

# Effects of prolonged-release melatonin, zolpidem, and their combination on psychomotor functions, memory recall, and driving skills in healthy middle aged and elderly volunteers

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**Background** Melatonin is an important regulator of the sleep–wake cycle. A prolonged-release formulation of melatonin (PR-M) that essentially mimics the profile of the endogenous production of the hormone is effective in the treatment of insomnia in patients aged 55 years and older. Because hypnotics result in impairments of various cognitive skills, it is important to examine the cognitive effects associated with the use of PR-M.

**Objectives and methods** The effects of therapeutic oral doses of PR-M (2 mg), zolpidem (10 mg) and their combination administered at bedtime on cognitive functions in healthy subjects aged 55 years and older (12 males + 4 females, age  $59.4 \pm 3.2$  years) were assessed in a randomized, double-blind, placebo-controlled, and four-way crossover study. Psychomotor functions, memory recall, and driving skills were assessed at 1 and 4 h following administration and the next morning.

**Results** Compared to placebo, PR-M alone did not impair performances on any cognitive tasks. Zolpidem significantly impaired psychomotor and driving performance 1 h and 4 h post-dosing, and early memory recall; these impairments were exacerbated with PR-M co-administration. No effects on next morning psychomotor or driving performance were observed except that the decline in memory recall after zolpidem was more pronounced in the next day. No pharmacokinetic interactions were found.

**Conclusions** This study extends previous researches showing impairment of cognitive functions by zolpidem within 5 h post-administration. Further, PR-M use was not found associated with impairment of psychomotor functions, memory recall, and driving skills, and points to a pharmacodynamic interaction between melatonin and GABA-A modulators. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS — melatonin; zolpidem; cognitive functions; driving simulator; elderly

## INTRODUCTION

Changes in sleep–wake patterns occur with age, resulting in an increased risk for insomnia in elderly (Pandi-Perumal *et al.*, 2002). The elderly are particularly liable to suffer from insomnia. Whereas the prevalence of sleep problems in 18–35 year olds is about 10–15%, it rises to 30% in the over-65 year olds (Foley *et al.*, 1995; Ohayon, 1997; Ustun *et al.*, 1996;

Walsh and Engelhardt, 1999). This is reflected in the usage of hypnotic medication with roughly 10–15% of the elderly population.

Melatonin, a hormone produced by the pineal gland at night, has a role in the regulation of the sleep–wake cycle and promotes sleep in humans (Zisapel, 1999). Nocturnal melatonin production declines with age (Dori *et al.*, 1994; Iguchi *et al.*, 1982; Kennaway *et al.*, 1999; Siegrist *et al.*, 2001). Excretion of the main melatonin metabolite 6-Sulfatoxymelatonin in insomniacs aged  $\geq 55$  years is about one third of that in healthy adults aged 20–35 years and about half of that in healthy adults  $\geq 55$  years without sleep complaints (Haimov *et al.*, 1994; Leger *et al.*, 2004). Exogenous

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melatonin was consistently found to decrease sleep latency and in certain cases to increase sleep efficiency in elderly insomniac patients (Garfinkel *et al.*, 1995; Haimov *et al.*, 1995; Leger *et al.*, 2004; Monti *et al.*, 1999; Wurtman and Zhdanova, 1995). Melatonin acts via its own receptors (MT1, MT2), which are members of the G protein-linked receptor family and are involved in the regulation of circadian rhythms and soporific function (Witt-Enderby *et al.*, 2003). In addition, lower affinity melatonin binding sites have been described (Laudon *et al.*, 1988). A melatonin binding site termed MT3 has recently been identified as quinone reductase 2 (Nosjean *et al.*, 2000; Witt-Enderby *et al.*, 2003) but its physiological role has not been elucidated.

An important aspect of hypnotics use is the decrement in cognitive functioning following intake (Glass *et al.*, 2005). Furthermore, recent studies highlight the major role of sleep in memory consolidation (Gais and Born, 2004; Stickgold and Walker, 2007). Impairment in psychomotor functioning, driving performance and memory consolidation may be specifically deleterious for daytime functioning at older age. Zolpidem, a hypnotic of second generation, is the most commonly prescribed hypnotic in the United States and Europe (Grunstein, 2002). Zolpidem binds selectively to the  $\alpha$  1-GABA(A) receptor in the brain. At its usual recommended doses (5–10 mg), zolpidem was proven as effective as benzodiazepines in the management of short-term insomnia but with few adverse effects (Holm and Goa, 2000; Vermeeren, 2004). Zolpidem is generally considered free of residual effects when taken at bedtime before 8 h of sleep (Allain *et al.*, 2003; Darcourt *et al.*, 1999; Hindmarch *et al.*, 2006; Rush *et al.*, 1998; Uden and Roth-Schechter, 1996; Verster and Volkerts, 2004). The lack of hangover effect is likely related to its short half-life (2.5–2.9 h) (Drover, 2004). Moderate to severe cognitive impairments can be observed within 5 h post-administration, that may be detectable until 7 h post-administration (Danjou *et al.*, 1999; Hindmarch *et al.*, 2001; Mintzer *et al.*, 1997; Patat *et al.*, 2001; Verster *et al.*, 2002).

