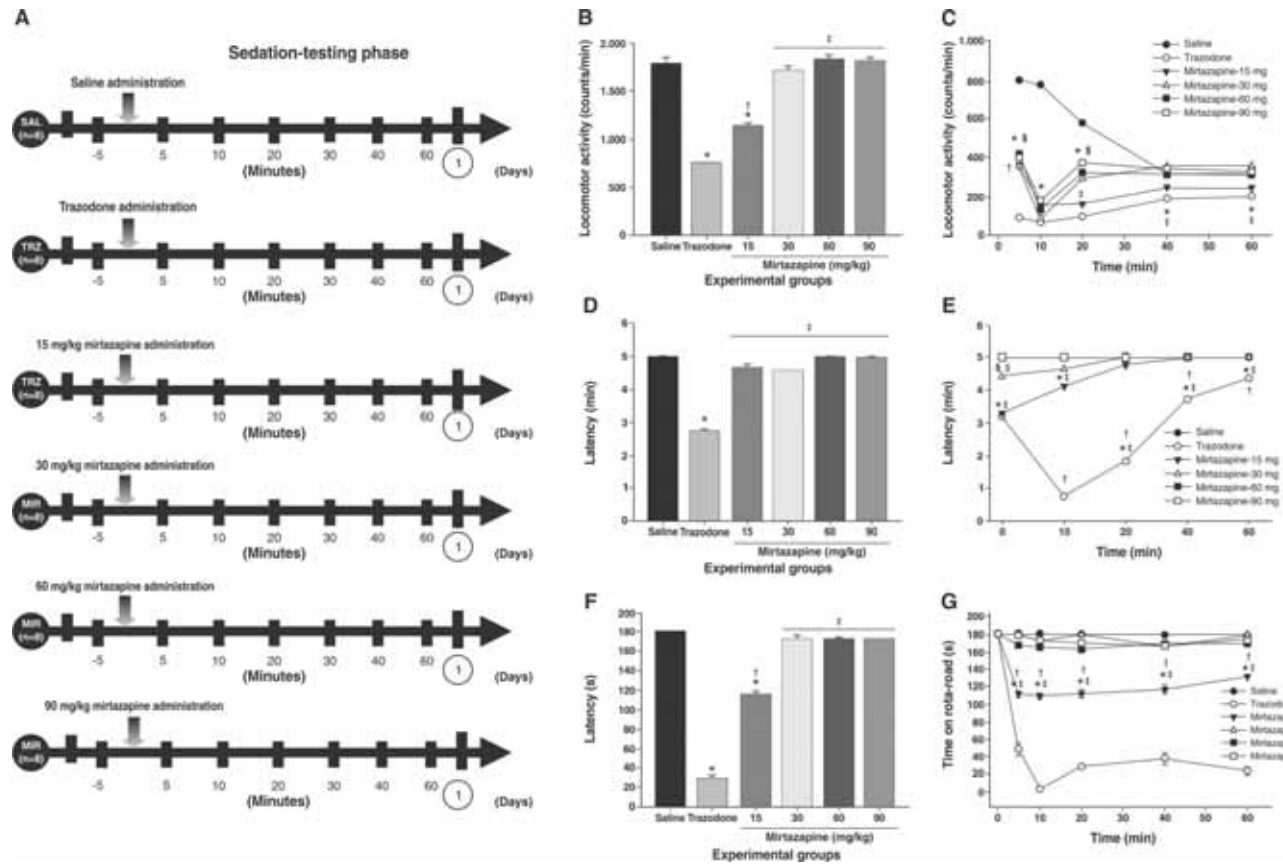
Figure 1 Characterization of sedation induced by single-dose administration of mirtazapine (MIR). A) Timeline of experiment 1. The experimental groups received saline solution (SAL), trazodone (TRZ), or MIR during the sedation-testing phase. B, C) In rats administered a single dose of TRZ or 15 mg/kg MIR , mean spontaneous locomotor activity decreased for up to 60 minutes. MIR doses ⩾ 30 mg/kg did not affect mean spontaneous locomotor activity. A decrease occurred only within 20 minutes of administration. D, E) TRZ decreased the time on the bar for up to 60 minutes. MIR at different doses did not reduce time on the bar. A decrease occurred only within 10 minutes of administration. F, G) Single-dose administration of TRZ or 15 mg/kg MIR decreased time on the rotarod for up to 60 minutes. MIR at ⩾ 30 mg/kg did not affect time on the rotarod. * $p < 0.01$, significant effect of TRZ and MIR_{15mg} treatment on locomotor activity compared to the SAL group. † $p < 0.01$, significant effect of MIR_{15mg} treatment on locomotor activity compared to the TRZ group. ‡ $p < 0.01$, significant effect of MIR at ⩾ 30 mg on locomotor activity compared to TRZ or MIR_{15mg} groups. § $p < 0.01$, significant effect of MIR at ⩾ 30 mg on locomotor activity compared to the SAL group. ‖ $p < 0.01$, significant effect of MIR_{30mg} treatment on locomotor activity compared to the MIR_{60mg} or MIR_{90mg} groups (one-way ANOVA with Tukey's test).

