TABLE 2. Cerebral Parameters in Diabetic and Nondiabetic Hypertension Groups and NTs

Variable	DHT Group (n=20)	HT Group (n=20)	NT Group (n=12)	*P* Value*
SCI				
No./person	2.2±2.4‡¶	0.9±1.3	0.5±0.8	0.015
Any infarct, n (%)	12 (60)	8 (40)	4 (33)	0.279
Multiple infarcts*, n (%)	10 (50)	5 (25)	3 (25)	0.191
White matter lesion				
Advanced lesion, n (%)	7 (35)	4 (20)	2 (17)	0.426
Cerebral metabolites				
NAA, mmol/kg	8.35±1.42†§	9.58±1.31	10.5±0.84	<0.001
NAA/creatine ratio	1.28±0.13§	1.39±0.13	1.50±0.20	0.001
Creatine, mmol/L per kg	6.49±0.86	6.90±0.97	7.03±0.64	0.171
Choline, mmol/L per kg	2.01±0.31	2.05±0.45	1.84±0.22	0.253
Cerebral volume flow, mL/min				
ICAs	292±73	263±49	288±56	0.289
MCAs	172±36	155±32¶	186±33	0.040
CVR, %				
ICAs	24.9±14.2‖	35.3±15.6	43.7±13.1	0.003
MCAs	20.1±13.5§	30.9±12.2	41.1±19.7	0.001

Data are shown as the mean±SD or the No. (percentage).
*Overall *P* values for 3 group comparisons of means (ANOVA F-test) or percentages (χ^2 test).
†$P<0.01$, ‡$P<0.05$ vs HT group; §$P<0.001$, ‖$P<0.01$, ¶$P<0.05$ vs NT group.

the DHT than in the NT group, and it tended to be lower in the DHT than in the HT group ($P=0.06$) (Table 2).

Cerebral Hemodynamics

Baseline quantitative volume flows in the ICAs and the MCAs were comparable among the 3 groups, except that there was lower MCA flow in the HT group than in the NT group (Table 2). The CVRs in ICAs (25% versus 35%; $P=0.07$) and MCAs (20% versus 31%; $P=0.06$) tended to be lower in the DHT than in the HT group, and CVR in the DHT group was significantly lower than that in the NT group ($P<0.05$). Neither baseline cerebral blood flow nor CVR in ICAs and MCAs was significantly correlated with a reduction in cerebral NAA (data not shown).

Diabetes and 24-Hour BP Level Effects on Cerebral NAA and CVR

We studied the effect of diabetes and 24-hour BP on the cerebral NAA and CVR in the total subjects (n=52) in the DHT, HT, and NT groups. After adjusting for other clinical characteristics (age, sex, BMI, and status of smoking and hyperlipidemia), cerebral NAA was independently associated with diabetes (standardized $\beta=-0.466$; partial $R^2=0.182$; $P<0.001$), but it was not significantly associated with 24-hour SBP level ($P=0.279$). After adjusting for other clinical characteristics, CVR in ICAs (standardized $\beta=-0.389$; partial $R^2=0.127$; $P=0.011$) and CVR in MCAs (standardized $\beta=-0.380$; partial $R^2=0.121$; $P=0.007$) were independently associated with diabetes, and CVR in MCAs was marginally associated with 24-hour SBP level (standardized $\beta=-0.261$; partial $R^2=0.059$; $P=0.055$).

Candesartan Therapy

Although candesartan therapy was well tolerated in 37 patients, 3 patients developed dizziness during candesartan therapy; however, because the BP reduction in these patients was mild, we did not discontinue medication. The data were successfully obtained from all 40 patients after candesartan therapy.

After candesartan therapy, CVRs in ICAs and MCAs were significantly increased in the DHT and HT groups, and these increases were significantly greater in the DHT group than in the HT group, even after controlling 24-hour systolic BP (ICAs $P=0.03$; MCAs $P=0.015$; Table 3). On the other hand, the cerebral NAA level did not change. The increases in CVRs in ICAs and MCAs were independent of the reduction of for the 24-hour BP level (Figure 3).

Discussion

This is the first study that assessed the cerebral metabolism and hemodynamics simultaneously in DHTs and HTs, and clarified that in DHT patients, brain damage is more advanced than that in HTs. The reduced levels of neuronal mass and CVR found in DHTs were predominantly determined by the presence of diabetes and independent of 24-hour BP level; however, they were independent of each other.