The effects of exogenous melatonin on neurobehavioral performances were also studied and appear time-dependent. Several studies reported alterations of alertness and performances by acute melatonin when administered during daytime (Atkinson *et al.*, 2005; Cajochen *et al.*, 1996; Dollins *et al.*, 1993; Graw *et al.*, 2001; Lieberman *et al.*, 1984; Neville and McNaughton, 1986; Rogers *et al.*, 1998) but not when administered during late afternoon or at bedtime in young and middle-aged (20–57 years) healthy

volunteers (Paul *et al.*, 2003; Stone *et al.*, 2000; Suhner *et al.*, 1998; Wynn and Arendt, 1988). Furthermore, melatonin administered nightly for 4 weeks improved certain aspects of cognition in healthy elderly subjects (Peck *et al.*, 2004). Fainstein *et al.* (1997) noted that melatonin at bedtime augmented the quality of alertness that is impaired with hypnotics (benzodiazepines). Most of these studies have used immediate-release melatonin, which induced elevated melatonin level for a short duration due to its short half-life (34–50 min) (Waldhauser *et al.*, 1984; Vakkuri *et al.*, 1985). In order to better mimic the endogenous melatonin release profile of healthy subjects, a prolonged-release formulation of melatonin (PR-M, Circadin<sup>®</sup>), has been developed, which allows elevated melatonin levels throughout the night sleep. A 2-mg oral dose was found safe and effective in improving sleep initiation and sleep quality in elderly insomniacs (Garfinkel *et al.*, 1997; Haimov *et al.*, 1995; Leger *et al.*, 2004). PR-M appears as a pharmacological agent which may induce a more physiological sleep than currently prescribed hypnotic drugs in elderly insomniac patients, and thus may have a better neurobehavioral profile.

Because PR-M and zolpidem have different primary mechanisms of action and both appear relatively safe, physicians may be tempted to combine both compounds in elderly insomniac patients. However, previous studies indicated that melatonin may potentiate the hypnotic effects of GABA(A) receptor modulators (i.e., benzodiazepine and non-benzodiazepine hypnotics) (Ferini-Strambi *et al.*, 1995; Garfinkel *et al.*, 1997; Wesensten *et al.*, 2005). Furthermore, co-administration of melatonin during the withdrawal period is thus useful to facilitate discontinuation of hypnotic drugs (Garfinkel *et al.*, 1999). Thus, it appears important to be aware about the safety profiles of the combination of zolpidem and PR-M in elderly subjects. The objective of the present study was to assess the effects of PR-M, zolpidem and their combination versus placebo on psychomotor functions memory and driving skills, at different time points following a bedtime administration in healthy elderly volunteers. Possible pharmacokinetic interaction was also investigated.

## MATERIALS AND METHODS

### *Subjects*

Sixteen healthy subjects (12 males, 4 females) aged 55–65 years (mean  $\pm$  SD: 59.4  $\pm$  3.2 years) participated in this randomized, double-blind and placebo-

controlled study conducted at a single centre (Forenap, France) after having given their written informed consent. To be eligible, they needed to have a body mass index between 20 and 32 kg/m<sup>2</sup> (mean: 25.5 ± 2.3 kg/m<sup>2</sup>), and a physical examination, cardiovascular examination and laboratory tests within normal ranges for their age. Exclusion criteria included hypertension, psychiatric disorders or any serious other disorders, intake of any drug with central effects within 45 days prior to experiment, excessive amount of alcohol (≥40 g/day) or xanthines, or smoking more than 10 cigarettes per day. All subjects possessed a valid driving license. The protocol was granted ethical approval by a local ethics committee (CCPPRB, Alsace I, and Strasbourg) and complied with the ethical principles stated in the revised version of the Declaration of Helsinki (Edinburgh, 2000).

#### *Study treatments*

After having successfully completed the screening procedure, subjects were randomized to receive capsules containing either placebo, PR-M (prolonged-release melatonin, Circadin<sup>®</sup> 2 mg), zolpidem (Stilnox<sup>®</sup> 10 mg) or both PR-M and zolpidem (PR-M + zolpidem) according to a double-blind, four-way crossover design. All treatment periods lasted 2 days and were separated by washout periods of 2–10 days. Study medications were administered in the evening of day 1 (around 20:00 h in order to test the immediate effects) during a standard dinner. Volunteers were not allowed to nap or to use caffeine or nicotine when hospitalized (day 1–2) and they were asked to maintain their usual habits during the washout periods.

#### *Cognitive tests*

A selection of tasks from the Cognitive Drug Research (CDR) computerized assessment system (see Wesnes *et al.*, 1989 for detailed description of the tasks included in this battery) was administered in the following order:

- Picture presentation.
- Simple reaction time: speed of reaction (millisecond) was recorded.
- Digit vigilance task: speed of reaction (millisecond) and number of correct responses were recorded.
- Choice reaction time: speed of reaction (millisecond) and percentage of correct responses were recorded.
- Tracking: the average distance off-target per second was recorded.
- Delayed picture recognition: speed of reaction (millisecond) was recorded and a sensitivity index (SI), which takes into account the ability to correctly identify previously presented pictures and to correctly reject novel items, was calculated.

A power of attention factor, which is a composite of the speed scores in simple reaction time, choice reaction time and digit vigilance, was also calculated.

Two training sessions, each consisting of two administrations of the test system, were completed by each volunteer during the screening period. On each treatment period, the tasks were administered in the morning following arrival in the clinical unit (baseline) and then at several time-points following the study drug administration: +1 h, +4 h, +12.5 h, and +15 h.

#### *Rivermead story*

In this memory test (Wilson *et al.*, 1985), the subject was asked to listen a short story and to recall this story again, without a second reading. The test took place 2 h (immediate recall) and 12.5 h (next morning/delayed recall) after drug intake.

