Aspirin in the prevention of cardiovascular disease in type 2 diabetes

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Time-dependent effects of low-dose aspirin on plasma renin activity, aldosterone, cortisol and catecholamines

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Abstract

Objective: Studies have shown that aspirin may decrease blood pressure when given at bedtime but not when administered upon awakening. However, until now, a biologically plausible mechanism of this striking phenomenon was not revealed. We investigated the effect of 100 mg aspirin administered at bedtime compared with upon awakening on plasma renin activity and aldosteron levels over 24 hours and excretion of cortisol and catecholamines in 24h-urine.

Research design: A randomized, placebo controlled, double-blind, crossover trial was performed in 16 grade I hypertensive subjects. During two periods of 2 weeks separated by a 4-week washout period, participants used both aspirin at morning and at night, which was blinded with placebo. After both periods, subjects were admitted for 24 hours to measure the aforementioned parameters.

Results: Aspirin intake at bedtime compared with upon awakening reduced average (24h) plasma renin activity by 0.08 µg/L/h (95% confidence interval (95%CI) 0.03–0.13, *P*=0.003) without affecting aldosterone levels (95%CI -0.01–0.01 nmol/L, *P*=0.93). Cortisol excretion in 24h-urine was 52 nmol/24h (95%CI 5–99, *P*=0.05) lower and dopamine and norepinephrine excretion were 0.25 µmol/24h (95%CI 0.01–0.48, *P*=0.04) and 0.22 µmol/24h (95%CI -0.03–0.46, *P*=0.02) lower in patients treated with bedtime aspirin.

Conclusions: Aspirin taken at bedtime compared with upon awakening significantly diminished 24h plasma renin activity and excretion of cortisol, dopamine and norepinephrine in 24h-urine. Decreased activity of these pressor systems forms a biologically plausible explanation for the finding that aspirin at night may reduce blood pressure whereas aspirin at morning does not.

Introduction

One of the most important modifiable risk factors for cardiovascular disease is the presence of arterial hypertension. Therefore, modern guidelines recommend treatment of hypertension with both lifestyle measures and medication to prevent cardiovascular events (1). A modest reduction of blood pressure (*i.e.* 3-5 mmHg) in the population will already produce a dramatic fall in serious events as myocardial infarction and stroke (2).

Nowadays, low-dose aspirin (acetylsalicylic acid) forms a cornerstone in the secondary prevention of cardiovascular events, particularly because its inhibitory effects on platelet aggregation, which is predominantly based on the irreversible inhibition of cyclooxygenase-1 mediated thromboxane A2 production by platelets (3). The clinical effectiveness of aspirin on the secondary prevention of cardiovascular events has been well established (4). Although aspirin is a potent vasoprotective drug, it is generally believed to have no effects on blood pressure (3). Notably, in studies addressing this association, it was not reported at what time of the day aspirin was ingested by the participants (5-7). In contrast, two recent randomized controlled trials of Hermida *et al.* have shown that 100 mg aspirin strongly decreases blood pressure in subjects with grade I essential hypertension when it was administered at bedtime, whereas – if anything – blood pressure might slightly increase when aspirin was taken upon awakening (8, 9). In the subjects allocated to aspirin at evening, reductions of systolic and diastolic blood pressure of respectively 7.2/4.9 and 6.8/4.6 mmHg (systolic/diastolic blood pressure) were recorded, whereas in the participants using aspirin at morning a slight elevation of respectively 1.5/1.0 and 2.6/1.6 mmHg was observed (8, 9).

An important yet unanswered question is by which mechanism(s) aspirin could time-dependently lower blood pressure (10). As the main regulators of blood pressure behave according to circadian rhythms, these may be potential targets of time-dependent aspirin therapy. The renin-angiotensin-aldosterone system (RAAS) is more active during early morning hours as a response to the nocturnal fall in blood pressure and renal perfusion pressure (11, 12). Aspirin given at night may decrease this nocturnal rise of RAAS activity and concomitantly attenuate the nocturnal drop in

nitric oxide production (10, 13). Furthermore, among others, hypothalamic-pituitary-adrenal (HPA) activity and sympathetic autonomous nervous activity, which are well-known regulators of blood pressure (14-16) also display circadian variation (12, 17). Several studies demonstrate that aspirin could influence various regulators of blood pressure (18-23). Moreover, other studies suggest that aspirin has time-dependent effects on lipoperoxides, beta-adrenergic receptors and angiotensin II (24, 25).
To assess the underlying mechanism of the reported time-dependent effect of aspirin on blood pressure, we conducted a randomized placebo-controlled crossover trial. We investigated the effects of 100 mg aspirin administered at bedtime compared with 100 mg aspirin given upon awakening, on the levels of plasma renin activity and aldosterone over 24 hours as well as excretion of cortisol and catecholamines in 24h urine among subjects with grade I hypertension. We hypothesized that intake of aspirin at bedtime compared to intake upon awakening attenuates RAAS activity as well as the other measured determinants of blood pressure, which would provide an explanation of previously found time-dependent effects of aspirin on blood pressure.