Statistical analysis

Data were expressed as mean ± standard error. Total photobeam interruption counts were used to measure locomotor activity during the testing sessions. The results for locomotor activity in each group during both experimental phases were analyzed with two-way ANOVA followed by Tukey's test for post-hoc comparisons. The time (in seconds) that the animals remained on the rod or wheel was recorded and subsequently analyzed with one-way ANOVA. Significance was set at $p < 0.05$.

Results

Experiment 1: effect of acute MIR dosing on sedation

Spontaneous locomotor activity test

The acute administration of 100 mg/kg TRZ ($p < 0.002$, Tukey's test) or MIR_{15mg} ($p < 0.001$, Tukey's test) to conscious rats produced a significant decrease ($F_{5,24} = 4.86$, $p < 0.003$, one-way ANOVA) in spontaneous locomotor activity (58 and 35% respectively compared to the control group). In contrast, in rats treated with MIR_{30mg} ($p = 0.56$), MIR_{60mg} ($p = 0.33$), and MIR_{90mg} ($p = 0.32$), Tukey's test found no significant differences in spontaneous locomotor activity. Motor activity in the MIR_{30mg}, MIR_{60mg}, and MIR_{90mg} groups was significantly different from that in the MIR_{15mg} group ($p < 0.0001$, Tukey's test) (Figure 1B). The latter group showed a 4% decrease with respect to baseline activity in the SAL group.

TRZ and MIR at all doses produced a significant decrease ($F_{25,210} = 3.22$, $p < 0.0006$, two-way repeated measures ANOVA) in spontaneous locomotor activity from minute 5 ($p < 0.0001$, Tukey's test) to minute 20 ($p < 0.0002$, Tukey's test) after administration. No significant difference was observed in the groups treated with different doses of MIR 40 minutes after administration
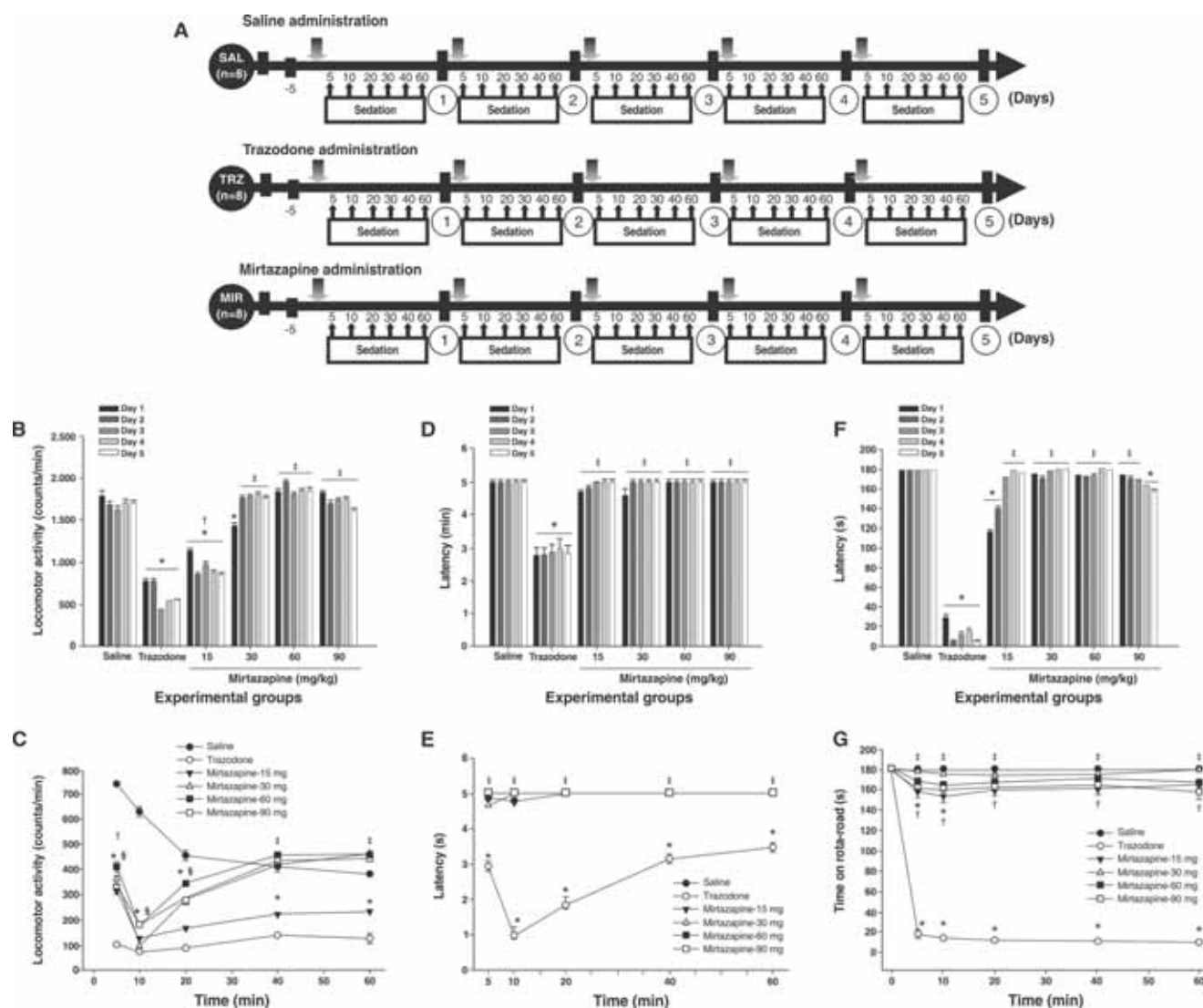
Figure 2 Characterization of sedation induced by repeated mirtazapine (MIR) administration. A) Timeline of experiment 2. The experimental groups received saline solution (SAL), trazodone (TRZ), and MIR (MIR) during the sedation-testing phase. B, C) Repeated administration of TRZ or 15 mg/kg MIR decreased mean spontaneous locomotor activity for up to 60 minutes. Doses $\geqslant$ 30 mg/kg did not affect mean spontaneous locomotor activity. A decrease occurred only within 20 minutes of administration. D, E) TRZ decreased time on the bar for up to 60 minutes. Repeated administration of MIR at different doses did not reduce time on the bar. F, G) Repeated administration of TRZ decreased time on the rotarod for up to 60 minutes. MIR doses $\geqslant$ 15 mg/kg did not affect time on the rotarod. * $p < 0.01$, significant effect of TRZ and MIR_{15mg} treatment on locomotor activity compared to the SAL group. [†] $p < 0.01$, significant effect of MIR_{15mg} treatment on locomotor activity compared to the TRZ group. [‡] $p < 0.01$, significant effect of MIR at $\geqslant$ 30 mg on locomotor activity compared to TRZ or MIR_{15mg} groups. [§] $p < 0.01$, significant effect of MIR at $\geqslant$ 30 mg on locomotor activity compared to the SAL group (one-way ANOVA with Tukey's test).