#### *Grooved pegboard*

The grooved pegboard is a manipulative dexterity test (Mathews and Klove, 1964). It consists of 25 holes with randomly positioned slots. Pegs, which have a key along one side, must be rotated to match the hole before they can be inserted. This test is designed to measure a timed unilateral motor response. The subject was asked to place 25 pegs in round holes as quickly as possible across the rows. The task was executed in the morning (baseline) and then at several time-points following the study drug administration: +1 h, +4 h, +12.5 h, and +15 h.

#### *Pentobarbital chlorpromazine alcohol group (PCAG)/sedation scale*

This scale is composed of 15 questions (Martin *et al.*, 1971). The subject was asked to read each of them and indicate if they appeared “true” or “false” based on his feelings at the time of the evaluation. If the subject answered “true” at one of these propositions, the score “+1” was attributed to this item. If the answer was “false”, the score “-1” was allocated for this item. A mean score of sedation was then calculated.

This evaluation was performed on Day 1, 1 h and 4 h after drug intake and on Day 2, 12.5 h, and 15 h after drug intake just before and after the driving test.

### *Simulated car driving*

The driving simulation was performed on a FAROS driving simulator that reproduces a current 57.6-hp medium-sized car (Renault Clio) in several aspects (dashboard, five-speed gearbox, pedals, and steering wheel). The roadway display video projection system subtends a 120° visual field (3-D cinema screen version). The simulator produces engine and tire squeal sound effects that correlate with the speed of the vehicle but has no system that enables simulation of the movements of the car. The test involved driving for 60 min during the day along a motorway in monotonous condition (light traffic, occasional long, wide curves, and repetitive landscape) during which the driver had rarely to react. For the test scenario, subjects were instructed to abide by the highway code (i.e., to drive on the right lane and to respect the speed limit set at 130 km/h during the complete test session) and to drive in the same way as they usually drive in real situations. After completion of the driving test, the program calculated the number of collisions (against other vehicle or crash barriers) (primary parameter), the median and standard deviation of absolute speed (kilometers per hour), of deviation from the speed limit (i.e., from -50 to +50), and of deviation from the ideal route (meters, -10 right to +10 left).

The driving simulator test took place 2 h and 13 h (next morning) after drug intake).

### *Zolpidem/PR-M assays and pharmacokinetic assessments*

Venous blood samples (5 ml) were collected in sterile tubes containing EDTA-Na at the following time points: prior to drug administration, 1 h post-dosing, just before and at the end of the two driving tests (+2 h, +3 h, +13 h, and +14 h) as well as +4 h, +5 h (except when drug intake was at 21:00 h to avoid disturbing the subject after midnight) and +12.5 h post-dosing (next morning).

Plasma levels of zolpidem were measured by high-performance liquid chromatography (HPLC) coupled to UV detection. Plasma levels of PR-M were measured by a double-antibody radioimmunoassay (RIA) using a commercial kit (Bühlmann) based on the Kennaway G280 antmelatonin antibody (Vaughan, 1993).

Three pharmacokinetic parameters were determined for zolpidem and melatonin using standard

non compartmental methods (WinNonlin V4 software). Maximal plasma level (C<sub>max</sub>) and the time to reach C<sub>max</sub> (T<sub>max</sub>) were directly derived from the visual inspection of the time-plasma concentration curves. The area under the time-concentration curve from zero to the last measurable time-point (AUC<sub>0-14</sub>) was determined by the linear trapezoidal method.

### *Statistics*

For the primary variables, CDR tests and driving simulator, for each period, the predose (baseline) score was subtracted from each post-dosing score to create “a difference from baseline score” for each time point. Repeated measure ANOVAs were performed using SAS Proc Mixed for each task measure (SAS<sup>®</sup> system v6.12). All studied parameters were analyzed in order to determine the effect “treatment”, “period”, “time,” and “treatment × time interaction.” Contrasts between each active treatment and placebo were evaluated at each time point if the effect “treatment” or the “treatment × time interaction” was significant. When, significant, contrasts between PR-M and zolpidem alone or in combination were indicated in the text. Statistical significance for all analyses was  $p < 0.05$ , trends ( $p < 0.1$ ) were reported.

Pharmacokinetic comparisons between treatments (zolpidem versus PR-M + zolpidem or PR-M versus PR-M + zolpidem) were made by Student's *t* tests (level of significance:  $p < 0.05$ ).

## RESULTS

### *Cognitive tests*

A main “treatment” effect was found for all variables ( $p < 0.05$ ). “Treatment” significantly impacted with “time” on the digit vigilance test (percentage of correct detection), on the picture recognition test (sensitivity index and speed), and on the power of attention ( $p \leq 0.05$ ).

Comparisons between treatment effects in these tests indicated that PR-M treatment did not affect the cognitive performance compared to placebo at any time after administration. Several variables were impaired at the 1 and 4 h time points following zolpidem alone or in combination with PR-M compared to placebo and PR-M. Percentage of correct detection (accuracy) in the digit vigilance task at 1 h and 4 h after administration was significantly altered with zolpidem compared to placebo ( $p = 0.017$  and  $0.0003$  respectively) and to PR-M ( $p = 0.037$  and  $0.0008$  respectively) (Figure 1). The PR-M + zolpidem

combination decreased percentage of correct detection significantly at both at 1 h and 4 h compared to placebo ( $p=0.0001$  and  $0.01$  respectively) and to PR-M ( $p=0.0001$  and  $0.023$  respectively) and at 1 h post-dosing also compared to zolpidem ( $p=0.002$ ) (Figure 1). Speed of detection in the digit vigilance task was significantly slower 1 h after zolpidem ( $p=0.013$ ) and the PR-M + zolpidem combination ( $p=0.0001$ ) compared to placebo as well as compared to PR-M ( $p=0.032$  and  $0.0001$  respectively). The difference between the effect of zolpidem and PR-M remained significant at 4 h post-dosing ( $p=0.009$ ). Slower speeds were detected at 1 h post-dose with PR-M + zolpidem in the simple reaction time and the choice reaction time compared to placebo ( $p=0.004$  and  $0.0002$  respectively) as well as to PR-M ( $p=0.002$  and  $0.0002$  respectively). The effect of PR-M + zolpidem combination at 1 h post-dosing on choice reaction time was significantly higher than that with zolpidem ( $p=0.002$ ).