Methods

Subjects

Subjects were eligible for participation if they had an untreated grade I hypertension, defined as systolic blood pressure between 140 and 159 mmHg and/or diastolic blood pressure between 90 and 99 mmHg (1), and were above 18 years old and capable to give informed consent, which are the same inclusion criteria as used by Hermida et al (8, 9). Subjects were excluded if they had grade II or III hypertension (blood pressure ≥160/100 mmHg), evidence of secondary arterial hypertension, any history of cardiovascular disease, diabetes mellitus or rheumatoid arthritis or known contraindications to use of aspirin (defined as history of asthma, any bleeding disorder, gastrointestinal tract bleeding or known allergy to acetylsalicylic acid). Other exclusion criteria were severe renal or hepatic dysfunction, pregnancy, concurrent participation in other research projects or blood donation and use of blood pressure lowering medication, non-steroidal anti-inflammatory drugs or anticoagulant

medication. We also excluded shift workers, since working in shifts may influence circadian rhythms.

Participants were recruited from general practitioners affiliated to the Leiden University Medical Center and from those who participated earlier in trials of our department. All subjects gave written informed consent and the study was approved by the Leiden University Medical Center medical ethics committee and performed in accordance with the Declaration of Helsinki.

Design

The study had a prospective, randomized, placebo-controlled, double-blind, crossover design (figure 1). After a first screening visit, all subjects (n=16) were assigned to both one 2-week period 100 mg aspirin upon awakening and one 2-week period 100 mg aspirin at bedtime, in a randomized order. To guarantee that participants and investigators were blinded for study medication, the trial was placebo-controlled. In the period in which subjects were allocated to receive aspirin at morning, placebos were provided to take at night and vice-versa. The first intervention period of 2 weeks was followed by a washout period of 4 weeks. When subjects received aspirin upon awakening in the first period, they were treated by aspirin at bedtime in the second intervention period, and subjects received aspirin upon awakening when they were first assigned to aspirin at bedtime. Double-blind study medication was prepared and stored at the department of clinical pharmacy of the Leiden University Medical Center. A computer-generated randomization code was also prepared by an independent person at this department.

The participants visited the research site after an overnight fast at the beginning of each 2-week intervention period for instructions, blood sampling, and to receive both aspirin and placebo for that period. After each treatment period, subjects visited the research site after an overnight fast for a 24h admission (table 1). Thus, each subject was admitted twice for 24 hours, both after intervention with aspirin upon awakening and aspirin at bedtime. By a structured interview, we asked for compliance, possible adverse events and changes in medication. To further assess compliance, remaining pills were counted. Non-compliance was defined as remaining pill count of three or more or the subject's acknowledgement of non-compliance.

The study has been designed and reported in accordance with the CONSORT guidelines for randomized controlled trials.

Table 1 - Day scheme 24h admissions

Time	Action
8:30 AM	Insertion intravenous catheter
	Start 24h ABPM
	Start 24h urine collection
	Blood sampling
9:00 AM	Breakfast
	Verified intake of study medication
10:30 AM	Blood sampling
12:30 AM	Blood sampling
1:00 PM	Lunch
2:30 PM	Blood sampling
4:30 PM	Blood sampling
5:30 PM	Diner
7:30 PM	Blood sampling
10:00 PM	Verified intake of study medication
10:30 PM	Blood sampling
11:00 PM	Bedtime – lights off
1:30 AM	Blood sampling
4:30 AM	Blood sampling
7:30 AM	Blood sampling
	Awakening – lights on
	30 minutes ambulation
8:15 AM	Blood sampling
8:30 AM	Stop 24h urine collection
	Stop ABPM