Reduced Neuronal Mass

Cerebral NAA, an indicator of functional neuronal mass and axons,[13,14,22,23] was significantly lower in DHT patients than in HT patients and NT subjects. In previous studies on cerebral metabolism in congestive heart failure patients, occipital NAA was found to be decreased in patients with

TABLE 3. Comparison of Changes in Cerebral Parameters After Angiotensin Receptor Blockade (candesartan) Therapy

Variables	DHT Group (n=20)	HT Group (n=20)
BP, mm Hg		
Clinic SBP	−13.0±−19.7†	−7.7±−13.7‡
Clinic DBP	−5.1±−8.9‡	−0.4±−6.7
24-hour SBP	−7.4±−11.9‡	−2.6±−13.3
24-hour DBP	−3.5±−5.7‡	−3.0±−6.5‡
Cerebral metabolites, mmol/L per kg		
NAA	−0.05±0.53	0.04±0.42
Creatine	−0.13±0.91	0.04±0.51
Choline	0.01±0.19	0.09±0.21
Cerebral volume flow, mL/min		
ICAs	5.5±57.4	6.5±46.4
MCAs	−7.0±42.0	4.3±31.4
Cerebrovascular reserve, %		
ICAs	14.8±13.6*§	5.7±11.9‡
MCAs	20.2±19.0*§	7.3±11.2†

Changes were calculated as the values after candesartan therapy minus the baseline values, and data are shown as the mean±SD.

*$P<0.001$, †$P<0.01$, ‡$P<0.05$ are the values after candesartan therapy minus the baseline values, analyzed by the paired *t* test within each group; §$P<0.05$ vs the HT group by repeated-measures ANOVA with Bonferroni test.

severe heart failure with systolic dysfunction.[22,23] This reduction of cerebral NAA was significantly associated with poor prognosis.[23] The area we investigated in the brain was the deep white matter, which is an ischemia-prone watershed area between the cortical circulation and perforator circulation of the brain. The NAA in this area is predominantly located in axons, and hypertensive ischemic morphological change detected by brain MRI occurs most frequently in this area.[6,14] Previous MRI studies showed that ischemic white matter lesions are associated with cognitive dysfunction, depression, gait disturbance, and future stroke.[6,14] Thus, the reduced NAA in DHTs found in the present study seems to indicate a higher risk for psychocognitive dysfunction as well as cerebrovascular events in these patients. Actually, previous population studies showed that diabetes is associated with either an accelerated cognitive decline or an increased incidence of dementia.[4] The prevalence of advanced white matter lesions tended to be higher in DHTs than in the HTs and NTs; however, there was no statistical significance. The reduction of NAA in deep white matter precedes this morphological change in DHTs.

The mechanism of the alteration of cerebral metabolism in DHTs remains unclear. Because the NAA concentration in white matter was reported to be significantly reduced in patients with symptomatic ICA,[24] we speculated that an impaired cerebral circulation may contribute to neuronal damage. However, neither baseline cerebral blood flow nor CVR in ICAs and MCAs was significantly correlated with the reduction in cerebral NAA. In addition, reduced cerebral NAA was predominantly determined by the presence of diabetes and was independent of 24-hour BP level. The reduced NAA in DHTs may not be directly attributable to impaired cerebral microvessel function or elevated BP level, per se, but rather, may be predominantly attributable to direct adverse effects of diabetes-related activation of the apoptotic cell death pathway that exaggerate brain damage.[25]

Impaired CVR

CVR was significantly lower in the DHT group than in the NT group and marginally lower in the DHT group than in the HT group. The lower CVR in the MCA was determined not only by the presence of diabetes but also tended to be associated with higher 24-hour BP level. CVR is the capacity of cerebral microarteriolar dilation to occur in response to decreased cerebral perfusion pressure to maintain constant cerebral blood flow. Diminished CVR is considered to be a risk factor for stroke,[26,27] Persistent high BP and other factors, such as the RAS and inflammatory reactions, all of which are activated in DHTs, may directly impair cerebral microvessel function.

