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CHRONOTHERAPY WITH VALSARTAN/AMLODIPINE FIXED COMBINATION: IMPROVED BLOOD PRESSURE CONTROL OF ESSENTIAL HYPERTENSION WITH BEDTIME DOSING

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Administration of valsartan at bedtime as opposed to upon wakening improves the sleep-time relative blood pressure (BP) decline towards a more normal dipper pattern without loss of 24-h efficacy. Amlodipine, however, has been shown to be effective in reducing BP throughout the day and night, independent of dosing time. A large proportion of hypertensive subjects cannot be properly controlled with a single medication. However, no study has yet investigated the potential differing effects of combination therapy depending of the time-of-day of administration. Accordingly, the authors investigated the administration-time-dependent BP-lowering efficacy of valsartan/amlodipine combination. The authors studied 203 hypertensive subjects (92 men/111 women), 56.7 ± 12.5 yrs of age, randomized to receive valsartan (160 mg/day) and amlodipine (5 mg/day) in one of the following four therapeutic schemes: both medications on awakening, both at bedtime, either one administered on awakening and the other at bedtime. BP was measured by ambulatory monitoring for 48 consecutive hours before and after 12 wks of treatment. Physical activity was simultaneously monitored every min by wrist actigraphy to accurately determine the beginning and end of daytime activity and nocturnal sleep. BP-lowering efficacy (quantified in terms of reduction of the 48-h mean of systolic/diastolic BP) was highest when both hypertension medications were ingested at bedtime, as compared to any one of the three other tested therapeutic schemes (17.4/13.4 mm Hg reduction with both medications on awakening; 15.1/9.6 mm Hg with valsartan on awakening and amlodipine at bedtime; 18.2/12.3 mm Hg with valsartan at bedtime and amlodipine on awakening; 24.7/13.5 mm Hg with both medications at bedtime; $p < .018$ between groups). The sleep-time relative BP decline was significantly increased towards a more normal dipper pattern *only* when both medications were jointly ingested at bedtime ($p < .001$). Bedtime dosing of the combination of the two medications also resulted in the largest percentage of controlled subjects among all the assessed therapeutic schemes ($p = .003$ between groups). In

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subjects requiring combination therapy to achieve proper BP control, the association of amlodipine and valsartan efficiently reduces BP for the entire 24 h independent of dosing time. However, the greater proportion of controlled patients, improved efficacy on lowering asleep BP mean, and increased sleep-time relative BP decline suggest valsartan/amlodipine combination therapy should be preferably administered at bedtime. (Author correspondence: rhermida@uvigo.es).

Keywords Amlodipine; Chronotherapy; Combination therapy; Essential hypertension; Valsartan

INTRODUCTION

Elevated blood pressure (BP) increases the risk for stroke, myocardial infarction, heart failure, and renal disease (Chobanian et al., 2003; Mancia et al., 2007). Pharmacologic treatment of hypertension reduces the incidence of these events and prolongs life (Blood Pressure Lowering Treatment Trialists' Collaboration, 2008). International guidelines recommend a target conventional clinic BP goal of <140/90 mm Hg for uncomplicated essential hypertension, and even lower one (<130/80 mm Hg) for high-risk patients, e.g., history of stroke, myocardial infarction, diabetes, or renal disease (Chobanian et al., 2003; Mancia et al., 2007). To obtain these target goals, most patients require treatment with more than a single BP-lowering medication (Dahlöf, 2009; Milani, 2005). Among them, both angiotensin-receptor blockers (ARBs) and calcium channel blockers (CCBs) are well established as hypertension monotherapies. The ARB/CCB combinations offer advantages, because they exert complementary effects and provide safe, effective, and well-tolerated BP control (Chobanian et al., 2003; Mancia et al., 2007). Thus, combination treatment appears to offer a number of advantages over high-dose therapy with single medications, including better BP control and improved patient tolerability and adherence than single-medication components (Giles, 2003; Liebson, 2006). For instance, the BP-lowering efficacy of the combination of the ARB valsartan and CCB amlodipine is greater than either monotherapy, alone, and the occurrence of peripheral edema is lower with the combination than amlodipine, alone (Allemann et al., 2008; Brachmann et al., 2008; Ferri et al., 2008; Flack et al., 2009; Frampton & Scott, 2009; Philipp et al., 2007; Schunkert et al., 2009; Sinkiewicz et al., 2009; Smith et al., 2007).