ABPM indicates ambulatory blood pressure monitoring

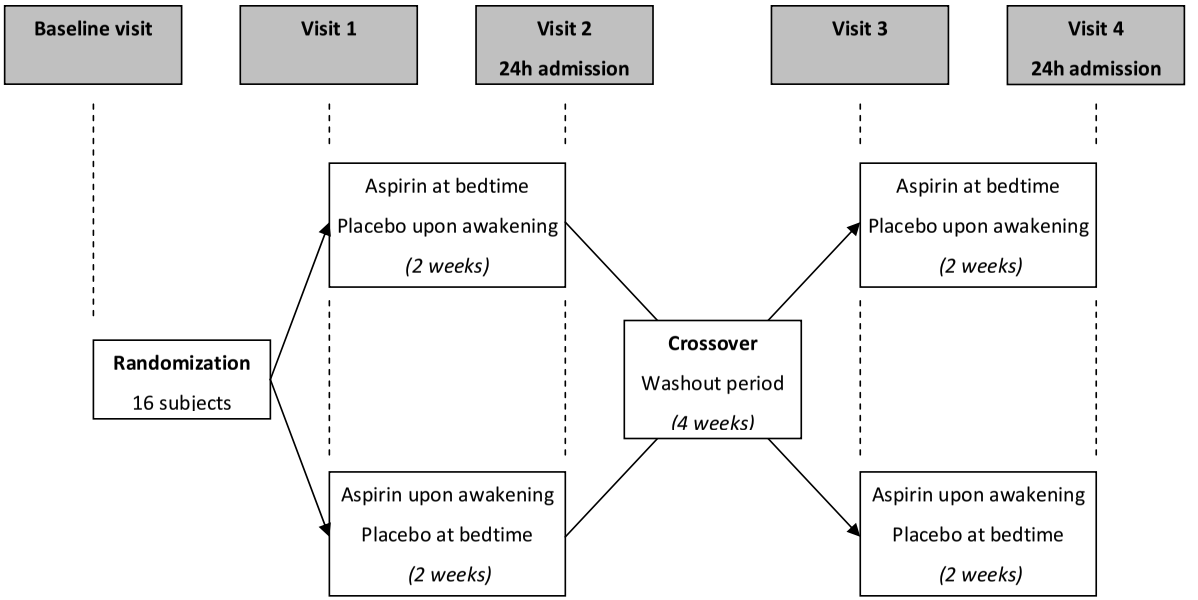

Figure 1 – Study design
Study subjects were randomly assigned to receive aspirin upon awakening in the first period and at bedtime in the second period, or vice versa. After both intervention periods, subjects were admitted for 24 hours.

24-hour admission periods

After each intervention period, subjects were admitted from 8:00 AM to 9:00 AM the next day. A summary of the day schedule is given in table 1. Meals were given at 8:30 AM, 13:00 PM, 5:30 PM and 8:30 AM, respectively. From 11:00 PM to 7:30 AM, lights were turned off so that the subjects could sleep. After awakening at 7:30 AM, participants were asked to ambulate for half an hour. Both upon arrival and at bedtime, subjects took their study medication under supervision of the investigators. During admission, blood samples were regularly collected in a supine position (during the day each two hours, during the night each three hours) for measurement of plasma renin activity and aldosterone. Subjects were asked to stay in a supine position each half hour before blood sampling. With this exception, subjects were allowed to behave according to their personal preference during the 24h admission, provided that they did not leave our Center. Moreover, blood was sampled before and after the walking exercise after awakening. Blood was sampled through a plastic intravenous catheter inserted in an antecubital vein. Saline was slowly infused to keep the catheter patent, usually with 10 mL/h and with a maximum of 20 mL/h. EDTA blood samples were taken from the catheter after discarding the first milliliters of saline-diluted blood and kept on ice. The first sample was collected at least half an hour after insertion of the catheter. After blood sampling, plasma was directly separated and aliquots with plasma were kept in liquid nitrogen until storage at -80 °C. In addition, the subjects were asked to collect their urine produced during the day in order to determine levels of cortisol and catecholamine excretion in 24h urine. As a secondary endpoint, 24h ambulatory blood pressure monitoring (ABPM) was performed during the two admissions of the subjects. The systolic and diastolic blood pressure and heart rate were automatically gauged every 15 minutes from 7:00 AM to 11:00 PM and every 30 minutes during the night. During both admissions, blood pressure was measured at the same arm with a suitable cuff using a validated Mobil-O-Graph® ABPM device (IEM GmbH, Stolberg, Germany) (26).

Laboratory measurements

At baseline, routine hematological and chemical variables were determined according to standard procedures. Renin activity in plasma was quantified by transformation of the generated angiotensin I to angiotensin II by converting enzyme and measured by a radioimmunoassay (DiaSorin, Brussels, Belgium). Aldosterone was measured with a solid phase radioimmunoassay, (DPC, Los Angeles, CA, USA). Urinary cortisol samples were purified over a C-18 column and measured with a fluorescence polarization immunoassay on a TDx analyzer (Abbott, Abbott Park, IL, USA). Urinary catecholamines (dopamine, norepinephrine and epinephrine) concentrations were assessed by high-performance liquid chromatography with electron capture detection (ESTA-Coulochem, Chelmsford, MA, USA). All assays for plasma renin activity, aldosterone, cortisol and catecholamines were performed blinded after completion of the study.