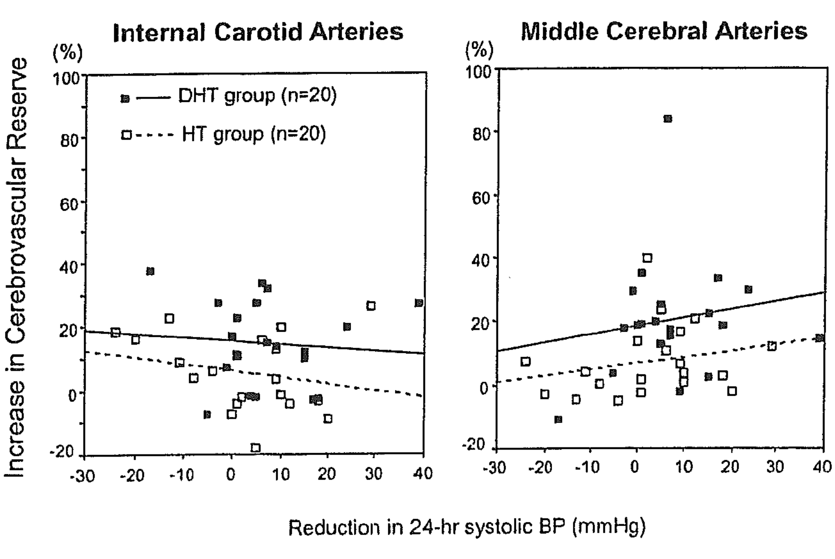

Figure 3. Association between changes of 24-hour systolic BP and CVR after candesartan therapy in DHT and HT groups.

In a recent report, despite effective antihypertensive treatment, resistance arteries from DHT patients showed marked remodeling that was greater than that of vessels from untreated HT subjects.[28]

Effect of ARB on CVR

Candesartan therapy for 3 to 4 months improved the reduced CVRs in ICAs and MCAs in the DHT and HT groups. This favorable effect was significantly greater in the DHT group than in the HT group. This result indicates that the RAS in cerebral microvessels might have some pathogenic role in the impaired CRV in hypertensives, particularly those with diabetes. The increases in CVR in ICAs and MCAs were independent of the reduction of the 24-hour BP level, indicating the BP-independent direct brain-protective effect of ARB. Clinically, this result appears to be in accord with the results of large clinical trials.[29,30] In the Losartan Intervention For Endpoint reduction in hypertension study (LIFE) of high-risk hypertensives, the stroke reduction by ARB was more marked in DHTs than HTs, independent of the BP-lowering effect.[29] The Study on Cognition and Prognosis in the Elderly (SCOPE) demonstrated that nonfatal stroke is reduced by candesartan treatment.[30] In NT rats and spontaneous hypertensive rats, candesartan restored cerebrovascular autoregulation without any influence on baseline cerebrovascular blood flow.[31]

Perspectives

The AT_1 receptor is known to be involved in cognitive function. However, the potential role of ARB in neuroplasticity remains unclear. Oral candesartan treatment very effectively inhibits the centrally mediated effects of angiotensin II, indicating that candesartan is an effective ARB in terms of crossing the blood–brain barrier.[32] In addition, because previous animal studies have shown that ARBs enable endogenous angiotensin II to stimulate neuronal regeneration via activation of AT_2 receptors,[11] we speculated that candesartan treatment might also restore the reduced cerebral NAA level, particularly in DHT patients. However, candesartan treatment for 3 to 4 months did not significantly alter the NAA level. Because NAA was measured in a small area of the brain, only changes in that small area would have been detected. This may have reduced the sensitivity of our ability to detect a change of NAA by candesartan therapy. A longer follow-up study of these patients may be required to demonstrate the potential beneficial effect of ARB on neuronal damage that has already occurred.

Because of the study limitation that the present study was an open one with ARB, a double-blind randomized controlled trial using renin-angiotensin-aldosterone system inhibitors and other antihypertensives of different classes will be necessary to confirm our results under similar levels of BP lowering. The results of this study provide a rationale for a long-term randomized controlled trial using RAS inhibitors for the prevention of stroke and cognitive dysfunction in preidentified DHT patients.

Conclusion

In hypertensive patients, the existence of diabetes is closely associated with advanced brain damage (reduced functional neuronal mass and CVR). ARB partly improved impaired cerebral microcirculation. This might provide an explanation if ARBs are found to have a benefit in improving clinical outcomes. This beneficial effect should be compared with the effect of a different class of antihypertensive with similar levels of BP lowering as a control in the future.