All of the previously studies conducted on the valsartan/amlodipine combination have relied on conventional clinic BP, without proper evaluation of possible changes after therapy on the circadian BP pattern determinable by ambulatory BP monitoring (ABPM). Most important, time of administration of the combination, presumably morning (de la Sierra et al., 2009; Hermida et al., 2002), has not been specifically stated in

previous combination trials. However, a number of prospective studies have consistently documented clinically meaningful morning-evening, treatment-time differences in BP-lowering efficacy, duration of action, safety profile, and/or effects on the circadian BP pattern of different classes of hypertension medications (Hermida et al., 2007a). We previously documented a significant change in the dose-response curve, increased proportion of controlled patients, and improved efficacy on lowering asleep BP when valsartan was ingested at bedtime, as compared to upon awakening, in subjects with essential hypertension (Hermida et al., 2003, 2005a, 2005b). Similar time-dependent effects on ambulatory BP, namely a more efficient nighttime BP control without any loss in efficacy during the diurnal span of wakefulness and a corresponding significant decrease in the prevalence of nondipping with bedtime as compared to morning dosing, were also documented for telmisartan (Hermida et al., 2007b) and olmesartan (Hermida et al., 2009a), ARBs with different terminal plasma half-life but all providing BP-lowering efficacy beyond 24 h after the last dose, as shown by clinical ABPM studies. On the other hand, several studies have also investigated the effects of morning versus evening administration of amlodipine (Mengden et al., 1993; Nold et al., 1998; Qiu et al., 2003). Similar to other dihydropyridine CCBs (Hermida et al., 2007a), amlodipine seems to reduce BP homogenously during the day and night, independent of dosing time.

So far, no study has investigated the potential differing effects of combination therapy as a function of the time of administration. Accordingly, this prospective trial was designed to compare, using BP data collected by 48-h ABPM, the BP-lowering efficacy in essential hypertension of combination valsartan/amlodipine therapy when ingested either upon awakening or at bedtime.

METHODS

Inclusion and Exclusion Criteria

Inclusion criteria were age ≥18 yrs, a diurnal activity and nocturnal sleep routine, and a diagnosis of previously untreated uncomplicated essential hypertension according to the guidelines of the European Society of Hypertension–European and Society of Cardiology (Mancia et al., 2007), as determined by repeated (within the 3 months previous to recruitment) conventional clinic BP measurements (systolic BP [SBP] ≥140 mm Hg and/or diastolic BP [DBP] ≥90 mm Hg), and corroborated by 48-h ABPM at the time of recruitment. The diagnosis of hypertension based on 48-h ABPM required an awake BP mean of ≥135/85 mm Hg or an asleep BP mean of ≥120/70 mm Hg (Mancia et al., 2007; Pickering et al., 2005).

Exclusion criteria were pregnancy, history of drug or alcohol abuse, employment in shift- or night work, diagnosis of acquired immunodeficiency syndrome (AIDS), type 1 diabetes, evidence of secondary hypertension, cardiovascular disorders (including concomitant unstable angina pectoris, heart failure, life-threatening arrhythmia, nephropathy, grade III–IV retinopathy or prior [within the last year] myocardial infarction, or coronary revascularization), intolerance to ABPM measurement, and inability to communicate and comply with all study requirements.

Study Design

This was a prospective, randomized, open-label, parallel-group, blinded-endpoint (PROBE) study. We identified 207 subjects who met the inclusion/exclusion criteria. Among these, 203 (92 men/111 women), 56.7 ± 12.5 (mean ± SD) yrs of age, completed the study and provided all required information for this trial. Subjects were participants in a larger prospective study (www.clinicaltrials.gov, code NCT00295542) (Hermida, 2007) approved by the Ethics Committee of Clinical Research. The study respected the criteria set forth for ethical medical research as outlined in the Helsinki Declaration and the journal (Portaluppi et al., 2008). All subjects gave written informed consent.

Subjects were assigned to receive valsartan (160 mg/day) and amlodipine (5 mg/day) in one of the following therapeutic schemes: both medications upon awakening, both medications at bedtime, either one of the medications upon awakening and the other at bedtime. The 160 mg valsartan dose was the highest and most frequently used in Spain at the time of the study. Moreover, the combination of this dose of valsartan with 5 mg amlodipine is currently available as a single formulation, i.e., fixed dose combination therapy. At each of the two visits to the medical setting, before and after 12 wks of timed therapy, respectively, six clinic BP measurements were obtained, after the subject had rested in a seated position for at least 10 min, using a validated automatic oscillometric device (HEM-705IT; Omron Health Care Inc., Vernon Hills, Illinois, USA). Blood samples were also obtained at each clinic visit from the antecubital vein, between 08:00 and 09:00 h, after nocturnal fasting and were analyzed using routine automatic techniques in the hospital laboratory.

ABPM Assessment

The SBP and DBP of each participant were automatically measured every 20 min from 07:00 to 23:00 h and every 30 min during the night for 48 consecutive hours, before and after timed therapy, with a calibrated SpaceLabs 90207 device (SpaceLabs Inc., Issaquah, Washington, USA). Participants were instructed to go about their usual activities with

minimal restrictions but to follow a similar schedule during the 2 days of ABPM and to avoid daytime napping. During monitoring, subjects maintained a diary listing the times of retiring to bed at night and awakening in the morning. BP series were not considered valid for analysis if >30% of the measurements were missing, if data were lacking for an interval of >2 h, if data were obtained while subjects had an irregular rest-activity schedule during the 2 days of monitoring, or if the nighttime sleep period was <6 h or >12 h during ABPM. BP monitoring was repeated within the same week in case of invalid recording (three subjects). Protocol-correct ABPM series were collected at baseline and after treatment from 203 subjects and therefore included in the study. Baseline BP profiles of four additional subjects were eliminated from this efficacy trial due to lack of follow-up: the subjects from whom they were derived failed to return for the second ABPM after treatment. No one withdrew from the trial due to adverse effects.