($p = 0.75$, Tukey's test) compared to the saline group (Figure 1C).

Moreover, as shown in Figure 1C, Tukey's test showed a significant decrease in locomotor activity of rats treated with 100 mg/kg TRZ at 5 minutes compared to those in groups treated with different doses of MIR ($p < 0.00032$). Nevertheless, at 10 minutes, we found no difference between groups ($p = 0.99$, Tukey's test). Twenty minutes after administration, Tukey's test revealed differences in locomotor activity in the MIR_{30mg}, MIR_{60mg}, and MIR_{90mg}

groups compared to the TRZ ($p < 0.0002$) and MIR_{15mg} ($p < 0.004$) groups.

Horizontal fixed bar test

Control animals injected with SAL solution were able to balance on the horizontal bar and support their own weight during the 5-minute test (Figure 1D). TRZ administration significantly decreased time on the bar ($F_{5,24} = 7.34$, $p < 0.0002$, one-way ANOVA) by 45% compared to

the SAL group (p < 0.0007, Tukey's test), whereas the different doses of MIR did not affect ability to stay on the bar (p = 0.97, Tukey's test). The post-hoc Tukey's test also revealed differences in time on the bar between the TRZ group and the groups treated with different doses of MIR (p < 0.0003) (Figure 1D).

Five minutes after administration, treatment with TRZ (p < 0.0002, Tukey's test), MIR_{15mg} (p < 0.0004, Tukey's test), and MIR_{30mg} (p < 0.0008, Tukey's test) significantly decreased stay on the bar, compared to the SAL group ($F_{25,210}$ = 0.50, p < 0.0001, two-way repeated measures ANOVA). Nonetheless, 10 minutes after treatment with TRZ, the ability of rats to stay on the bar decreased significantly compared to the SAL group (p < 0.0003, Tukey's test) and to those treated with different doses of MIR (p < 0.0002, Tukey's test). Furthermore, 20 minutes after administration, MIR, at all doses tested, did not affect the ability of the animals to stay on the bar (p = 0.89, Tukey's test) (Figure 1E).

Rotarod test

A 100 mg/kg dose of TRZ (p < 0.0001, Tukey's test) or MIR_{15mg} (p < 0.0001, Tukey's test) significantly reduced ability to stay on the rotarod, by 84 and 35% respectively ($F_{5,24}$ = 207.31, p < 0.0001, one-way ANOVA). In contrast, MIR_{30mg} (p = 0.84, Tukey's test), MIR_{60mg} (p = 0.56, Tukey's test), and MIR_{90mg} (p = 0.79, Tukey's test) did not affect time on the rotarod (Figure 1F). Tukey's test also found differences in time on the rotarod between the TRZ and MIR_{15mg} groups (p < 0.0001).

Treatment with TRZ or MIR_{15mg} produced a sustained decrease in time on the rotarod from minute 5 ($F_{25,210}$ = 7.10, p < 0.0001, two-way repeated measures ANOVA). Furthermore, Tukey's post-hoc test showed significant differences between the TRZ (p < 0.0003) and MIR_{15mg} (p < 0.0002) groups for all tested time points, compared to the SAL group and to those treated with different doses of MIR (Figure 1G).