There were no significant effects of the drugs at 12.5 h and 15 h post-dose with the exception of a trend ( $p=0.0916$ ) for an improvement in the speed of detection in the digit vigilance task at 15 h post-dose with PR-M compared to placebo.

The power of attention factor score is a composite of the speed scores in simple reaction time, choice reaction time and digit vigilance. This score broadly reflected the results from the individual tasks, as would

be expected. There was no effect for PR-M compared to placebo ( $p>0.05$ ), but there were impairments with zolpidem 4 h after dosing ( $p=0.033$ ) compared to placebo. The PR-M + zolpidem combination impaired power of attention significantly at 1 h and 4 h post-dosing compared to placebo ( $p=0.0001$  and  $0.03$  respectively) and at 1 h compared to PR-M ( $p=0.0001$ ) and zolpidem ( $p=0.004$ ) (Figure 2).

The sensitivity index in picture recognition was not affected by PR-M or zolpidem alone compared to placebo ( $p>0.05$ ), whereas the PR-M + zolpidem combination induced a significant impairment at 1 h compared to placebo, to PR-M as well as to zolpidem ( $p=0.0001$  for all). PR-M did not affect the speed of responses in the picture recognition task whereas zolpidem significantly impaired the speed of responses in this task at 1 h and 4 h post-dosing compared to placebo ( $p=0.0001$  and  $0.023$  respectively) and to PR-M ( $p=0.0001$  and  $0.0005$ , respectively) (Figure 3). The PR-M + zolpidem combination also impaired picture recognition speed at 1 h and 4 h post-dosing compared to both placebo and PR-M and at 1 h also compared to zolpidem ( $p=0.0001$  for all) (Figure 3).

Performance in the tracking task [average distance off-target per second] was also adversely affected by the administration of zolpidem alone and in combination with PR-M, but not by PR-M alone. For zolpidem, there was a significant impairment at 4 h

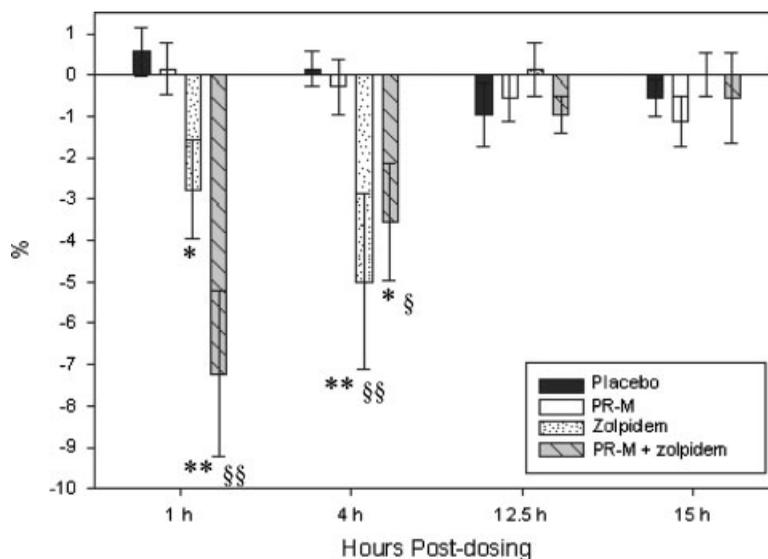


Figure 1. Percentage of correct detection in the digit vigilance task as a function of the treatment and the hours post-dosing (means  $\pm$  standard errors; \* $p < 0.05$ ; \*\* $p < 0.001$  compared to placebo; § $p < 0.05$ ; §§ $p < 0.001$  compared to PR-M)

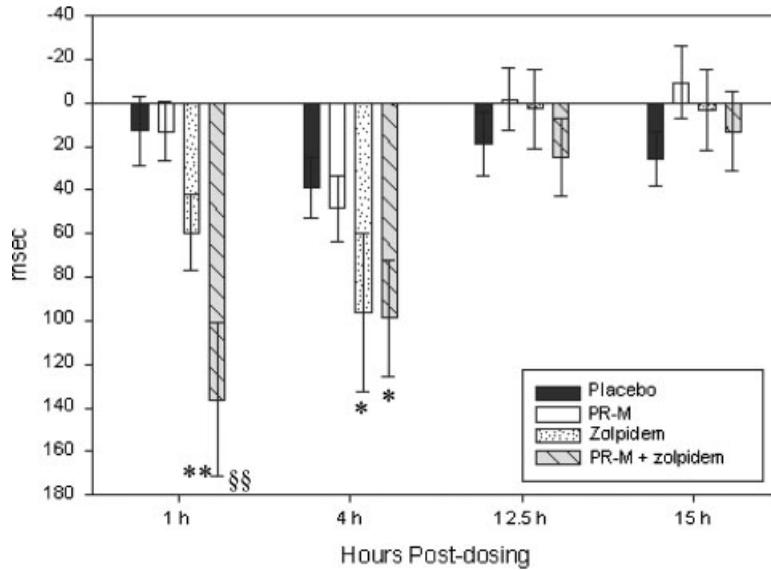


Figure 2. Power of attention factor score in the cognitive tests as a function of the treatment and the hours post-dosing (means  $\pm$  standard errors; \* $p < 0.05$  compared to placebo; §§ $p < 0.001$  compared to PR-M)

( $p = 0.046$ ) compared to placebo. PR-M + zolpidem produced significant impairments at 1 h compared to placebo ( $p = 0.0001$ ), to PR-M ( $p = 0.006$ ) and to zolpidem ( $p = 0.015$ ).