Actigraphy

All subjects wore an actigraph (Mini-Motion-Logger; Ambulatory Monitoring Inc., Ardsley, New York, USA) on the dominant wrist to monitor physical activity every min during both 48-h ABPM sessions. This compact (about half the size of a wristwatch) device works as an accelerometer. We synchronized the internal clocks of the actigraph and the ABPM device through their respective interfaces using the same computer. The actigraphy data, combined with patient diaries, were used to corroborate absence of daytime napping and to accurately define the beginning and end of the diurnal awake and nocturnal asleep spans so the respective BP means for each subject could be precisely determined.

Statistical Methods

Each individual's clock hour BP and heart rate (HR) values were first referenced to hours after awakening from nocturnal sleep, based on data obtained by wrist actigraphy. This transformation avoided the introduction of bias due to slight differences among subjects in their sleep/activity routine. To correct for measurement errors and outliers, BP was edited according to conventional criteria (Staessen et al., 1991). Thus, readings of SBP >250 or <70 mm Hg, DBP >150 or <40 mm Hg, and pulse pressure (PP, difference between SBP and DBP) >150 or <20 mm Hg were automatically discarded. The "48-h BP mean" was calculated as the average of all valid readings obtained during the 48-h sampling. The sleep-time relative BP decline (an index of BP dipping), defined as the percent decrease in mean BP during the hours of nocturnal sleep relative to the mean BP during the hours of diurnal activity, was calculated as:

[(awake BP mean − asleep BP mean)/awake BP mean] × 100, using all the data sampled by ABPM for 48 consecutive hours. The changes from baseline in the awake, asleep, and 48-h BP means as well as in the sleep-time relative BP decline were compared among groups by analysis of variance (ANOVA). The demographic and clinical characteristics were compared between groups by ANOVA (quantitative variables) or nonparametric χ^2 test. Within-group comparisons of clinical and ABPM characteristics before and after treatment were performed using a paired *t* test.

RESULTS

Demographic Characteristics and Clinic BP

The demographic characteristics of the four treatment-time groups were comparable at baseline, and they remained unchanged after treatment. Clinic BP measurements, including PP, were significantly reduced after treatment ($p < .001$) and to a comparable extent in all four groups. The serum values of glucose, creatinine, cholesterol, triglycerides, uric acid, and fibrinogen were comparable at baseline between the four treatment-time groups, and they were not significantly changed after treatment (Table 1).

Changes in Ambulatory BP

There was lack of statistically significant differences in ambulatory BP at baseline between the four treatment-time groups (Table 2). After 12 wks of timed treatment, the BP reduction from baseline was largest when both medications were administered at bedtime (17.4/13.4 mm Hg reduction in the 48-h mean of SBP/DBP with both medications upon awakening; 15.1/9.6 mm Hg reduction with valsartan upon awakening and amlodipine at bedtime; 18.2/12.3 mm Hg with valsartan at bedtime and amlodipine upon awakening; 24.7/13.5 mm Hg with both medications at bedtime; $p < .001/.018$ for SBP/DBP between groups; Figures 1 and 2). Moreover, asleep BP regulation was significantly better achieved in the group of subjects who jointly ingested valsartan and amlodipine at bedtime as compared to the groups of subjects who ingested the two medications according to the three other schedules ($p < .001$; Table 2). With combination-treatment at bedtime, 69.2% of the subjects achieved controlled ABPM values after intervention, i.e., values below all the diagnostic thresholds for hypertension mentioned above (Mancia et al., 2007). The percentage of controlled subjects was significantly lower in the other three therapeutic schemes (58.3% with both medications upon awakening; 43.6% with valsartan upon awakening and amlodipine at bedtime; and 54.2% with valsartan at bedtime and amlodipine upon awakening;

