SUPPLEMENTARY DATA

Research Design and Methods

Inclusion and Exclusion Criteria

The sample represents a population of Spanish hypertensive patients with type 2 diabetes of both sex, ≥18 years of age. Exclusion criteria were pregnancy, history of drug/alcohol abuse, night/shift-work employment, diagnosis of AIDS, type 1 diabetes, secondary hypertension, cardiovascular (CVD) disorders (unstable angina pectoris, heart failure, life-threatening arrhythmia, nephropathy, and grade III-IV retinopathy), intolerance to ambulatory blood pressure (BP) monitoring (ABPM), and inability to communicate and comply with all study requirements. Participants represent a consecutive series of patients fulfilling the exclusion/inclusion criteria. Patients were recruited among those referred to the hospital (within the Social Security Health Care System) for ABPM evaluation. After recruitment, all follow-up visits were performed at the same hospital by the MAPEC investigators in keeping with the protocol described below. This prospective single-center study (registered at www.clinicaltrials.gov, with identifier code NCT00295542) was approved by the state Ethics Committee of Clinical Research. All patients gave written informed consent.

Subjects and Diagnostic Criteria

For the specific hypothesis tested here (influence of time of hypertension treatment on CVD risk in diabetes), we assessed 480 patients fulfilling the inclusion/exclusion criteria. Among these, 448 (255 men/193 women, 62.5±10.8 (mean±SD) years of age) provided all required information for the study. We established a priori a minimum time of follow-up of ≥6 months for each patient and a minimum median follow-up of 5 years (1). A total of 32 patients evaluated by ABPM for potential inclusion were not randomized due to their lack of consent for additional ABPM evaluations. Diagnosis of hypertension was based on accepted ABPM criteria -- an awake BP mean of ≥135/85 mmHg for systolic (SBP)/diastolic BP (DBP), or an asleep BP mean ≥120/70 mmHg (2).

Study Design

This was a prospective, randomized, open-label, blinded endpoint (PROBE) trial. Participants were randomized to ingest were randomized to ingest all their prescribed BP-lowering medications upon awakening (232 patients) or ≥1 of them at bedtime (216 patients). The MAPEC study did not specify or require a unique investigational hypertension medication; rather, participating physicians were given the choice of prescribing, as first-line therapy, one of the recommended therapeutic classes (2). The allowed choices were the angiotensin-receptor blockers valsartan, telmisartan, and olmesartan; the angiotensin-converting enzyme inhibitors ramipril and spirapril; and the calcium channel blockers (CCB) amlodipine and nifedipine GITS. Randomization of participants to treatment-time (awakening or bedtime) was done separately for each allowed individual hypertension medication. This ensured that the proportion of patients treated with each medication was similar across the morning and bedtime treatment arms of the study. If patients were uncontrolled based on ABPM criteria after 3 months of monotherapy, additional medications could be added in keeping with current clinical practice. The diuretic hydrochlorothiazide (up to 25 mg/day) or a dihydropyridine CCB were the primary choices as second-line therapy, and either one of these medications or the α-blocker doxazosin were the primary choices as third-line therapy. Adherence to the time-of-day (awakening or bedtime) and medications of the prescribed treatment was evaluated by personal interview at each follow-up visit. The prescription and administration-time of medications other than hypertension ones, such as statins, aspirin, and/or diabetes medications, were not part of the randomized protocol and were prescribed as needed in keeping with clinical practice

Blood samples were obtained the same week when each 48h ABPM session was initiated. Participants reported to the hospital between 08:00 and 09:00h, after overnight fasting, for blood withdrawal from the antecubital vein. Samples were analyzed using routine automatic techniques in the hospital laboratory. Just before commencing ABPM, six clinic BP measurements were always obtained

by the same investigator with a validated automatic oscillometric device (HEM-705IT, Omron Health Care Inc., Vernon Hills, IL) after the patients had rested in a seated position for ≥10 min.

ABPM Assessment

At inclusion, as well as at each scheduled visit for ABPM during follow-up (see below), the SBP and DBP of each patient were automatically measured every 20 min between 07:00 and 23:00h and every 30 min during the night for 48 consecutive hours with a calibrated SpaceLabs 90207 ABPM monitor (SpaceLabs Inc., Issaquah, WA). Participants were instructed to do their usual activities with minimal restrictions but to adhere to a similar schedule during the two days of ABPM and 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 considered invalid for analysis if ≥30% of the measurements were missing, if data were lacking for an interval of >2h, if data were obtained while patients had an irregular rest-activity schedule during the two days of monitoring, or if the nighttime sleep period was <6h or >12h during ABPM.

Actigraphy

All patients wore an actigraph (Mini-Motion-Logger, Ambulatory Monitoring Inc., Ardsley, NY) on the dominant wrist to monitor physical activity every min during ABPM. We synchronized the internal clocks of the activity and ABPM devices through their respective interfaces using the same computer. The actigraphy data, combined with patient diaries, were used to corroborate the absence of daytime napping and to define the commencement and termination of the diurnal awake and nocturnal asleep spans so the respective BP means for each subject could be accurately determined.