*Rivermead story*

There was a main “treatment” effect ( $p < 0.0001$ ) on memory recall and no “time” or “treatment  $\times$  time interaction” effects.

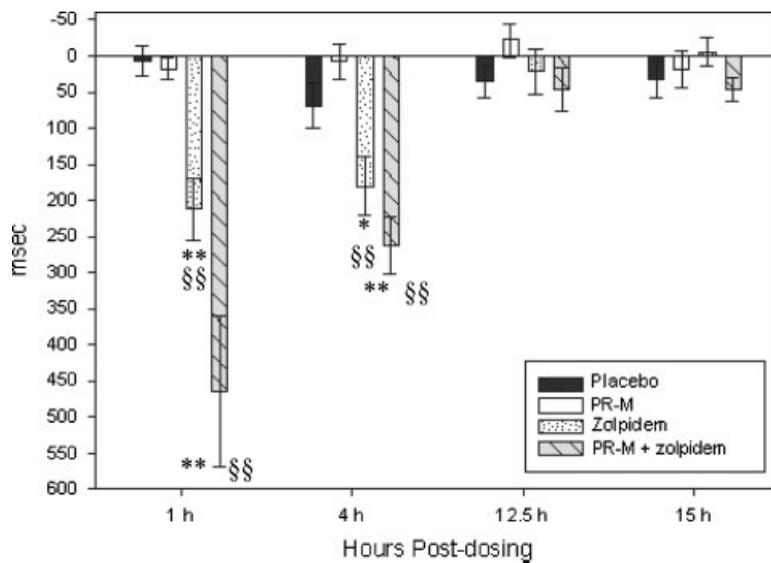


Figure 3. Speed of response in the picture recognition task as a function of the treatment and the hours-post-dosing (means  $\pm$  standard errors; \* $p < 0.05$ ; \*\* $p < 0.001$  compared to placebo; §§ $p < 0.001$  compared to PR-M)

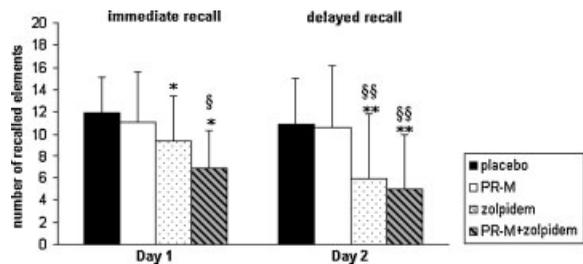


Figure 4. Number of recalled elements as a function of the treatment and the day (day 1 = +2 h, day 2 = +12.5 h post-dosing) (means  $\pm$  standard errors \* $p$  < 0.05, \*\* $p$  < 0.001 compared to placebo; § $p$  < 0.05; §§ $p$  < 0.01 compared to PR-M)

PR-M did not affect the immediate or delayed memory recall. A significant decrease in the number of recalled elements (assessing memory efficiency) was observed with zolpidem and PR-M + zolpidem for both type of recall compared to the placebo (immediate,  $p = 0.011$  and  $p = 0.004$ , respectively and delayed,  $p < 0.001$  for both). Compared to PR-M, zolpidem alone or in combination induced a significant alteration of delayed recall ( $p = 0.029$ ), which was even more pronounced after PR-M + zolpidem ( $p = 0.005$ ). In addition, PR-M + zolpidem induced a significant alteration of immediate recall compared to PR-M ( $p = 0.006$ ) (Figure 4).

### Grooved pegboard

For both conditions (ipsilateral and contralateral), the mean duration was affected by “treatment” ( $p < 0.0001$ ) and the “treatment  $\times$  time interaction” ( $p < 0.0001$ ). There was no effect of PR-M anytime post-dosing. A significant increase of this duration (slowing down of the task execution) was observed 1 and 4 h post-dosing with zolpidem (1 h,  $p < 0.0001$  for ipsi and contra; 4 h, ipsi:  $p = 0.03$  and contra:  $p = 0.0015$ ) and PR-M + zolpidem (1 h,  $p < 0.0001$  for ipsi and contra; 4 h, ipsi:  $p < 0.0001$  and contra:  $p = 0.0002$ ) compared to placebo. Compared to PR-M, the mean duration was significantly longer with zolpidem for both conditions at 1 h (ipsi:  $p = 0.0004$ ; contra:  $p < 0.0001$ ) and 4 h (ipsi:  $p = 0.01$ ; contra:  $p = 0.03$ ) as well as with PR-M + zolpidem (1 h,  $p < 0.0001$  for ipsi and contra; 4 h, ipsi:  $p = 0.0001$  and contra:  $p = 0.008$ ) (Figure 5). No difference was observed regarding the number of drops.

### Pentobarbital chlorpromazine alcohol group (PCAG)/sedation scale

The mean score of sedation was affected by “treatment” ( $p < 0.001$ ), “time” ( $p < 0.001$ ), and the “treatment  $\times$  time interaction” ( $p = 0.001$ ).