TABLE 1 Demographic and analytical characteristics of subjects investigated

Variable	Valsartan + amlodipine upon awakening	Valsartan upon awakening amlodipine at bedtime	Valsartan at bedtime amlodipine upon awakening	Valsartan + amlodipine at bedtime
Subjects, n	50	53	48	52
Sex, % men	46.0	46.2	36.7	51.9
Age, yrs	54.0 ± 12.3	54.0 ± 12.4	59.2 ± 14.5	59.7 ± 9.3
Height, cm	161.8 ± 8.3	162.1 ± 7.6	158.2 ± 7.5	161.6 ± 9.7
Before treatment				
Weight, kg	73.4 ± 11.8	72.1 ± 11.1	70.4 ± 14.7	72.1 ± 12.5
BMI, kg/m^2	28.2 ± 3.6	27.4 ± 3.9	28.2 ± 4.8	27.6 ± 3.6
Waist, cm	92.8 ± 11.0	91.7 ± 9.0	90.2 ± 13.2	92.5 ± 11.7
Hip, cm	103.9 ± 6.3	102.2 ± 6.3	103.9 ± 10.0	102.1 ± 6.5
SBP, mm Hg*	162.9 ± 18.7	163.5 ± 20.0	161.1 ± 21.6	161.0 ± 12.3
DBP, mm Hg*	95.4 ± 10.3	91.2 ± 11.9	91.6 ± 11.9	92.3 ± 10.5
PP, mm Hg*	67.5 ± 14.2	72.3 ± 17.0	69.5 ± 16.1	68.7 ± 8.9
HR, beats/min*	81.6 ± 11.8	77.2 ± 13.6	75.4 ± 12.8	67.9 ± 7.7
Glucose, mg/dL	108.6 ± 18.1	95.5 ± 13.2	103.0 ± 21.4	107.7 ± 21.8
Creatinine, mg/dL	1.05 ± 0.33	0.94 ± 0.18	0.93 ± 0.10	0.99 ± 0.16
Cholesterol, mg/dL	230.8 ± 37.0	216.5 ± 42.1	223.6 ± 32.3	213.7 ± 23.8
Triglycerides, mg/dL	126.1 ± 54.3	103.8 ± 51.8	111.9 ± 43.2	117.2 ± 30.5
Uric acid, mg/dL	5.6 ± 1.5	5.6 ± 1.3	5.5 ± 1.7	5.5 ± 1.3
Fibrinogen, mg/dL	310.5 ± 75.8	306.6 ± 77.9	303.2 ± 69.7	297.8 ± 60.1
After treatment				
Weight, kg	74.0 ± 11.1	73.4 ± 11.7	70.6 ± 14.6	72.6 ± 12.3
BMI, kg/m^2	28.5 ± 3.3	28.0 ± 4.1	28.3 ± 4.9	27.8 ± 3.6
Waist, cm	93.3 ± 11.8	94.0 ± 9.8	92.0 ± 12.7	92.6 ± 10.4
Hip, cm	105.0 ± 5.8	103.3 ± 6.4	104.9 ± 10.3	102.3 ± 5.1
SBP, mm Hg*	137.9 ± 17.3	144.8 ± 22.8	136.6 ± 14.9	139.0 ± 15.0
DBP, mm Hg*	79.1 ± 9.3	79.2 ± 10.9	78.8 ± 7.6	79.2 ± 10.3
PP, mm Hg*	58.8 ± 14.3	65.6 ± 17.3	57.8 ± 11.0	59.8 ± 9.1
HR, beats/min*	77.2 ± 9.8	76.8 ± 13.9	73.2 ± 8.6	69.5 ± 8.8
Glucose, mg/dL	108.7 ± 22.5	105.7 ± 17.6	106.8 ± 34.1	111.9 ± 22.7
Creatinine, mg/dL	0.96 ± 0.28	0.95 ± 0.17	0.91 ± 0.18	1.05 ± 0.22
Cholesterol, mg/dL	234.9 ± 33.4	209.4 ± 35.5	212.4 ± 37.1	203.1 ± 27.3
Triglycerides, mg/dL	120.8 ± 54.0	96.8 ± 41.4	114.3 ± 45.5	106.5 ± 29.4
Uric acid, mg/dL	5.7 ± 1.7	5.5 ± 1.2	5.7 ± 1.4	5.5 ± 1.5
Fibrinogen, mg/dL	319.0 ± 73.7	325.2 ± 71.7	329.5 ± 69.1	279.7 ± 50.2

Note. All values are shown as mean ± SD.

*Values correspond to the average of six conventional BP measurements obtained for each subject at the clinic before starting 48-h ABPM.

$p = .003$ between treatment-time groups). Combination treatment with valsartan/amlodipine did not exert any significant effect on HR at any time of treatment.

Comparison of Figures 1 and 2 indicates that the differences in efficacy between the treatment-time groups were significantly larger for SBP than DBP. Accordingly, there was a highly significant administration-time-dependent effect of combination therapy on ambulatory PP

TABLE 2 Blood pressure characteristics of subjects investigated

Variable	Valsartan + amlodipine upon awakening	Valsartan upon awakening amlodipine at bedtime	Valsartan at bedtime amlodipine upon awakening	Valsartan + amlodipine at bedtime
Subjects, n	50	53	48	52
Before treatment				
Awake SBP mean, mm Hg	144.6 ± 15.0	143.2 ± 15.0	142.6 ± 14.7	143.7 ± 12.2
Asleep SBP mean, mm Hg	131.1 ± 12.6	131.1 ± 17.8	131.6 ± 14.1	131.1 ± 12.7
48-h SBP mean, mm Hg	140.3 ± 13.5	139.5 ± 14.9	138.0 ± 13.6	138.4 ± 9.2
Sleep-time relative SBP decline, %	9.3 ± 7.0	8.4 ± 8.0	7.7 ± 6.3	8.8 ± 10.2
Awake DBP mean, mm Hg	90.2 ± 10.6	86.5 ± 11.5	85.9 ± 12.1	85.4 ± 12.5
Asleep DBP mean, mm Hg	77.3 ± 8.0	75.8 ± 11.1	75.9 ± 9.3	76.0 ± 7.6
48-h DBP mean, mm Hg	86.5 ± 9.3	83.2 ± 10.8	82.9 ± 11.0	80.6 ± 10.3
Sleep-time relative DBP decline, %	13.9 ± 8.7	12.0 ± 9.0	11.2 ± 6.6	11.0 ± 9.2
After treatment				
Awake SBP mean, mm Hg	126.3 ± 14.5	127.9 ± 14.0	124.6 ± 14.1	121.1 ± 12.1
Asleep SBP mean, mm Hg	116.7 ± 11.9	116.5 ± 14.3	113.1 ± 12.9	103.0 ± 10.7
48-h SBP mean, mm Hg	123.0 ± 13.2	124.4 ± 13.7	119.8 ± 13.0	113.6 ± 11.0
Sleep-time relative SBP decline, %	8.1 ± 4.8	8.9 ± 4.9	9.0 ± 6.5	14.3 ± 7.9
Awake DBP mean, mm Hg	75.7 ± 7.6	76.7 ± 9.6	73.1 ± 7.9	72.7 ± 9.4
Asleep DBP mean, mm Hg	67.1 ± 6.9	66.6 ± 8.4	64.8 ± 5.9	61.3 ± 6.9
48-h DBP mean, mm Hg	73.1 ± 6.7	73.6 ± 9.0	70.6 ± 7.2	67.1 ± 8.3
Sleep-time relative DBP decline, %	11.7 ± 7.7	13.0 ± 5.9	11.0 ± 6.9	16.2 ± 8.2