Follow-up

The same evaluation procedure described above, including conventional clinic BP measurement, 48h ABPM and wrist activity monitoring, blood sampling, plus other complementary tests as ordered by the physicians (e.g., electrocardiogram, funduscopic evaluation, and echocardiogram), was scheduled annually, or more frequently (after 3 months of any change in treatment) if the therapeutic scheme was modified to improve ambulatory BP control. All follow-up visits scheduled for this trial were performed at the referral hospital by the registered investigators of the MAPEC study. The same leading investigator (D.E.A.) evaluated each ABPM profile obtained during the course of this trial using dedicated software for ABPM evaluation (3), and these reports were used to guide changes in treatment, as described above. With this previously described application, each ABPM profile is analyzed by comparison to both upper and lower time-specified reference limits; use of a lower reference threshold allows avoidance of nocturnal hypotension (3).

Investigators blinded to the timed-treatment scheme of each participant (thus excluding those performing clinic evaluation at each visit to the hospital, clinic and ambulatory BP measurement, and/or statistical analyses) reviewed at least annually the complete clinical records of all enrolled subjects to assess CVD morbidity and mortality. The clinical records, currently in electronic format, include the complete medical history of any given subject within the Social Security Health Care System in our region (Northwest Spain). Verification and categorization of CVD events listed in the patient records were accomplished following customary medical practice using previously reported diagnostic criteria (1) of the corresponding hospital services, including cardiology, neurology, and nephrology, by personnel not participating in the MAPEC study and who were thus unaware of the patients randomization and treatment schedule. Registered events for the primary outcome included: death from all causes, myocardial infarction, angina pectoris, coronary revascularization, heart failure, acute arterial occlusion of lower extremities, rupture of aortic aneurisms, thrombotic occlusion of the retinal artery, hemorrhagic stroke, ischemic stroke, and transient ischemic attack.

SUPPLEMENTARY DATA

Statistical Methods

To correct for measurement errors and outliers, ABPM profiles were edited according to conventional criteria. Thus, SBP readings >250 or <70 mmHg, DBP >150 or <40 mmHg, and pulse pressure (difference between SBP and DBP) >150 or <20 mmHg were automatically discarded. The "48h BP mean" was calculated as the average of all valid readings obtained during the 48h ABPM sampling. The sleep-time relative BP decline (an index of BP dipping), defined as the percent decrease in mean BP during nocturnal sleep relative to the mean BP during diurnal activity, was calculated as: [(awake BP mean – asleep BP mean)/awake BP mean] x 100, using all the data sampled by 48h ABPM. For comparative purposes, a subject was defined as dipper if the sleep-time relative SBP decline was ≥10%, and as non-dipper otherwise.

The primary outcomes study endpoint was total CVD morbidity and mortality, which included all the events listed above. In keeping with previous literature in the field, we also used as an additional primary endpoint major CVD events, i.e., a composite of CVD deaths, myocardial infarction, and stroke. Demographic and clinical characteristics were compared on an intention-to-treat basis among groups of subjects randomized to the two treatment-time groups -- (i) all hypertension medications ingested upon awakening or (ii) ≥1 BP-lowering medication ingested at bedtime -- by t-test (quantitative variables) or nonparametric chi-square test (proportions). The Cox proportional-hazard model was used to estimate relative risks (with 95% confidence intervals) for events associated with time of treatment, with adjustment for significant confounding variables. Event-rates for fatal and non-fatal CVD events during follow-up were also expressed as the number/1000 patient-years, i.e., ratio of the observed number of events to the total number of patient-years of exposure. For survival analysis, follow-up was established as either the time to the first documented event or the time to the last evaluation in event-free subjects. Survival curves were generated using the Kaplan-Meier product-limit method and compared by the Mantel log-rank test. Statistical analyses were performed using SPSS, version 13.0 (SPSS Inc, Chicago, IL) and KaleidaGraph version 3.6.4 (Synergy Software, Reading, PA).

Limitations and strengths of the study

Our study has some potential limitations. First, compared to other larger multi-center clinical trials on hypertensive patients entailing only clinic BP measurement during follow-up, the sample size of the ABPM-based, single-center MAPEC study might seem a limitation. However, the number of patients participating in our study was considerably greater than that of most other published trials on the prognostic value of ABPM in patients with diabetes, and sufficient according to the statistical significance of the reported results. Second, in keeping with usual clinical practice, the design of the MAPEC study allowed treatment with BP-lowering medications of different classes. Thus, the sample size of the trial is limited to derive conclusions from the comparison between classes of medications and their combinations on the benefits, in terms of CVD risk reduction, of bedtime treatment. Finally, the use of PROBE design might also be considered a limitation. However, such design, that closely reflects usual clinical practice, was specifically developed for the conduct of long-term morbidity and mortality trials. Nonetheless, the design of the MAPEC study also incorporated several strengths. While all previous trials on the prognostic value of ABPM relied on a single baseline profile from each subject, the MAPEC study is the first to provide results that are based on systematic periodic evaluations by ABPM throughout the median 5.4 years of follow-up. This so-far unique approach allowed first-time determination of the influence on CVD risk of specific changes during follow-up in ambulatory BP. Further strengths of the MAPEC study are the use of: (i) 48h, instead of the most common 24h ABPM sampling, to increase the reproducibility of the BP findings (4); and (ii) wrist actigraphy to precisely and individually determine the beginning and end of the activity and sleep spans for each subject to enable accurate calculation of the awake and asleep BP means, sleep-time relative BP decline, and type of dipping pattern.

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