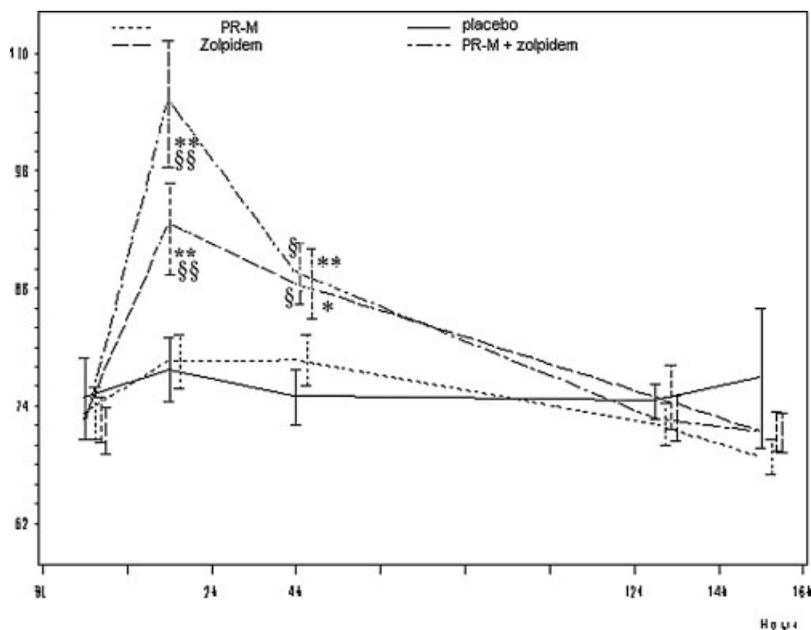


Figure 5. Test duration in the contralateral condition in the grooved pegboard test as a function of the treatment and the hours post-dosing (means  $\pm$  standard errors; \* $p$  < 0.05 compared to placebo; § $p$  < 0.05; §§ $p$  < 0.001 compared to PR-M)

Zolpidem and PR-M + zolpidem significantly increased sedation compared to placebo at 1 h ( $p = 0.0011$ ,  $p < 0.0001$ , respectively) and 4 h post-dosing ( $p < 0.0001$  for both) and compared to PR-M at 1 h ( $p = 0.03$ ,  $p < 0.0001$ , respectively) and 4 h ( $p = 0.015$  for both).

### Simulated car driving

Regarding the number of collisions (primary parameter for safety concern) and the standard deviation (SD) of all driving indexes, overall significant effects were detected for “treatment” ( $p \leq 0.04$ ), “time” ( $p \leq 0.0008$ ), and “treatment  $\times$  time interaction” ( $p < 0.03$ ). Planned contrasts indicated that these differences were due to zolpidem either alone or in combination with PR-M and to the earliest time of driving test (2 h).

The number of collisions was significantly increased with zolpidem and PR-M + zolpidem compared to placebo ( $p = 0.03$  and  $p < 0.0001$ , respectively) and to PR-M ( $p = 0.03$  and  $p < 0.0001$ , respectively) (Figure 6).

PR-M did not have any significant effects in this task. A significant increase of the SD of absolute speed was noticed with zolpidem and PR-M + zolpidem compared to placebo ( $p = 0.007$  and  $p < 0.0001$ , respectively) as well as compared to PR-M ( $p = 0.006$  and  $p < 0.0001$  respectively). A significant increase of the SD of the difference from the speed limit was also noticed with zolpidem and PR-M + zolpidem compared to placebo ( $p = 0.005$  and  $p = 0.0007$  respectively) as well as compared to PR-M ( $p = 0.01$  and  $p = 0.002$  respectively). For the deviation from the ideal route, a significant increase of the SD of this parameter was noticed with zolpidem and PR-M + zolpidem compared to placebo ( $p < 0.0001$  for both) and

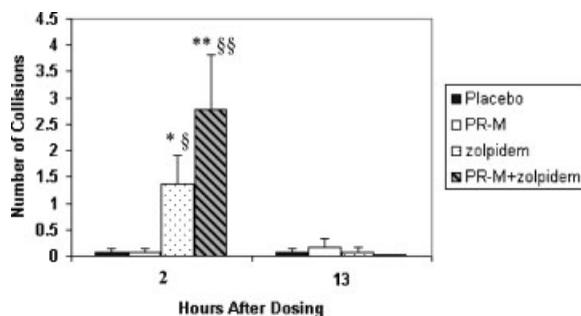


Figure 6. Number of collision in the simulated driving task as a function of the treatment and the hours post-dosing (means  $\pm$  standard errors; \* $p < 0.05$ ; \*\* $p < 0.001$  compared to placebo; § $p < 0.05$ ; §§ $p < 0.001$  compared to PR-M)

compared to PR-M ( $p = 0.0002$  and  $p < 0.0001$ , respectively).

The ANOVAs failed to reveal any significant “treatment” or “treatment  $\times$  time interaction” effects for the median of all driving indexes, excepted for deviation from the ideal route (treatment effect:  $p = 0.037$ ). The latter was related to an impairment induced by zolpidem compared to placebo ( $p = 0.004$ ).

### Safety

There were no safety concerns during the study. PR-M, zolpidem, and their combination did not affect cardiovascular variables (heart rate, blood pressure, and ECG parameters) and no clinically relevant physical or laboratory anomaly considered to be related to PR-M, zolpidem, or the combination of PR-M and zolpidem were observed.