Experiment 2: effect of chronic MIR dosing on sedation

Spontaneous locomotor activity

Repeated administration of TRZ and MIR_{15mg} gradually decreased (by 63 and 43%, respectively) spontaneous locomotor activity for up to 5 days. As shown in Figure 2B, a two-way repeated measures ANOVA revealed significant differences in group × time interaction ($F_{20,96}$ = 2.14, p < 0.007). Tukey's test showed significant differences in locomotor activity in the TRZ (p < 0.0001) and MIR_{15mg} (p < 0.0001) groups for the 5 days of the test, compared to the SAL group. In the MIR_{30mg} group, Tukey's post-hoc revealed differences (p < 0.0001) in locomotor activity on the first day of treatment, compared to the control group, but no difference (p = 0.67) from the second day of treatment onward. For MIR_{60mg} (p = 0.44) or MIR_{90mg} (p = 0.24), Tukey's test indicated no differences compared to the SAL group. In contrast, Tukey's test revealed differences during the 5 days of treatment between the TRZ (p < 0.0001) and MIR_{15mg} (p < 0.0001)

groups compared to the MIR_{30mg}, MIR_{60mg}, and MIR_{90mg} groups (Figure 2B).

Rats repeatedly treated with TRZ (p < 0.003, Tukey's test) or MIR_{15mg} (p < 0.001, Tukey's test) exhibited a significant decrease in locomotor activity for up to 60 minutes, whereas MIR_{30mg} (p < 0.0001, Tukey's test), MIR_{60mg} (p < 0.0002, Tukey's test), and MIR_{90mg} (p < 0.0002, Tukey's test) only caused a decrease in motor activity within 20 minutes of administration ($F_{25,120}$ = 4.87, p < 0.0001, two-way repeated measures ANOVA). Forty minutes after administration, the post-hoc test revealed no differences between the MIR_{30mg} (p = 0.21), MIR_{60mg} (p = 0.1), and MIR_{90mg} (p = 0.99) groups, compared to the SAL group. Nevertheless, there were differences between the latter groups with respect to the TRZ (p < 0.0001) and MIR_{15mg} (p < 0.0001) groups. Tukey's post-hoc test did not reveal differences in motor activity between the TRZ and MIR_{15mg} groups (p = 0.98) (Figure 2C).

Horizontal fixed bar test

During the 5-day trial, a 100-mg dose of TRZ produced a significant (p < 0.0003, Tukey's test) 43% decrease in the ability of animals to stay on the bar ($F_{20,96}$ = 2.61, p < 0.0009, two-way repeated measures ANOVA). The post-hoc test found no significant differences in time spent on the bar in animals treated with different doses of MIR (p = 1) in each of the 5 days of the test, compared to the control group (Figure 2D).

Two-way repeated measures ANOVA revealed significant differences in the ability of animals to stay on the bar ($F_{25,120}$ = 11.90, p < 0.0001). There were significant differences (p < 0.0001) between rats treated with TRZ and those in the SAL group from 5 minutes and onward. Tukey's test, however, found no differences (p = 1) in time on the bar between rats in the SAL group and those treated with different doses of MIR during the test period (Figure 2E).

Rotarod test

Two-way repeated measures ANOVA revealed significant differences in group × time interaction ($F_{20,96}$ = 18.76, p < 0.00001). Compared to control (saline) administration, daily TRZ administration significantly decreased time on the rotarod by 93% on test days (p < 0.0001, Tukey's test). Differences were also found in the ability of animals in the MIR_{15mg} group to stay on the rotarod during the first (p < 0.0001, Tukey's test) and second (p < 0.007, Tukey's test) days of treatment, but no differences in time spent on the rotarod in the MIR_{30mg} (p = 0.94), MIR_{60mg} (p = 0.98), or MIR_{90mg} (p = 0.89) groups during the 5 days of the test (all Tukey's test; Figure 2F).

Moreover, ANOVA revealed differences in group × time interaction ($F_{25,120}$ = 53.102, p < 0.0001, two-way repeated measures ANOVA). In animals treated with TRZ during the 5-day trial, decreased time on the rotarod within 5-60 minutes of testing was observed (p < 0.0001, Tukey's test). In contrast, the MIR_{30mg} (p = 1), MIR_{60mg} (p = 0.92), and MIR_{90mg} (p = 0.50) groups showed no significant differences in ability of the animals to stay on
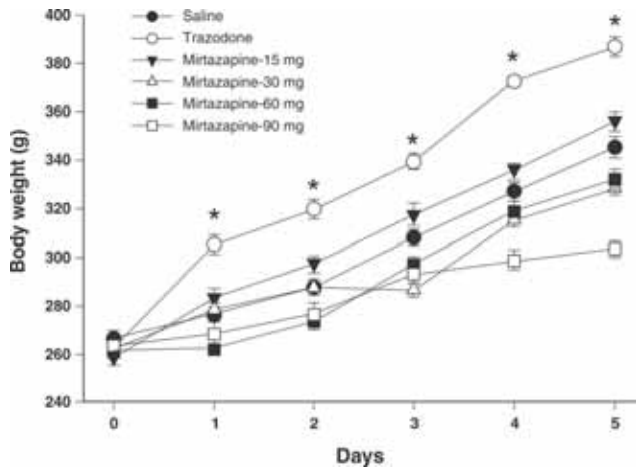
Figure 3 Characterization of changes in body weight of rats dosed with mirtazapine. Repeated administration of trazodone increased body weight. Doses $\geqslant$ 15 mg/kg did not affect body weight. * Significant effect ($p < 0.01$) of trazodone treatment on body weight compared to the saline solution group (one-way ANOVA followed by Tukey's test).

the rotarod compared to the SAL group. Tukey's test indicated that treatment with MIR$_{15mg}$ reduced time on the rotarod at 5 ($p < 0.003$) and 10 ($p < 0.005$) minutes after administration. At 20 minutes, no differences were found ($p = 0.62$) (Figure 2G).