Statistical analysis

We calculated a required sample size for this crossover trial of 14 patients to have 90% power at the 5% significance level to detect a 10% plasma renin activity reduction by aspirin at bedtime versus aspirin upon awakening, assuming a within-patient standard deviation of plasma renin activity of 8%. Since our sample size calculation was based on our primary endpoint (i.e. differential effects of aspirin according to time of intake on underlying mechanisms of blood pressure, primarily plasma renin activity), our study was not powered to find effects on the secondary endpoint blood pressure.

To describe the characteristics of the subjects included in our study, continuous variables are presented as mean values ± standard deviation (SD) and categorical variables as frequencies (percentages). To describe the outcome characteristics according to time of intake of aspirin, continuous variables are presented as mean values ± standard error of the mean (SEM). To estimate the effects of aspirin taken at bedtime versus aspirin taken upon awakening on plasma renin activity, aldosterone, cortisol, catecholamines and blood pressure and their 95% confidence intervals (95%CI), we employed mixed models, in order to deal accurately with the repeated measurements we had from each participant. More specifically, a unique ID for each

subject was entered as a random effect in the models, while both a variable denoting aspirin use at morning or at night and a variable for each time a measurement of the relevant outcome variable was done, were added as fixed effects. In the case of cortisol and catecholamine levels in 24h urine, of which we of course had only one measurement per intervention period, these models equal simple paired samples t tests. In the latter case, next to the effect estimates from the mixed models, we also provide *P*-values calculated with the non-parametric Wilcoxon signed rank test, because these comparisons are based on relatively few numbers and the differences in those parameters tended to be skewed.
All statistical analyses were performed using SPSS version 14.0 (SPSS, Chicago, IL, USA). All analyses were two-sided, with a level of significance of α=0.05.

Results

We included a total of 16 subjects in our crossover trial. Subjects were included between March 2007 and January 2008. All subjects were compliant to study medication and no adverse events occurred. Subject characteristics are summarized in table 2. Thirty-one percent of the participants was female and the mean age of all subject was 58 years old (ranging from 47 to 68). Baseline office systolic and diastolic blood pressure levels were 147 ± 12 mmHg and 86 ± 6 mmHg, respectively. None of the participants used any relevant co-medication, especially no non-steroidal anti-inflammatory drugs or antihypertensive drugs. The total volumes of urine collected during the 24h admissions were 2191 ± 591 mL and 2286 ± 612 mL for intervention with aspirin upon awakening and at bedtime, respectively, indicating complete urine collection after both interventions (*P*=0.49 for difference).

Table 2 - Baseline characteristics

Variable	Included subjects (*n* = 16)
Age (yrs)	58.4 ± 6.8
Female sex	5 (31.3)
Systolic tension (mmHg)	147 ± 12
Diastolic tension (mmHg)	86 ± 6
Heart rate (n/min)	64 ±7
Smoking	2 (12.5)
BMI (kg/m^2)	29.6 ± 3.1
Glucose (mmol/L)	5.4 ± 1.0
HbA_{1c} (%)	5.2 ± 0.4
Total cholesterol (mmol/L)	5.8 ± 0.6
HDL-cholesterol (mmol/L)	1.4 ± 0.4
LDL-cholesterol (mmol/L)	4.0 ± 0.7
Triglycerides (mmol/L)	1.8 ± 0.7

Data are *n* (%) or means ± standard deviation (SD).

BMI indicates body mass index, calculated as weight (kg) divided by square height (m); HbA_{1c}, glycosylated hemoglobin A; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Effects on mechanisms underlying blood pressure

The effects of 100 mg aspirin intake at bedtime versus 100 mg aspirin intake upon awakening are summarized in table 3 and figures 2 and 3. Aspirin intake at bedtime compared with upon awakening reduced average plasma renin activity over 24 hours by 0.08 µg/L/h (95%CI 0.03 to 0.13, *P*=0.003) versus aspirin upon awakening, which is a 14% (95%CI 5 to 22) reduction (figure 1). The reduction was observed during the whole 24 hours of observation. At 8:15 AM, after thirty minutes of ambulation after awakening, the reduction of plasma renin activity by aspirin at night compared to aspirin at morning was even 0.24 µg/L/h (95%CI 0.00 to 0.48, *P*=0.05), which is a 31% (95%CI 0 to 60) reduction.

Overall, time of intake of aspirin did not influence plasma levels of aldosterone (mean difference 0.00 nmol/L, 95%CI -0.01 to 0.01, *P*=0.93). Observing the patterns over 24 hours (figure 3), aldosterone levels seem to be lower at daytime in favor of aspirin intake at bedtime, whereas during the night (particularly early morning) the levels seem to have a reverse pattern.