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BP Measurement

Determinants of Exaggerated Difference in Morning and Evening Blood Pressure Measured by Self-measured Blood Pressure Monitoring in Medicated Hypertensive Patients: Jichi Morning Hypertension Research (J-MORE) Study

Joji Ishikawa, Kazuomi Kario, Satoshi Hoshide, Kazuo Eguchi, Masato Morinari, Ruri Kaneda, Yuji Umeda, Shizukiyo Ishikawa, Toshio Kuroda, Yukihiro Hojo, Kazuyuki Shimada, on Behalf of the J-MORE Study Group

Background: Morning blood pressure (BP) surge in ambulatory BP monitoring was a risk factor for stroke in our previous study. We studied the determinants of the morning minus evening systolic BP difference (ME difference) in self-measured BP monitoring, as a possible risk factor for stroke in medicated hypertensive patients.

Methods: Nine hundred sixty-nine hypertensive outpatients receiving stable antihypertensive drug treatment were studied using self-measured BP monitoring in the morning and evening.

Results: The ME difference ranged from −37.3 to 53.3 mm Hg (mean 7.9 mm Hg). The highest quartile (Q4) of the ME difference group (>15.0 mm Hg) had older age (68.0 ± 9.8 years *v* 66.2 ± 10.3 years, $P = .01$) and higher prevalence of men (48.3% *v* 39.9%, $P = .02$), regular alcohol drinkers (34.7% *v* 26.0%, $P = .01$) and β-blocker use (26.9% *v* 19.9%, $P = .03$) than the other quartile groups (Q1 to Q3), whereas there was no significant difference in the average of morning and evening (ME average) BP. In logistic regression analysis controlling for ME average and other confounding factors, independent risks for Q4 of ME difference were older age (10 years older: odds ratio [OR] 1.21, $P = .01$, 95% confidence interval (CI) 1.04–1.42), regular alcohol drinker (OR 1.51, $P = .04$, 95% CI 1.01–2.26), and β-blocker use (OR 1.50, $P = .02$, 95% CI 1.06–2.12).

Conclusions: Older age, β-blocker use, and regular alcohol drinking were significant determinants of the exaggerated ME difference in medicated hypertensive patients.
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Key Words: Self-measured blood pressure monitoring, hypertension, morning surge.

Cardiovascular events tend to occur most frequently in the morning.[1] Elevated morning blood pressure (BP) level was shown to be associated with target organ damage, such as left ventricular hypertrophy[2] and microalbuminuria,[3,4] in some cross-sectional studies. The Ohasama study, a prospective study in the northern part of Japan, showed that morning BP measured by self-measured BP monitoring was an independent predictor of future stroke[5] and mortality.[6] Therefore, morning BP level plays an important role in the incidence of cerebrovascular disease; however, clear evidence about the risk of BP surge in the morning has hitherto been lacking.

Recently, we reported that exaggerated morning systolic BP surge (the morning BP [average of 4 to 5 BP readings during the first 2 h after wake-up time] minus the lowest BP [average of 3 BP readings centered on the lowest night-time reading]) evaluated by ambulatory BP monitoring was an independent risk factor for the prevalence of silent cerebral infarcts and the incidence of stroke events independently of the 24-h BP level.[7] Moreover, morning minus evening systolic BP difference (ME difference) from ambulatory BP monitoring was also shown to be an independent predictor of stroke.[8]

Self-measured BP monitoring is a possible substitute

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From the Division of Cardiology and the Department of Community and Family Medicine, Jichi Medial School, Tochigi, Japan.

Address correspondence and reprint requests to Dr. Kazuomi Kario, Division of Cardiovascular Medicine, Department of Medicine, Jichi Medical School, 3311-1 Yakushiji, Minamikawachi, Kawachi, Tochigi, 329-0498, Japan; e-mail: kkario@jichi.ac.jp

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for ambulatory BP monitoring.[9] In the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure guidelines (JNC7),[10] the self-measured BP level was evaluated as the average of all BPs measured in the morning and in the evening. However, ME difference may have additional clinical usefulness for the management of hypertensive patients, and exaggerated ME difference with high morning BP and low evening BP may be a risk factor for cardiovascular disease even in medicated hypertensive patients with a well-controlled ME average.

In this study, we investigated ME difference as a possible alternative to the morning ambulatory BP surge, and examined its determinants in medicated hypertensive patients.

Methods

Patients

We studied 1027 hypertensive outpatients with stable antihypertensive drug treatment for at least 3 months. They were consecutively recruited from 43 doctors in 32 different clinics and hospitals in Japan.