### Pharmacokinetics

From the plasma concentration collected at T (0 h) to T + 14 h, C<sub>max</sub>, T<sub>max</sub>, and AUC<sub>(0-14)</sub> of melatonin did not differ significantly whether PR-M was administered alone or in combination with zolpidem treatments ( $p = 0.69$ ; 0.54 and 0.94, respectively). Similarly, pharmacokinetic parameters of zolpidem did not vary significantly after co-administration with PR-M ( $p = 0.24$ , 0.26, and 0.16, respectively). Noteworthy, large inter-individual variations were observed for all parameters and all treatment groups.

### DISCUSSION

The present study investigated the early and residual effects of a prolonged-release melatonin formulation (PR-M, 2 mg) on attention, memory and driving abilities of healthy old subjects, in comparison with a well established hypnotic drug, zolpidem (10 mg), given in the evening at their recommended clinical doses. Possible behavioral and pharmacokinetic interactions between both drugs were also assessed.

The main findings of this study indicated that PR-M alone had no effect on any cognitive measures, including memory recall and driving performances whereas zolpidem induced performance decrements on various cognitive tasks and driving simulator 1 h and 4 h post-dosing and on memory recall 2 h and 12.5 h post-dosing, which are even more pronounced when zolpidem was co-administered with PR-M. In agreement with the short half life of zolpidem and lack of deterioration in cognitive functioning in the morning following intake of this drug (Allain *et al.*,

2003; Darcourt *et al.*, 1999; Hindmarch *et al.*, 2006; Rush *et al.*, 1998; Uden and Roth-Schechter, 1996), no residual effects on next morning were observed except that the decline in memory recall after zolpidem persisted in the next day for the zolpidem and PR-M + zolpidem treated groups.

The results from the different cognitive tasks, including the validated computerized CDR assessment system, the Rivermead story and the grooved peg-board indicated that evening PR-M (2 mg) alone did not alter motor skills, attention and memory either before (1 h and 4 h post-dose) or after the night of sleep (12–15 h post-dose). These findings are complementary and in agreement with the results obtained in the study conducted by Paul *et al.* (Paul *et al.*, 2003), which reported that prolonged-released melatonin (Circadin<sup>®</sup> 6 mg) did not change performances on various cognitive tasks (serial reaction time, logical reasoning, serial subtraction multitask) for 7 h after its morning ingestion in healthy subjects (21–53 years). Comparison with other studies was more delicate due to the use of fast-release melatonin. However, Stone *et al.* (2000) found no effects of fast-release melatonin (0.1, 0.5, 1.0, and 5.0 mg) on performance (digit symbol substitution (DSST), letter memory recall, picture memory recall) 6.5–8.5 h after evening administration (18 h and 23 h) in healthy young subjects. Thus, exogenous melatonin administered in the evening did not affect cognitive performances of healthy subjects whatever their age range. On the contrary, daytime administration of fast-release melatonin was shown to impair neurobehavioral performances of young healthy volunteers from 1 h and up to 6 h post-dosing depending on the cognitive tasks (Atkinson *et al.*, 2005; Dollins *et al.*, 1993; Graw *et al.*, 2001; Rogers *et al.*, 1998). However, neurobehavioral impairments produced by daytime melatonin appeared less pronounced than those induced by a benzodiazepine (Rogers *et al.*, 2003). These results are consistent with the Suhner's ones (Suhner *et al.*, 1998), which showed no effect of melatonin (5 mg) on driving skill assessed by a driving computer test battery 90 min after a daytime administration. Altogether, these results suggest that PR-M administered at bedtime is devoid of deleterious effects on cognitive performances and driving abilities of elderly subjects even early after its administration. The difference in cognitive effects of melatonin reported may be explained by the melatonin formulation (prolonged versus fast released) and the circadian factor which could modulate the melatonin receptors sensitivity during daytime (Zhdanova, 2005).

Zolpidem caused several acute neurobehavioral performance impairments at the earliest post-dosing time points (1 h–4 h), which resolved by 12.5 h except the decline in memory recall after zolpidem that persisted in the next day. These effects were seen across measures of attention, memory, and motor coordination. They are consistent with previous studies, where 10 mg of zolpidem at daytime or night-time was found to produce significant impairments of psychomotor performances, attention, and memory from 1–5 h (Berlin *et al.*, 1993; Danjou *et al.*, 1999; Hindmarch *et al.*, 2001) but no residual effects at 8 h or more after administration (Berlin *et al.*, 1993; Fairweather *et al.*, 1992) although delayed memory might be impaired up to 8.25 h after a bedtime administration (Troy *et al.*, 2000). Driving abilities were also impaired 2 h after zolpidem intake, with standard deviations of both speed limit deviation, absolute speed deviation and ideal route deviation, and the number of collisions being significantly affected. These results are consistent with previous studies reporting zolpidem-induced driving impairments in real car driving up to 4 h post-administration (Verster *et al.*, 2002; Volkerts *et al.*, 2000). These effects were not present during the second driving session at 13 h post-dose. Several authors have described the absence of effect of 10-mg zolpidem on driving performance in healthy volunteers after 8 h or more (Berthelon *et al.*, 2003; Bocca *et al.*, 1999) in healthy subjects. Lack of residual effects of zolpidem (10 mg) on driving performances was also reported in insomniac patients when assessed in the morning following bedtime administration (Staner *et al.*, 2005; Vermeeren *et al.*, 1995). Even, a modified-release formulation of zolpidem administered in the evening showed no residual functional impairment in the morning in psychometric and cognitive tests in elderly subjects (Hindmarch *et al.*, 2006).