Note. All values are shown as mean ± SD.

The sleep-time relative BP decline, an index of BP dipping, is defined as the percent decline in mean BP during the hours of nocturnal sleep relative to the mean BP during the hours of diurnal activity, and calculated as: [(awake BP mean − asleep BP mean)/awake BP mean] × 100.

(Figure 3). The effect on PP by valsartan/amlodipine combination when ingested at bedtime was almost double than it was for each one of the other therapeutic schemes tested (Figure 3).

The differences between the treatment-time groups were larger on the asleep than awake BP mean (Figures 1 and 2). These differing treatment-time effects on asleep BP are reflected in differences in the sleep-time relative BP decline between groups (Figure 4). There was a significant ($p < .001$) increase of this relative decline towards a more normal dipping pattern *only* when valsartan and amlodipine were jointly ingested at bedtime. However, no subject increased his sleep-time BP decline to an extreme-dipper pattern (sleep-time relative BP decline ≥20%) after treatment with the combination at bedtime. The sleep-time relative BP decline was not significantly modified in any of the other three groups by the valsartan/amlodipine combination therapy ($p < .001$ between treatment-time groups; Figure 4).

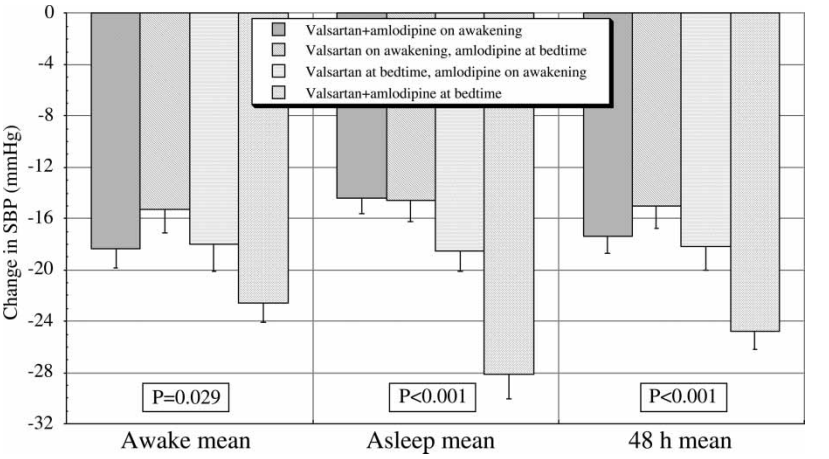

FIGURE 1 Changes from baseline (in mm Hg) in awake (active hours), asleep (nocturnal resting hours), and 48-h mean of SBP after 12 wks of daily ingestion of valsartan/amlodipine combination therapy at different circadian times in subjects with essential hypertension studied by 48-h ABPM before and after treatment. Probability values are shown for comparison of the effects between the four groups of subjects by ANOVA.

The effects of combination therapy on BP for the two groups of subjects ingesting the two tested medications jointly at the same circadian time, i.e., either both medications upon awakening or both medications at bedtime, are illustrated in Figure 5. This figure shows the efficacy of BP-lowering in terms of the duration in hours from the last timed dose of the valsartan/amlodipine therapy. As shown in Figure 5, SBP reduction was