Body weight

Two-way repeated measures ANOVA revealed significant differences in group × time interaction ($F_{25,215} = 4.71$, $p < 0.0001$). During the 5-day trial, TRZ was associated with a gradual increase in body weight compared to the SAL group ($p < 0.0003$, Tukey's test). Tukey's test found no significant difference in body weight across the MIR$_{15mg}$ ($p = 0.84$), MIR$_{30mg}$ ($p = 1$), MIR$_{60mg}$ ($p = 0.50$), and MIR$_{90mg}$ ($p = 0.90$) groups with respect to SAL (Figure 3).

Discussion

Use of MIR, an atypical antidepressant approved for the treatment of moderate to severe depression with comorbid anxiety disorders,[25] is associated with significant weight gain, sedation/somnolence, and fatigue, especially in short-term therapy.[26,27] The results of this study showed that a 15 mg/kg dose of MIR induces sedative effects for up to 60 minutes, whereas 30 mg/kg or more produces sedation within minutes and only during the first few days of administration in rats.

Experiment 1: effect of acute MIR dosing on sedation

The results of experiment 1 revealed that single-dose MIR administration in rats decreased spontaneous locomotor activity, balance, and motor coordination at a relatively low dose ($\leqslant$ 15 mg/kg) up to 60 minutes after administration. At higher doses ($\geqslant$ 30 mg/kg), MIR altered these behavioral parameters within 20 minutes of administration;

subsequently, sedation gradually decreased and reached baseline levels at 60 minutes.

These results are consistent with previous reports in humans showing that a single dose of 7.5 or 15 mg/kg of MIR impaired psychomotor performance transiently in healthy men.[10] Additionally, Poyurovsky et al.[15] have reported that MIR given in doses of 15 mg/kg is well tolerated and causes only mild and transient sedation. Other authors reported that MIR at a dose of 30 mg/kg or more has no adverse effects of sedation[28,29] in patients with major depression on the first day of treatment, compared to other antidepressants such as TRZ.[16,19] This is in line with the results of our experiment.

Others clinical studies have indicated that one of the main therapeutic effects of MIR is its ability to improve sleep in depressed patients within minutes of administration.[30-32]

In this study, we found that a single 15-mg dose of MIR caused sedation for up to 60 minutes after administration, which is consistent with previous results that indicate that the dose of MIR is an important factor in its ability to improve sleep.[29] MIR given in low doses at the onset of treatment has been reported to increase sleep duration in patients with major depression, which suggests that the ability of low-dose MIR to promote sleep may be caused by sedation-related side effects.[29]

TRZ is an effective and well tolerated antidepressant, and is widely used for the treatment of patients with depression with or without anxiety and/or primary or secondary insomnia, due its sedative action and its ability to normalize sleep patterns.[33]

In this study, we used TRZ for its pharmacological profile similar to that of MIR[33] and because several studies have indicated that, at doses different from those used for the treatment of depression, the sedative action of TRZ can be attributed primarily to antagonism of 5-HT$_{2A}$ receptors, H1Rs, and α1-adrenergic receptors,[34] as has been suggested to occur with MIR.

In this study, we found that TRZ at a dose of 100 mg mg/kg significantly decreased spontaneous locomotor activity and the ability of the rats to stay on a fixed bar or rotarod for up to 60 minutes after administration.

These results are consistent with those of several clinical studies of patients with depression treated with different doses of TRZ, in which this agent was found to cause significant sedation/somnolence[33] after administration of a single night-time dose.[35,36] In fact, several clinical trials reported that patients with severe insomnia associated with deep depression were initially treated with TRZ as monotherapy and as part of a combination strategy to induce an immediate improvement in sleep.[33,37] In fact, doses higher than 100 mg/kg given to geriatric patients with severe insomnia associated with major depression significantly improved sleep and antidepressant efficacy,[36] which was attributed to its powerful sedative side effect.

Experiment 2: effect of chronic MIR dosing on sedation

The efficacy of an antidepressant depends on its dosing regimen, side effects, patient acceptance, intake adherence, and safety.[36]

In this experiment, a 5-day dosing regimen with 15 mg/kg MIR decreased spontaneous locomotor activity for up to 5 days. The same dose did not affect time on the bar (balance), although it had a negative impact on time spent on the rotarod (motor coordination) in the first 2 days. This finding suggests that repeated MIR administration at this dose produced only partial sedative effects (with significant impairment in spontaneous locomotor activity) without altering other behavioral parameters. In contrast, daily dosing of MIR (30 mg/kg or more) did not permanently affect any of the three behavioral parameters tested in our study. Conversely, TRZ decreased spontaneous locomotor activity, balance, and motor coordination for up to 5 days.