Smoking was defined as having a current smoking habit. Chronic renal disease was defined as overt proteinuria or elevated serum creatinine level more than 176.8 μmol/L (2.0 mg/dL). Diabetes mellitus was defined as more than 7.0 mmol/L (126 mg/dL) of fasting blood glucose or more than 11.1 mmol/L (200 mg/dL) casual glucose level in patients who were not treated or treated for diabetes mellitus. Glucose intolerance was defined as fasting blood glucose level in the range of 6.1 to 6.9 mmol/L (110 to 125 mg/dL). Hyperlipidemia was defined as more than 5.7 mmol/L (220 mg/dL) total cholesterol level or more than 1.7 mmol/L (150 mg/dL) triglyceride level. Clinical histories of the patients were obtained from interviews by the patient's own doctors.

All of the antihypertensive medications were classified as calcium channel blockers (CCB), angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), β-blockers, diuretics, α-blockers, and others. Patients who were taking verapamil or diltiazem and dihydropyridine calcium channel blocker were classified as taking one CCB; αβ-blocker was classified as β-blocker. The institutional review board of Jichi Medical School approved this study, and informed consent was obtained from all patients.

Study Protocol

Morning and evening BP were measured using commercially available self-measured BP devices of which the accuracy was validated. All of the patients were instructed to measure BP using a cuff oscillometric device on the same upper arm position for 3 days. If the patients were not using their own self-measured BP devices in daily practice, cuff oscillometric semiautomatic devices (UA-631, A&D, Tokyo, Japan)[11] were given to them for this study. Self-measured BP was conducted twice on each occasion in a seated and relaxed position with the arm bare in the morning (within 1 h after waking, before having breakfast and taking medication) and evening (just before going to bed) for 3 consecutive days (total of six measurements). The first measurement was performed after more than 2 min of rest and the second measurement was performed after an interval of more than 30 sec. The patients were asked to document all of the self-measured BP value on the sheet and report them to their own physician.

Morning BP and evening BP were defined as the average of the first and the second self-measured BP values in the morning and in the evening, respectively, for 3 days (total of six BP measurements). The average of the morning and the evening systolic BP (ME average) was calculated. The ME difference was defined as morning systolic BP minus evening systolic BP.

Clinic BP was measured after resting for at least 5 min at two different clinic visits before and after the self-measured BP monitoring period. Clinic BP was defined as the average of the BPs measured at two visits (9 AM to 5 PM). We did not adjust the time of clinic BP measurements at trough time.

Statistical Methods

After excluding the 58 patients, those on night-shift work (25 patients) and incomplete data sets (33 patients), statistical analyses were conducted for 969 patients using the computer software SPSS version 11.0J (SPSS Inc., Chicago, IL). The comparisons of two parameters were performed by the two-tailed nonpaired t test and comparisons of categorical variables were performed by the χ^2 test. One-way analysis of variance (ANOVA) was performed to detect differences among groups, and Tukey's honestly significant differences (HSD) test was used for multiple pairwise comparisons of means among groups. Odds ratio (OR) and the 95% confidence interval (CI) were calculated by multiple logistic regression analysis. A probability value $< .05$ was considered statistically significant.

Results

Patient Characteristics

The age of the total study population ranged from 32 to 95 years (mean ± SD: 66.5 ± 10.2 years) and 407 men and 562 women were enrolled. All of the 969 patients were taking one or more antihypertensive medications: CCB (71.2%), ACEI (27.3%), ARB (31.6%), β-blockers (21.7%), α-blockers (10.6%), diuretics (12.6%), and others. Thirty-three percent of the patients were taking antihypertensive medication in the evening or before going to bed. Hyperlipidemia was observed in 40.9% of patients. Diabetes mellitus or impaired glucose was observed in 15.9% of patients. Regular alcohol drinkers constituted 28.3% of all patients. Current smokers constituted 12.2%. History of cardiovascular events included angina pectoris (8.3%), myocardial infarction (5.6%), and stroke (7.4%). Chronic renal disease was present in 5.0% of the patients.

BP Control Status

Clinic BP, morning BP, evening BP, and ME average were 143.0 ± 15.6/80.7 ± 10.1 mm Hg, 139.8 ± 14.6/81.7 ± 10.0 mm Hg, 131.8 ± 14.2/75.9 ± 9.8 mm Hg, and 135.8 ± 13.2/78.8 ± 9.3 mm Hg, respectively. Systolic ME average was controlled to less than 135 mm Hg in 472 patients (49.3% of all patients). We considered 140 mm Hg for clinic systolic BP and 135 mm Hg for self-measured systolic BP at home as the cutoff level, according to the JNC7.[10] Well-controlled clinic systolic BP was seen in 422 patients (43.6% of all patients). Masked morning systolic hypertension (clinic systolic BP <140 mm Hg and self-measured systolic BP in the morning ≥135 mm Hg) was present in 218 patients (22.5% of all patients and 51.7% of well-controlled clinic systolic BP patients).