Aspirin taken at bedtime compared with upon awakening also lowered excretion of cortisol in 24h urine. The absolute reduction was 52 nmol/24h (95%CI 5 to 99, *P*=0.05), while the relative reduction was 16% (95%CI 5 to 30).

Concerning catecholamines measured in 24h urine samples, aspirin at night was also favorable compared with intake at morning. Dopamine levels were lowered by 0.25 µmol/24h (95%CI 0.01 to 0.48, *P*=0.04), which is a 14% (1 to 28) reduction. The effect on norepinephrine was an absolute reduction of 0.22 µmol/24h (95%CI -0.03 to 0.46, *P*=0.02), while the relative reduction was as high as 40% (95%CI -1 to 88). The circadian timing of aspirin had no effect on excretion of epinephrine in 24h urine as the reduction by aspirin taken at night is 0.00 µmol/24h (95% -0.01 to 0.01, *P*=0.20).

Table 3 - Effects of circadian timing of aspirin intake on mechanisms underlying blood pressure

Variable	Aspirin upon awakening	Aspirin at bedtime	Difference (95%CI)	*P*-value
	(*n*=16)			
Plasma renin activity (µg/L/h)*	0.58 ± 0.11	0.50 ± 0.08	0.08 (0.03 to 0.13)	0.003
Aldosterone (nmol/L)*	0.12 ± 0.01	0.12 ± 0.02	0.00 (-0.01 to 0.01)	0.93
Cortisol (nmol/24h)	328 ± 34	276 ± 33	52 (5 to 99)	0.05
Dopamine (µmol/24h)	1.74 ± 0.10	1.49 ± 0.12	0.25 (0.01 to 0.48)	0.04
Norepinephrine (µmol/24h)	0.52 ± 0.11	0.31 ± 0.04	0.22 (-0.03 to 0.46	0.02
Epinephrine (µmol/24h)	0.02 ± 0.008	0.02 ± 0.006	0.00 (-0.01 to 0.01)	0.20

Data are means ± standard errors of the mean (SEM)) and mean differences (95% confidence interval). Positive differences denote an effect in favor of aspirin at bedtime, whereas a negative difference indicates an effect in favor of aspirin upon awakening.

*Levels and differences of plasma renin activity and aldosterone are average values of all measurements over 24 hours.

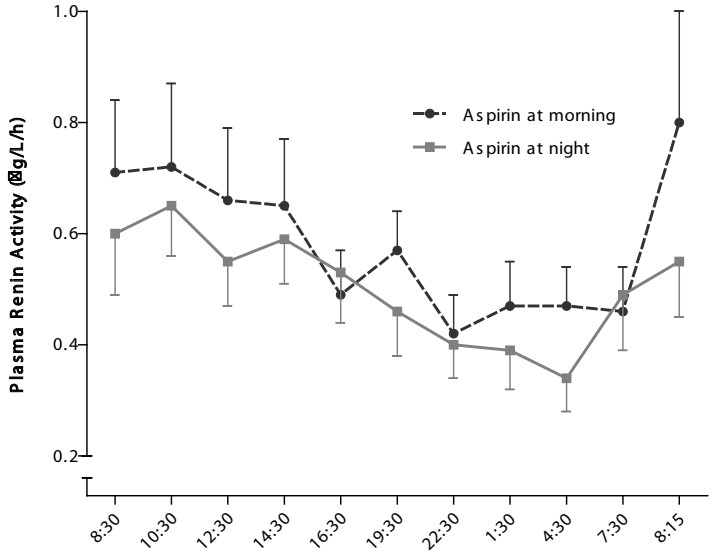

Figure 2 – Effects of circadian timing of aspirin on plasma renin activity

The dashed dark grey line denotes plasma renin activity after intervention with aspirin at morning, whereas the solid light grey line shows values after aspirin at night.

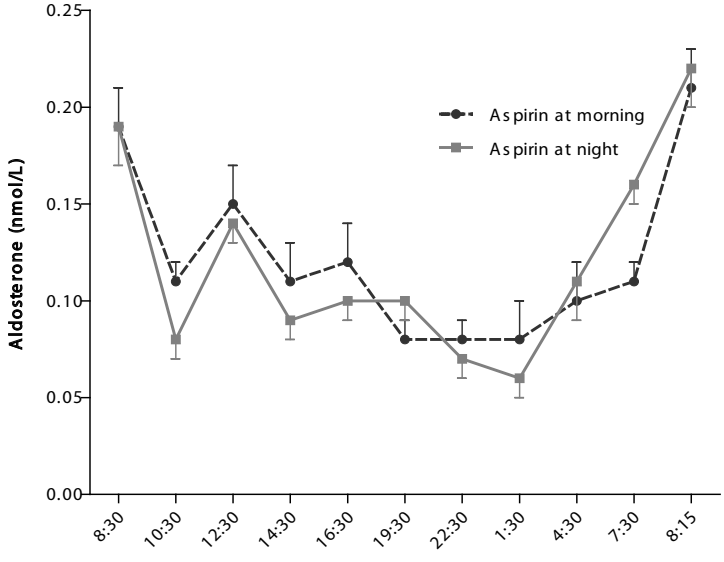

Figure 3 – Effects of circadian timing of aspirin on plasma aldosterone

The dashed dark grey line denotes plasma aldosterone levels after intervention with aspirin at morning, whereas the solid light grey line shows values after aspirin at night.