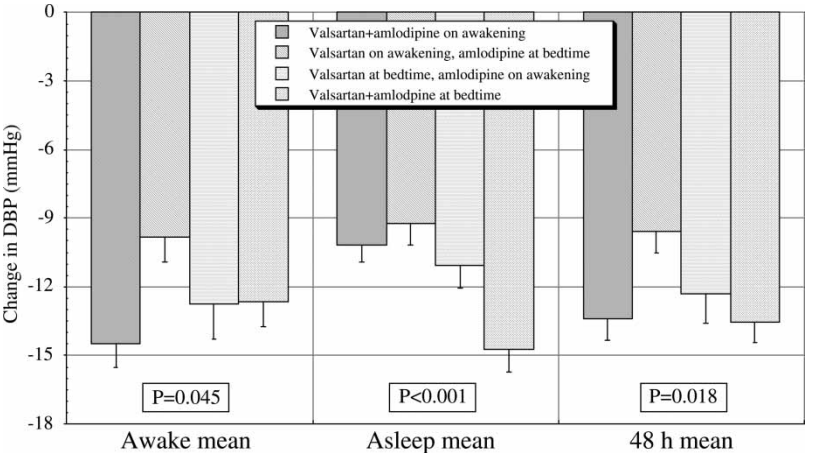

FIGURE 2 Changes from baseline (in mm Hg) in awake (active hours), asleep (nocturnal resting hours), and 48-h mean of DBP after 12 wks of daily ingestion of valsartan/amlodipine combination therapy at different circadian times in subjects with essential hypertension studied by 48-h ABPM before and after treatment. Probability values are shown for comparison of the effects between the four groups of subjects by ANOVA.

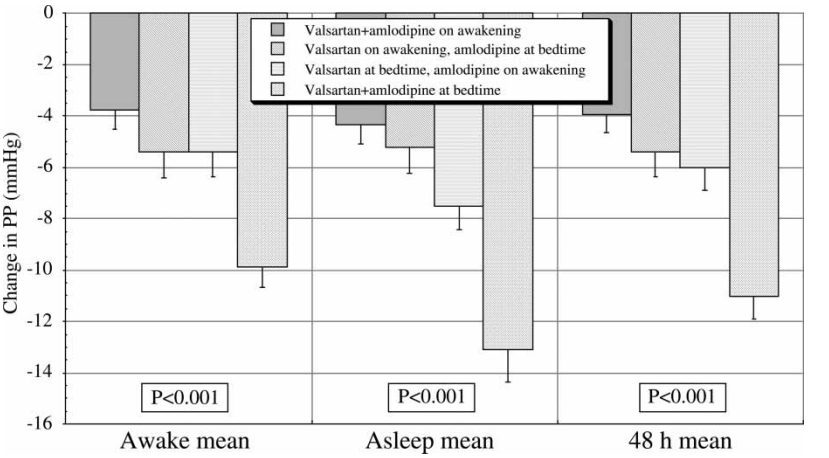

FIGURE 3 Changes from baseline (in mm Hg) in awake (active hours), asleep (nocturnal resting hours), and 48-h mean of PP after 12 wks of daily ingestion of valsartan/amlodipine combination therapy at different circadian times in subjects with essential hypertension studied by 48-h ABPM before and after treatment. Probability values are shown for comparison of the effects between the four groups of subjects by ANOVA.

significantly greater throughout the 24 h following the last dose when the combination was ingested at bedtime than upon awakening. For DBP, the BP-lowering effect was more homogeneous when the two medications were ingested at bedtime. However, administration of the valsartan/amlodipine upon awakening resulted in a significantly greater DBP reduction during the first than during the last 12 h of the dosing interval (Figure 5).

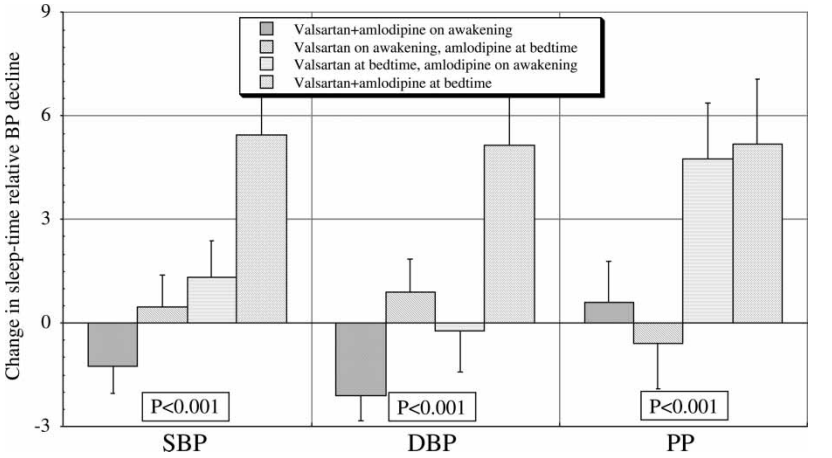

FIGURE 4 Changes from baseline in sleep-time relative decline of SBP, DBP, and PP after 12 wks of daily ingestion of valsartan/amlodipine combination therapy at different circadian times in subjects with essential hypertension studied by 48-h ABPM before and after treatment. Probability values are shown for comparison of the effects between the four groups of subjects by ANOVA.