As previously mentioned, a single dose of MIR (15 mg/kg or lower) causes mild and transient sedation.[15,38] Radhakishun et al.[39] reported that MIR treatment with initial doses of 15 mg/kg subsequently increased to 30 mg/kg for several weeks was well tolerated, with only 10% of patients reporting adverse effects during the first week and fewer patients doing so in subsequent weeks. This dosage regimen facilitated and increased sleep efficiency and improved depression, but did not affect daytime alertness or motor performance.[39]

Graves & Napier[40] found that chronic dosing with MIR did not alter motor performance in a rat study. We recently reported that chronic MIR dosing (30 mg/kg for 30 consecutive days) did not alter spontaneous locomotor activity in rats.[22] This finding is consistent with the present study, which indicates that a daily dosing regimen of 30 mg/kg or more does not alter spontaneous locomotor activity, balance, or motor coordination.

The use of different daytime dosing regimens of TRZ suggests that daily administration of this drug has a strong and long-term sedative effect that gradually improves sleep.[36,41] This is also consistent with the results of the present study, in which a sustained decrease in all three behavioral parameters of interest was observed for up to 5 days.

The differences between the TRZ-treated group and the groups treated with different doses of MIR indicate that MIR treatment at 30 mg/kg or higher doses is not associated with long-term sedative effects when given for several days.[19] This finding may point to the fact that the sedative effect of MIR at doses of 30 mg/kg or higher gradually decreases. Our statistical analyses revealed no difference between the different doses of MIR tested herein.

Furthermore, our study also showed that a daily dosing regimen of MIR at 15 mg/kg or more decreased spontaneous locomotor activity within 10 minutes of administration, with a gradual decrease in sedative effects,[10,15] while balance and motor coordination were unaffected. Conversely, TRZ impaired motor activity, balance, and motor coordination quickly (within 5 minutes) and for up to 60 minutes. Additionally, we found that chronic MIR dosing at 15 mg/kg slightly increased body weight; however, MIR doses $\geqslant$ 30 mg/kg did not affect body weight. These results are consistent with a recent mouse study in which chronic MIR dosing with 10 or 50 mg/kg failed to increased body weight relative to control animals.[42] This

suggests that the adverse effects of different daily dosing regimens of MIR disappear within minutes of administration, which is consistent with its sleep-promoting effects.[19,29] This pharmacological property of MIR increases its treatment adherence and substantially improves the symptoms of depression.

The lack of experiments aimed at determining the participation of H1, 5-HT$_2$, or 5-HT$_3$ receptors in the sedative effects of MIR and characterizing the pharmacological and molecular mechanisms (up or down regulation) through which chronic MIR dosing would alter functioning of these receptors is a limitation of this study. Nevertheless, it has been reported that MIR treatment induces a decrease in density of 5-HT$_{2A}$R,[43] a receptor which has been suggested to be involved in the sedative effects of MIR. Additionally, various reports indicate that the high binding affinity and agonist profile of MIR for H1 receptors seems to play an important role in its sedative effects.[11] Other authors suggest that tolerance to the sedative effects of MIR develops rapidly, as with H1 antihistamines.[44,45] This could explain at least in part the decrease in MIR sedation that accompanies long-term daily dosing.

Conclusion

The most common pharmacological therapies for patients with major depressive disorder are mood stabilizers, antidepressants, and atypical antipsychotics.[46] Side effects are common and vary among medications, with weight gain, metabolic dysregulation, sedation/somnolence, and akathisia among those observed most frequently. Some authors propose that the side effects of antidepressants are more prominent during the initial phase of treatment (particularly the first week).[16] These side effects, particularly weight gain and sedation/somnolence, have a negative effect on treatment adherence.[1,9,46] In particular, patients who do not develop tolerance to the sedating effects of the drug can experience a markedly negative effect on quality of life, with sedation/somnolence, weight gain, and cognitive dysfunction impairing social and occupational functioning.[47]

In this study, we found that acute or chronic MIR dosing in rate induced permanent sedative effects at low doses ($\leqslant$ 15 mg/kg) and transient sedative effects at higher doses ($\geqslant$ 30 mg/kg). These results are important from the perspective of patients with major depression. For patients who do not develop tolerance to the sedative effect, dosing with $\geqslant$ 30 mg/kg of MIR improves depression, but does not induce sedation. In patients with severe depression and sleep disturbances, initial dosing with 15 mg/kg of MIR can facilitate and increase sleep efficiency; a subsequent switch to $\geqslant$ 30 mg/kg would then improve sleep and depression, without affecting daytime alertness or motor performance. Our finding of no significant differences between groups dosed with 60 or 30 doses of 90 mg/kg indicate that, in patients with severe depression requiring high-dose therapy, MIR is safe and does not induce permanent sedation.

Our results also indicate that the behavioral procedures used in this study would be a good model to characterize

the pharmacological and molecular mechanisms of the side effects of MIR in detail, and support existing evidence that MIR may be a novel pharmacological agent that merits further testing in clinical trials for the treatment of different neuronal diseases. Recent studies indicate that chronic dosing with MIR (50 mg/kg) restores morphological alterations, normalizes physiological functions, and reestablishes normal levels of neurotransmission in animals with characteristic symptoms of Rett syndrome.[42] Other studies have suggested that MIR can be used to treat drug withdrawal symptoms and reduce drug use in substance abusers.[22,48]

These results support the clinical observation that chronic dosing with MIR does not produce sedation, a pharmacological property that can increase treatment adherence and substantially improve symptoms of depression.