Effects on blood pressure

As a secondary endpoint, we assessed the potential time-dependent effect of aspirin on blood pressure itself. The mean systolic and diastolic blood pressure measured by 24h ABPM respectively were 133.4 ± 3.1 mmHg and 85.2 ± 2.5 mmHg after treatment with aspirin at morning, while those values were 133.8 ± 3.1 mmHg and 85.8 ± 2.5 mmHg after the intervention period with aspirin at evening. This results in differences of respectively -0.5 mmHg (95%CI -1.4 to 0.4) and -0.7 mmHg (95%CI -1.4 to 0.6), thus timing of aspirin did not influence the average blood pressure over 24 hours measured by ABPM. Interestingly, in analogy to the 24h patterns of aldosterone, there seems to be a small but significant difference in systolic blood pressure during daytime in favor of aspirin at bedtime (1.3 mmHg, 95%CI 0.1 to 2.5, *P*=0.03, whereas the pattern seems to be reversed during the night (-2.4 mmHg, 95%CI -3.7 to 1.2, P<0.001).

Discussion

Previous studies showed that treatment with aspirin may decrease blood pressure when given at bedtime whereas administration of aspirin at morning slightly increased blood pressure (8, 9). However, until now, a biologically plausible explanation of this striking phenomenon was not revealed in a clinical study. We specifically conducted a randomized double-blind crossover trial among grade I hypertensive subjects to study the underlying mechanism of the potential blood pressure lowering effect of aspirin at evening, but not at morning. Our main finding is that aspirin administered at night compared to intake at morning statistically significantly diminished plasma renin activity over 24 hours, as well as excretion of cortisol, dopamine and norepinephrine in 24h urine.

Time-dependent effects on regulators of blood pressure

The results of our study corroborate past experiments that indicated that aspirin might influence various regulators of blood pressure (18-23). However, those studies did not address potentially differential effects according to timing of aspirin. A few

older reports already suggested that aspirin might have some time-dependent effects (24, 25, 27). For the first time, we demonstrated statistically significant differential effects according to time of intake of aspirin on plasma renin activity, cortisol and catecholamines in our clinical study.

Important questions to address are why and how aspirin intake at night confers those effects on underlying mechanisms of blood pressure compared with ingestion at morning. Probably, in the late evening there is a window of opportunity to influence those systems by aspirin, in contrast to the early morning. As RAAS activity and levels of cortisol and catecholamines all display a circadian pattern with an increase in the early morning hours, an explanation may be that administration of aspirin at morning is simply too late to influence the nocturnal rise in activity of those systems, whereas intake at bedtime might be the right timing to result in a longer lasting blood pressure lowering effect (11, 12, 17). Indeed, we showed that the activity of renin in plasma rises in the early morning hours, whereas it decreases spontaneously (independent of intervention) after the morning peak (figure 2). According to this potential explanation, the time-dependent effect of aspirin would be particularly based on the time-dependent bioavailability of its substrate. This is corroborated by an animal study in which oral intake of aspirin prevented the increase in blood pressure, which occurred in animals that were not treated by aspirin, induced by chronic angiotensin II infusion, whereas intake of aspirin alone, without angiotensin II, did not reduce blood pressure (22). In other studies aspirin also influenced the response (e.g. HPA activity or hydrogen peroxide-mediated toxicity) upon stimulation with various vasoactive substances (18-20, 28). Future studies are warranted to further elucidate these and other questions, e.g. which of the blood pressure regulating systems is primarily affected by aspirin.

We did not observe an effect on average plasma aldosterone levels over 24 hours. The most likely explanation is that potential RAAS-mediated effects of time of intake of aspirin on blood pressure do not act via aldosterone as an effector hormone, but e.g. via angiotensin II. Previous literature provides no evidence supporting effects of aspirin on aldosterone, whereas there are several reports that reported effects of aspirin on angiotensin II and downstream effects (22, 25). Unfortunately, we were not able to measure levels of angiotensin II levels to support or reject the latter

hypothesis. Therefore, the present results call for future studies to elucidate the time-dependent effects of aspirin on RAAS in more detail.