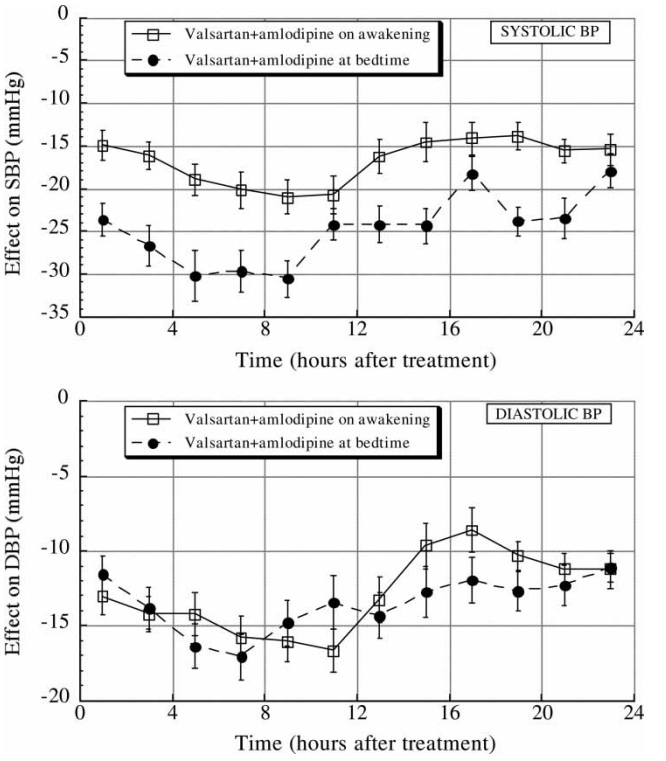

FIGURE 5 Changes (in mm Hg) from baseline along the 24 h after treatment in SBP (top) and DBP (bottom) after 12 wks of daily ingestion of valsartan/amlodipine combination therapy either upon awakening or at bedtime in subjects with essential hypertension studied by 48-h ABPM before and after treatment.

DISCUSSION

This prospective, randomized study is the first to document administration-time-dependent effects of combination therapy on ambulatory BP regulation in essential hypertension subjects. The results of this trial first corroborate the significant BP-lowering effect of valsartan/amlodipine combination therapy, independent of treatment-time, with over half the studied sample showing properly controlled BP according to current thresholds established for the diagnosis of hypertension based on ABPM (Mancia et al., 2007). The BP-lowering efficacy was stronger with the combination than the reported efficacy of each of the medications, alone, as also documented in several previous clinical trials relying on clinic BP measurements (Allemann et al., 2008; Brachmann et al., 2008; Flack et al., 2009; Philipp et al., 2007; Schunkert et al., 2009; Sinkiewicz et al., 2009; Smith et al., 2007). The present study further documents a significantly increased BP-lowering efficacy with the valsartan/amlodipine combination ingested at bedtime as compared to any one of the other assessed therapeutic schemes,

leading to a significantly higher percentage of properly controlled patients in this compared to the other three treatment groups. Moreover, results indicate that effects on BP regulation of the combination therapy cannot be directly extrapolated from the documented effects of each monotherapy, alone. Thus, the BP-lowering efficacy (Figure 5) and effects on the circadian BP pattern (Figure 4) were different when valsartan and amlodipine were jointly administered at bedtime than when they were administered at any one of the other tested treatment schedules.

Most important, differences in efficacy between the four treatment groups of this study were more pronounced on both the asleep SBP (Figure 1) and asleep DBP (Figure 2) means. On the other hand, the sleep-time relative BP decline was significantly increased towards a more normal BP pattern *only* when the valsartan/amlodipine combination was jointly ingested at bedtime (Figure 4). These findings may be clinically relevant, since nondipping and/or insufficient asleep BP control have been related to an increase in end-organ injury and cardiovascular events (Boggia et al., 2007; Brotman et al., 2008; Ohkubo et al., 2002; Staessen et al., 1999). Moreover, independent prospective studies have also concluded that the nighttime BP mean is a better predictor of cardiovascular mortality than the awake or 24-h BP mean (Ben-Dov et al., 2007; Dolan et al., 2005; Fagard et al., 2008; Kikuya et al., 2005; Staessen et al., 1999). Along these lines, the results shown in Figure 5 indicate, first, that the administration of the valsartan/amlodipine combination of medications at bedtime provides greater SBP reduction than their administration upon awakening during the 24-h postdosing interval, thus increasing the actual percentage of properly controlled subjects. The graphs of Figure 5 also indicate a change in the dose-response curve depending on timing of the two constituent medications of the combination therapy, leading to the remodeling of the circadian BP pattern towards a more dipping profile *only* when they were jointly administered at bedtime (Figure 4). Results also suggest the need to use ABPM for proper evaluation of subjects under timed combination treatment, including the detection of a potential nocturnal hypotension from the increased efficacy of bedtime therapy, in particular.

The results also document a greater efficacy in lowering ambulatory PP when the valsartan/amlodipine combination is ingested at bedtime. There is mounting evidence that an elevated PP, which is indicative of increased artery stiffness, is an independent marker of cardiovascular risk, mainly for myocardial infarction, congestive heart failure, and cardiovascular death (Benetos et al., 1997; Verdecchia et al., 2001). Epidemiologic studies have shown that cardiovascular mortality is positively related to SBP level (Blacher et al., 2000). However, at any given value of SBP, cardiovascular mortality is higher when DBP is lower (Blacher et al., 2000). Moreover, several studies have shown that the

attenuation of arterial stiffness is associated with an improvement in survival (Topouchian et al., 2007). Reduction of arterial stiffness and lowering PP by pharmacotherapy varies according to the medications, doses used, and duration of treatment (Alici et al., 2009; Cushman et al., 2001; Takami & Shigemasa, 2003). The results of the present study document the effects of hypertension treatment on PP might also be significantly dependent on the circadian time of combination therapy.