Furthermore and this is a new observation, we observed a pharmacodynamic interaction (potentiation of the central effects) between PR-M and zolpidem at 1 h following co-dosing which was partly attenuated by 4 h, this interaction not being after 12.5 h. Melatonin concentrations after administration of PR-M and PR-M + zolpidem were comparable with a peak at 1–2 h. The same scheme was observed for zolpidem concentration after administration of PR-M and PR-M + zolpidem. Thus, a pharmacokinetic interaction can be discarded. One may suggest that the melatonin-zolpidem potentiation results from the different and synergistic mode of action of these compounds. Indeed, it is known that the sleep inducing effect of melatonin results from inhibition of SCN

circadian related wake-promoting mechanism (reviewed in Dubocovich, 2007). For instance, the SCN has been shown to have an inhibitory influence on the ventro-lateral preoptic nuclei (VLPO) which is one of the main sleep-promoting neuronal group (reviewed in Mistleberger, 2005). One may thus speculate that the overall GABA-related sleep inducing effects of zolpidem are reinforced by the indirect effect of melatonin on the VLPO (i.e., through SCN inhibition). Could melatonin interact with GABA(A) receptor and increase the zolpidem effect? Wang *et al.* (2003) cited a certain number of studies which taken together, indicate that melatonin affects the function of the GABA(A) receptor, and some of the neuropharmacological actions of melatonin (including hypnotic activity) may be mediated through the GABA(A) receptor.

The observed impairments in cognitive and driving performance essentially matched the sedation caused by zolpidem and the PR-M + zolpidem treatments as shown by the scores at the PCAG scale. Impairment of immediate memory recall induced by zolpidem and PR-M + zolpidem is likely due to deleterious effects of zolpidem on encoding processes. Worsening of memory recall on the next day may represent inhibitory effect of zolpidem on memory consolidation processes, which occur during night's sleep (Born *et al.*, 2006; Gais and Born, 2004; Rauchs *et al.*, 2005; Stickgold and Walker, 2007). This explanation warrants further research.

Several possible limitations to this study must be discussed. First, the hypnotic drugs were given at 20:00 h while in clinical practice such drugs are more likely taken at bedtime (i.e., around 22:00–23:00 h). Therefore while no significant impairments in psychomotor and driving performance were observed with zolpidem at 20:00 h (12 h after intake), in clinical practice, if taken at a later hour, zolpidem might still have some residual effects in the morning. Since exogenous melatonin was shown to modify the circadian phase in a time-dependent manner, it cannot completely ruled out that the next-morning measurements may also be affected by the exact time of administration. However, as PR-M did not affect cognitive performance and driving at any time after administration, the exact time of intake may not have practical implications in this respect. Second, the driving performances were obtained with a driving simulator. Although the latter offers several advantages (elimination of external variables, controlled environment identical for all subjects), it is necessary to test on the road driving after PR-M intake at bedtime to confirm the present findings.

Third, one could argue that the tests used in the present study are not enough sensitive to observe deleterious cognitive effects with PR-M. This is quite unlikely for several reasons: firstly, all the tests used in the present studies are well validated and found suitable for such studies. Thus, the automatic CDR battery tests as well as specific neurobehavioral tasks which are part of the CDR battery (digit vigilance, tracking, and picture recognition) were demonstrated to be sensitive to drug-induced cognitive functions (both cognitive impairment and improvement) (Ebert *et al.*, 1998; Kennedy *et al.*, 2000; O'Neil *et al.*, 2000) including sedative compounds (e.g., alcohol, benzodiazepines, and zolpidem) (Berlin *et al.*, 1993; Hindmarch *et al.*, 2005; O'Neil *et al.*, 2000; Rush and Griffiths, 1996; Rush and Baker, 2001; Wesnes *et al.*, 2000). Using the same driving simulator device, our group has already demonstrated its sensitivity to several hypnotic drugs on the next morning (Staner *et al.*, 2005). Secondly, in the present study, five tests with a total of 20 variables (CDR, driving simulator, grooved pegboard, Rivermead story, and subjective sedative scale) were used to explore different aspects of cognition; all but four variables (CDR: accuracy of choice reaction time; driving simulator: 3 medians) were impaired by zolpidem alone or in combination with PR-M within the 4 h post-administration. Noteworthy, the standard deviation variables of all driving parameters appeared more sensitive to zolpidem than medians. Thus, the performance decrements observed with zolpidem in the present study confirm that the lack of effects with PR-M cannot be attributed to a lack of sensitivity of the measurements. Finally, we have to stress the fact that contrast comparisons between treatments were performed only if the ANOVAs revealed a main "treatment" or "treatment  $\times$  time" effect. Since the treatment effect was calculated on all time points, it could be that small effects of zolpidem at 1 h and 4 h post-dose could have been missed by the ANOVA due to the lack of effects at late time points. Hence, one may argue that we used a quite conservative approach of analysing drug effects warranting that positive zolpidem versus placebo contrasts are true differences.

Thus, the first conclusion from these data is that PR-M (Circadin<sup>®</sup>) has no effect on psychomotor functions, memory recall, and driving skills.

The second one is that zolpidem causes an acute impairment in psychomotor functions and driving skills, and in memory recall that persists in the next day for the latter.

The third conclusion is that clinicians should be aware that co-dosing PR-M with zolpidem can cause acute sedative effects, greater than those expected with

zolpidem alone, and which will have a significant impact on the patient's cognitive abilities. However, these effects are short-lived and when considered in use with an insomniac population, the evidence here strongly supports the conclusion that there will be no hangover effects on cognitive function next morning that will impair the ability to conduct the activities of daily living. No significant safety issues were observed. Further studies on the influence of the co-administration of PR-M and zolpidem, or other hypnotics on sleep (architecture, quality) and daytime vigilance of elderly subjects and insomniac patients and particularly the effects on memory consolidation are thus warranted.

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