Time-dependent effects on blood pressure

We did not observe a decrease in 24 hour blood pressure by administration of aspirin at bedtime compared with aspirin given upon awakening. There are several explanations for this finding. Our study was not designed and powered to find effects of timing of aspirin on blood pressure but to study effects on mechanisms underlying blood pressure. In addition, the intervention period in our study was only two weeks, whereas in earlier studies the participants received aspirin for a period of three months (8, 9). We hypothesize that treatment with aspirin at night for two weeks is long enough to obtain differences in blood pressure regulating systems, but not to translate those effects into blood pressure lowering. Interestingly, our results regarding both aldosterone and systolic blood pressure suggest an effect in favor of aspirin at bedtime during daytime, whereas these patterns were not observed or even tended to reverse during the night.

Study limitations and strength

There are limitations to our study. In our crossover study we compared aspirin given at bedtime with aspirin given upon awakening, which was blinded by use of placebos. We did not compare either aspirin at morning or aspirin at evening with only placebo. This would have required another crossover and a third intervention period, which would have been more aggravating for the participants. However, we do not consider this as a real limitation, because even if aspirin at morning also had some effects on our endpoints (which is not likely), this would only have underestimated our findings to some extent. Furthermore, we were not able to measure angiotensin II as effector hormone of the RAAS, as well as effects on nitric oxide metabolism, which pathway might also provide an explanation for the observed time-dependent effects of aspirin on blood pressure (13, 23). The major strength of our study is that we used a randomized double-blind crossover design. Using this design, the same patients were randomly exposed to both interventions in different treatment periods and therefore served as their own controls. This unique characteristic of crossover studies maximizes power to detect an effect and virtually excludes the risk of confounding.

Perspectives

Our study demonstrates that aspirin taken at bedtime compared with aspirin intake upon awakening results in a statistically significantly diminished activity of different biological regulators of blood pressure: plasma renin activity over 24 hours as well as excretion of cortisol, dopamine and norepinephrine in 24h-urine were found to be decreased. Reduced activity of these pressor systems forms a biologically plausible explanation for the finding that aspirin at night may reduce blood pressure whereas aspirin at morning does not. Before this strategy may be implemented in clinical practice, future studies are warranted to assess whether the blood pressure lowering effects of aspirin taken at bedtime sustain in patients who have a clinical indication to be treated with aspirin, i.e. patients at high risk of (recurrent) cardiovascular events. This may be challenging because these patients are likely treated with a variety of antihypertensive drugs which may dilute or interact with the time-dependent effects of aspirin on blood pressure. Another unanswered but important research question is whether effects of aspirin on platelet aggregation vary according to time of intake. Ultimately, clinical endpoint studies are needed to answer the final question whether aspirin given at bedtime will lead to incremental cardiovascular protection beyond treatment upon awakening.

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References

1. Parati G, Stergiou GS, Asmar R, Bilo G, de LP, Imai Y, Kario K, Lurbe E, Manolis A, Mengden T, O'Brien E, Ohkubo T, Padfield P, Palatini P, Pickering T, Redon J, Revera M, Ruilope LM, Shennan A, Staessen JA, Tisler A, Waeber B, Zanchetti A, Mancia G. European Society of Hypertension guidelines for blood pressure monitoring at home: a summary report of the Second International Consensus Conference on Home Blood Pressure Monitoring. J Hypertens 2008;26:1505-1526.
2. Turnbull F. Effects of different blood-pressure-lowering regimens on major cardiovascular events: results of prospectively-designed overviews of randomised trials. Lancet 2003;362:1527-1535.
3. Patrono C, Garcia Rodriguez LA, Landolfi R, Baigent C. Low-Dose Aspirin for the Prevention of Atherothrombosis. N Engl J Med 2005;353:2373-2383.
4. Antithrombotic Trialists' Collaboration. Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. BMJ 2002;324:71-86.
5. Hansson L, Zanchetti A, Carruthers SG, Dahlof B, Elmfeldt D, Julius S, Menard J, Rahn KH, Wedel H, Westerling S. Effects of intensive blood-pressure lowering and low-dose aspirin in patients with hypertension: principal results of the Hypertension Optimal Treatment (HOT) randomised trial. Lancet 1998;351:1755-1762.
6. Nawarskas JJ, Townsend RR, Cirigliano MD, Spinler SA. Effect of aspirin on blood pressure in hypertensive patients taking enalapril or losartan. Am J Hypertens 1999;12:784-789.
7. Avanzini F, Palumbo G, Alli C, Roncaglioni MC, Ronchi E, Cristofari M, Capra A, Rossi S, Nosotti L, Costantini C. Effects of low-dose aspirin on clinic and ambulatory blood pressure in treated hypertensive patients. Am J Hypertens 2000;13:611-616.
8. Hermida RC, Ayala DE, Calvo C, Lopez JE, Mojon A, Rodriguez M, Fernandez JR. Differing administration time-dependent effects of aspirin on blood pressure in dipper and non-dipper hypertensives. Hypertension 2005;46:1060-1068.
9. Hermida RC, Ayala DE, Calvo C, Lopez JE. Aspirin administered at bedtime, but not on awakening, has an effect on ambulatory blood pressure in hypertensive patients. J Am Coll Cardiol 2005;46:975-983.