Acknowledgements

Disclosure

The authors report no conflicts of interest.

References

1 Torrens M, Fonseca F, Mateu G, Farré M. Efficacy of antidepressants in substance use disorders with and without comorbid depression. A systematic review and meta-analysis. Drug Alcohol Depend. 2005;78:1-22.

2 de Boer T. The effects of mirtazapine on central noradrenergic and serotonergic neurotransmission. Int Clin Psychopharmacol. 1996;10:19-23.

3 Chanrion B, Mannoury la Cour C, Gavarini S, Seimandi M, Vincent L, Pujol JF, et al. Inverse agonist and neutral antagonist actions of antidepressants at recombinant and native 5-hydroxytryptamine2C receptors: differential modulation of cell surface expression and signal transduction. Mol. Pharmacol. 2008;73:748-57.

4 Nakayama K, Sakurai T, Katsu H. Mirtazapine increases dopamine release in prefrontal cortex by 5-HT1A receptor activation. Brain Res Bull. 2004;63:237-41.

5 Cipriani A, Furukawa TA, Salanti G, Geddes JR, Higgins JP, Churchill R, et al. Comparative efficacy and acceptability of 12 new-generation antidepressants: a multiple-treatments meta-analysis. Lancet. 2009;373:746-58.

6 Watanabe N, Omori IM, Nakagawa A, Cipriani A, Barbui C, McGuire H, et al. Mirtazapine versus other antidepressants in the acute-phase treatment of adults with major depression: systematic review and meta-analysis. J Clin Psychiatry. 2008;69:1404-15.

7 Watanabe N, Omori IM, Nakagawa A, Cipriani A, Barbui C, Churchill R, et al. Mirtazapine versus other antidepressive agents for depression. Cochrane Database Syst Rev. 2011;12:CD006528.

8 Bourin M, Briley M. Sedation, an unpleasant, undesirable and potentially dangerous side-effect of many psychotropic drugs. Hum Psychopharmacol. 2004;19:135-9.

9 Watanabe N, Omori IM, Nakagawa A, Cipriani A, Barbui C, McGuire H, et al. Safety reporting and adverse-event profile of mirtazapine described in randomized controlled trials in comparison with other classes of antidepressants in the acute-phase treatment of adults with depression: systematic review and meta-analysis. CNS Drugs. 2010;24:35-53.

10 Iwamoto K, Kawano N, Sasada K, Kohmura K, Yamamoto M, Ebe K, et al. Effects of low-dose mirtazapine on driving performance in healthy volunteers. Hum Psychopharmacol. 2013;28:523-8.

11 Anttila SA, Leinonen EV. A review of the pharmacological and clinical profile of mirtazapine. CNS Drug Rev. 2001;7:249-64.

12 Mann JJ. The medical management of depression. N Engl J Med. 2005;353:1819-34.

13 Linden M, Westram A. Prescribing a sedative antidepressant for patients at work or on sick leave under conditions of routine care. Pharmacopsychiatry. 2010;43:1-6.

14 Kasper S, Praschak-Rieder N, Tauscher J, Wolf R. A risk-benefit assessment of mirtazapine in the treatment of depression. Drug Saf. 1997;17:251-64.

15 Poyurovsky M, Bergman J, Pashinian A, Weizman A. Beneficial effect of low-dose mirtazapine in acute aripiprazole-induced akathisia. Int Clin Psychopharmacol. 2014;29:296-8.

16 Grasmäder K, Verwohlt PL, Kühn KU, Frahnert C, Hiemke C, Dragicevic A, et al. Relationship between mirtazapine dose, plasma concentration, response, and side effects in clinical practice. Pharmacopsychiatry. 2005;38:113-7.

17 Stahl SM, Kremer CME, Pinder RM. Tolerability of mirtazapine used in high or low initial dose. Eur Neuropsychopharmacol. 1997;7:S157-8.

18 Stimmel GL, Sussman N, Wingard P. Mirtazapine safety and tolerability: analysis of the clinical trials database. Prim Psychiatry. 1997;96:82-90.

19 Sasada K, Iwamoto K, Kawano N, Kohmura K, Yamamoto M, Aleksic B, et al. Effects of repeated dosing with mirtazapine, trazodone, or placebo on driving performance and cognitive function in healthy volunteers. Hum Psychopharmacol. 2013;28:281-6.

20 dela Peña IJ, Hong E, Kim HJ, de la Peña JB, Woo TS, Lee YS, et al. Artemisia capillaris Thunberg produces sedative-hypnotic effects in mice, which are probably mediated through potentiation of the GABA(A) receptor. Am J Chin Med. 2015;43:667-79.

21 Timmer CJ, Paanakker JE, Vrijmoed-deVries M. Mirtazapine pharmacokinetics with two dosage regimens and two pharmaceutical formulations. Pharm Res. 1997;14:98-102.