Many of the mechanisms regulating BP are circadian stage dependent (Hermida et al., 2007c; Portaluppi & Smolensky, 2000); thus, it is not surprising that hypertension medications display a circadian time dependency in their pharmacodynamics (Hermida et al., 2007a). Moreover, circadian rhythms, for example, in gastric pH and emptying; gastrointestinal motility, biliary function, and circulation; liver enzyme activity; blood flow to the duodenum, kidney, and other organs; and glomerular filtration rate, with a maximum in the daytime and a minimum at night (Koopman et al., 1989; Labrecque & Beauchamp, 2003), can give rise to well-known appreciable ingestion-time differences in the pharmacokinetics of conventional BP-lowering medications. Accordingly, one might expect the combination of valsartan/amlodipine to be cleared more slowly overnight, thus potentially prolonging their duration of action. Time-dependent differences in the pharmacodynamics of BP medications may also result from circadian rhythms in drug-free fraction, rate-limiting steps of key metabolic processes, receptor number and conformation, and/or second messenger and signaling pathways (Witte & Lemmer, 2003). Indeed, clinically relevant dosing-time differences in the beneficial and adverse effects of several different classes of BP-lowering medications, including ARBs, CCBs, angiotensin-converting enzyme inhibitors (ACEIs), α-blockers, β-blockers, and diuretics, are well known as reviewed elsewhere (Hermida et al., 2007a). Thus, one might expect the pharmacodynamics of the combination of valsartan/amlodipine to be more favorable for optimizing BP reduction when ingested at bedtime.

We previously compared the BP-lowering efficacy of the ARB valsartan (160 mg/day) when ingested by hypertensive subjects for 3 months as a monotherapy, either in the morning after awakening or at bedtime. Significant BP reduction was achieved throughout the entire 24 h, independent of the treatment-time (Hermida et al., 2003). However, valsartan administration at bedtime resulted in improved sleep-time relative BP decline, greater efficacy in decreasing asleep BP, and significant increase in the proportion of patients with controlled BP (Hermida et al., 2003, 2005a, 2005b). Two more recent studies extended these results by showing asleep BP regulation is significantly better achieved with bedtime than awakening dosing of two other ARBs, telmisartan (Hermida et al., 2007b) and olmesartan (Hermida et al., 2009). These relevant administration-time-dependent effects on BP regulation are similar for all three

ARBs, despite their markedly different terminal half-life (~24 h for telmisartan, 13 h for olmesartan, and 6–9 h for valsartan). In an independent study, Tofé and García (2009) also documented nighttime BP was significantly greater reduced with bedtime than awakening ingestion of olmesartan by hypertensives with type 2 diabetes and thus more prone to have a blunted nocturnal BP decline than the general population. Finally, Pechère-Bertschi et al. (1998) documented an almost doubling of the nighttime SBP reduction when yet another different ARB, irbesartan (terminal half-life 11–15 h), was ingested in the evening rather than in the morning for 6 wks by 10 subjects with uncomplicated essential hypertension. All these consistent findings showing the enhanced benefits associated with the bedtime dosing of ARBs, namely, an increased sleep-time relative BP decline and a greater efficacy in reducing asleep BP, may be a class-related feature.

In contrast to ARBs, most dihydropyridine CCBs, including amlodipine, seem to reduce BP homogenously during day and night, independent of dosing time (Hermida et al., 2007a). Mengden et al. (1993) evaluated the antihypertensive efficacy of 5 mg/day amlodipine administered either with breakfast or with dinner for 4 wks in their study of 20 subjects. Results of this rather small study indicated that amlodipine reduced BP to a comparable extent independent of dosing time. Nold et al. (1998) reported similar results from an even smaller study on 12 subjects who ingested the same 5 mg/day amlodipine dose for just 3 wks either in the morning (at 08:00 h) or in the evening (at 20:00 h). In contrast to these results, Qiu et al. (2003) reported the somehow surprising finding that morning (07:00 h) ingestion of amlodipine exerted a better effect on the circadian BP pattern than evening (21:00 h) administration, namely, a greater nocturnal BP fall and increased sleep-time relative BP decline after therapy.

Despite the impressive findings on the administration-time-dependent effects of individual ABR and CCB hypertension medications, the potential merit of the chronotherapy of ABR/CCB combination therapy had never been explored previously. The results of this study on subjects with essential hypertension treated with the combination of valsartan/amlodipine at different circadian times demonstrate a normalization of the circadian BP profile towards a more normal dipper pattern *only* when the combination is jointly administered at bedtime. Moreover, the greater proportion of controlled patients, improved efficacy in lowering the asleep BP mean, increased sleep-time relative BP decline, and enhanced reduction of ambulatory PP, all suggest valsartan/amlodipine combination therapy should be preferably administered at bedtime. This timed therapeutic scheme could also be associated with potential additional cardiovascular risk reduction beyond that associated with BP-lowering, itself, an issue that deserves prospective investigation.

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