10. Messerli FH. Aspirin: A Novel Antihypertensive Drug?: Or Two Birds With One Stone? J Am Coll Cardiol 2005;46:984-985.
11. Gordon RD, Wolfe LK, Island DP, Liddle GW. A diurnal rhythm in plasma renin activity in man. J Clin Invest 1966;45:1587-1592.
12. Angelli A, Gatti G, Masera R. Chronobiology of the hypothalamic-pituitary-adrenal and renin-angiotensin-aldosterone systems. In: Touitou Y, Haus E, editors. Biologic rhythms in clinical and laboratory medicine.Berlin/Heidelberg: Springer-Verlag; 1992. p. 292-314.
13. Kanabrocki EL, Third JL, Ryan MD, Nemchausky BA, Shirazi P, Scheving LE, McCormick JB, Hermida RC, Bremner WF, Hoppensteadt DA, Fareed J, Olwin JH. Circadian relationship of serum uric acid and nitric oxide. JAMA 2000;283:2240-2241.
14. De Wardener HE. The Hypothalamus and Hypertension. Physiol Rev 2001;81:1599-1658.
15. Noll G, Wenzel RR, Binggeli C, Corti C, Luscher TF. Role of sympathetic nervous system in hypertension and effects of cardiovascular drugs. Eur Heart J 1998;19 Suppl F:F32-F38.
16. Kaplan NM. Kaplan's Clinical Hypertension. 9th ed. Philadelphia: Lippincott Williams and Wilkins; 2005.
17. Linsell CR, Lightman SL, Mullen PE, Brown MJ, Causon RC. Circadian rhythms of epinephrine and norepinephrine in man. J Clin Endocrinol Metab 1985;60:1210-1215.
18. Nye EJ, Hockings GI, Grice JE, Torpy DJ, Walters MM, Crosbie GV, Wagenaar M, Cooper M, Jackson RV. Aspirin Inhibits Vasopressin-Induced Hypothalamic-Pituitary-Adrenal Activity in Normal Humans. J Clin Endocrinol Metab 1997;82:812-817.
19. Noon JP, Walker BR, Hand MF, Webb DJ. Impairment of forearm vasodilatation to acetylcholine in hypercholesterolemia is reversed by aspirin. Cardiovasc Res 1998;38:480-484.
20. Husain S, Andrews NP, Mulcahy D, Panza JA, Quyyumi AA. Aspirin improves endothelial dysfunction in atherosclerosis. Circulation 1998;97:716-720.
21. Wu R, Lamontagne D, de Champlain J. Antioxidative Properties of Acetylsalicylic Acid on Vascular Tissues From Normotensive and Spontaneously Hypertensive Rats. Circulation 2002;105:387-392.
22. Wu R, Laplante MA, De Champlain J. Prevention of angiotensin II-induced hypertension, cardiovascular hypertrophy and oxidative stress by acetylsalicylic acid in rats. J Hypertens 2004;22:793-801.

23. Taubert D, Berkels R, Grosser N, Schroder H, Grundemann D, Schomig E. Aspirin induces nitric oxide release from vascular endothelium: a novel mechanism of action. Br J Pharmacol 2004;143:159-165.
24. Cornelissen G, Halberg F, Prikryl P, Dankova E, Siegelova J, Dusek J. Prophylactic aspirin treatment: the merits of timing. International Womb-to-Tomb Chronome Study Group. JAMA 1991;266:3128-3129.
25. Abacioglu N. Effects of drugs and electroshock on blood angiotensin II concentrations. Ankara University: Department of Pharmacology, PhD dissertation; 1982.
26. Jones CR, Taylor K, Chowienczyk P, Poston L, Shennan AH. A validation of the Mobil O Graph (version 12) ambulatory blood pressure monitor. Blood Press Monit 2000;5:233-238.
27. Moore JG, Goo RH. Day and night aspirin-induced gastric mucosal damage and protection by ranitidine in man. Chronobiol Int 1987;4:111-116.
28. Grosser N, Schroder H. Aspirin Protects Endothelial Cells From Oxidant Damage Via the Nitric Oxide-cGMP Pathway. Arterioscler Thromb Vasc Biol 2003;23:1345-1351.