22 Salazar-Juárez A, Barbosa-Méndez S, Jurado N, Hernández-Miramontes R, Leff P, Antón B. Mirtazapine prevents induction and expression of cocaine-induced behavioral sensitization in rats. Prog Neuropsychopharmacol Biol Psychiatry. 2016;68:15-24.

23 Marinescu IP, Predescu A, Udriştoiu T, Marinescu D. Comparative study of neuroprotective effect of tricyclics vs. trazodone on animal model of depressive disorder. Rom J Morphol Embryol. 2012;53:397-400.

24 Deacon RM. Measuring motor coordination in mice. J Vis Exp. 2013;75:e2609.

25 Croom KF, Perry CM, Plosker GL. Mirtazapine: a review of its use in major depression and other psychiatric disorders. CNS Drugs. 2009;23:427-52.

26 Serretti A, Mandelli L. Antidepressants and body weight: a comprehensive review and meta-analysis. J Clin Psychiatry. 2010;71:1259-72.

27 Papakostas GI, Homberger CH, Fava M. A meta-analysis of clinical trials comparing mirtazapine with selective serotonin reuptake inhibitors for the treatment of major depressive disorder. J Psychopharmacol. 2008;22:843-8.

28 Davis R, Wilde MI. Mirtazipine: a review of its pharmacology and therapeutic potential in the management of major depression. CNS Drugs. 1996;5:389-402.

29 Dolder CR, Nelson MH, Iler CA. The effects of mirtazapine on sleep in patients with major depressive disorder. Ann Clin Psychiatry. 2012;24:215-24.

30 Castillo JL, Menendez P, Segovia L, Guilleminault C. Effectiveness of mirtazapine in the treatment of sleep apnea/hypopnea syndrome (SAHS). Sleep Med. 2004;5:507-8.

31 Carley DW, Olopade C, Ruigt GS, Radulovacki M. Efficacy of mirtazapine in obstructive sleep apnea syndrome. Sleep. 2007;30:35-41.

32 Wang D, Li Z, Li L, Hao W. Real-world, open-label study to evaluate the effectiveness of mirtazapine on sleep quality in outpatients with major depressive disorder. Asia Pac Psychiatry. 2014;6:152-60.

33 Fagiolini A, Comandini A, Catena B, Dell'Osso M, Kasper S. Rediscovering trazodone for the treatment of major depressive disorder. CNS Drugs. 2012;26:1033-49.

34 Stahl SM. Mechanism of action of trazodone: a multifunctional drug. CNS Spectr. 2009;14:536-46.

35 Orlando JM, Case BC, Thomson AE, Griffith E, Sherman BL. Use of oral trazodone for sedation in cats: a pilot study. J Feline Med Surg. 2016;18:476-82.

36 Fabre LF. Trazodone dosing regimen: experience with single daily administration. J Clin Psychiatry. 1990;51:23-6.

37 Nierenberg AA, Adler LA, Peselow E, Zornberg G, Rosenthal M. Trazodone for antidepressant-associated insomnia. Am J Psychiatry. 1994;151:1069-72.

38 Matreja PS, Badyal DK, Deswal RS, Sharma A. Efficacy and safety of add on low-dose mirtazapine in depression. Indian J Pharmacol. 2012;44:173-7.

39 Radhakishun FS, van den Bos J, van der-Heijden BC, Roes KC, O'Hanlon JF. Mirtazapine effects on alertness and sleep in patients as recorded by interactive telecommunication during treatment with different dosing regimens. J Clin Psychopharmacol. 2000;20:531-7.

40 Graves SM, Napier TC. Mirtazapine alters cue-associated metham-phetamine seeking in rats. Biol Psychiatry. 2011;69:275-81.

41 Davey A. A comparison of two oral dosage regimens of 150 mg trazodone in the treatment of depression in general practice. Psy-chopharmacology (Berl). 1988;95:S25-30.

42 Bittolo T, Raminelli CA, Deiana C, Baj G, Vaghi V, Ferrazzo S, et al. Pharmacological treatment with mirtazapine rescues cortical atro-phy and respiratory deficits in MeCP2 null mice. Sci Rep. 2016;6: 19796.

43 Muguruza C, Miranda-Azpiazu P, Díez-Alarcia R, Morentin B, González-Maeso J, Callado LF, et al. Evaluation of 5-HT2A and mGlu2/3 receptors in postmortem prefrontal cortex of subjects with major depressive disorder: effect of antidepressant treatment. Neuro-pharmacology. 2014;86:311-8.

44 Richardson GS, Roehrs TA, Rosenthal L, Koshorek G, Roth T. Tol-erance to daytime sedative effects of H1 antihistamines. J Clin Psy-chopharmacol. 2002;22:511-5.

45 Ramaekers JG. Antidepressants and driver impairment: empirical evi-dence from a standard on-the-road test. J Clin Psychiatry. 2003;64:20-9.

46 Kemp DE. Managing the side effects associated with commonly used treatments for bipolar depression. J Affect Disord. 2014;169:S34-44.

47 Kane JM, Sharif ZA. Atypical antipsychotics: sedation versus effi-cacy. J Clin Psychiatry. 2008;69:18-31.

48 Zueco-Pérez PL. [Mirtazapine in the treatment of cocaine-dependence in patients with methadone]. Actas Esp Psiquiatr. 2002;30:337-42.