ME Difference

The ME difference ranged from −37.3 to 53.3 mm Hg (mean: 7.9 mm Hg) and the highest quartile (Q4) of ME difference was more than 15.0 mm Hg (n = 240, median: 21.3 mm Hg). Even in the 472 patients (49.3%) with well-controlled systolic ME average (≤135 mm Hg), exaggerated ME difference (>15 mm Hg) was seen in 109 patients (23.1%) (Fig. 1). The ME difference was not correlated with the ME average (r = 0.04, P = .24), although morning BP and evening BP were correlated with ME difference (morning systolic BP: r = 0.43, P < .001; evening systolic BP: r = −0.37, P < .001).

Determinants of Exaggerated ME Difference

We compared the Q4 group of ME difference group with the other three quartile groups (Q1 to Q3) and evaluated the determinants of the exaggerated ME difference (Table 1). The patients in the Q4 group of ME difference were older (68.0 ± 9.8 v 66.2 ± 10.3 years, P = .01) and had a higher prevalence of male gender (48.3% v 39.9%, P = .02), regular alcohol drinkers (drinker) (34.7% v 26.0%, P = .01), and β-blocker users (26.9 v 19.9%, P = .03) than those in the Q1 to Q3 groups. The prevalence of smokers tended to be lower in the Q4 of ME difference patients than that in the Q1 to Q3 patients (9.5% v 13.1%, P = .17). There was no significant difference in the ME average between the two groups (Q4 v Q1 to Q3: 137.2 ± 14.2 v 135.3 ± 12.9 mm Hg for systolic BP, P = .06; 78.8 ± 8.7 v 78.9 ± 9.5 mm Hg for diastolic BP, P = 0.90) (Table 2). There was no significant difference in the prevalence of patients who were taking antihypertensive medication at night or before going to bed between the two groups (Q4 v Q1 to Q3: 32.2% v 33.6%, P = .753).

In multiple logistic regression analysis, the OR (95% CI) for the Q4 of ME difference were 1.21 (1.04–1.42) for age (10-year increase) (P = .013), 1.50 (1.06–2.12) for β-blocker use (P = .02), 1.51 (1.01–2.26) for drinkers (P = .04), and 0.52 (0.31–0.87) for smokers (P = .01) (Table 3).

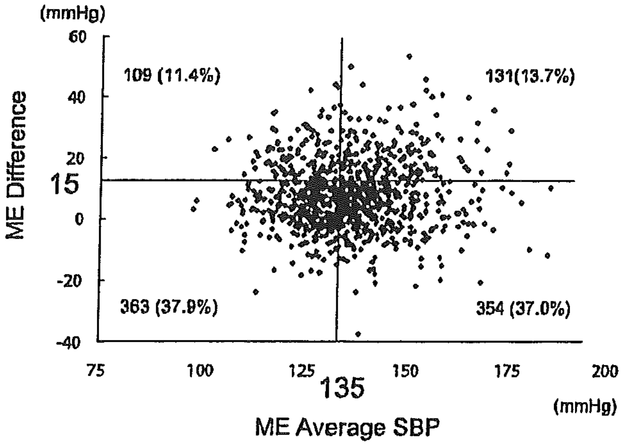

FIG. 1. Prevalence of exaggerated ME difference. ME difference = morning systolic blood pressure (SBP) − evening SBP; ME Average SBP = average of morning SBP and evening SBP.

Regular Alcohol Drinkers

Drinkers had significantly lower evening systolic BP (129.9 ± 14.0 v 132.6 ± 14.2 mm Hg, P = .01) and higher evening heart rate (70.6 ± 10.5 v 67.9 ± 8.6 beats/min, P < .001) than nondrinkers. Morning diastolic BP was significantly higher in drinkers (drinkers versus nondrinkers: 83.6 ± 10 v 81.0 ± 9.9 mm Hg, P < .001), whereas morning systolic BP did not show a significant difference (drinkers versus nondrinkers: 140.1 ± 14.4 v 139.6 ± 14.7 mm Hg, P = .66).

The increase of the ME difference in drinkers was more prominent in elderly patients (aged ≥65 years) than in younger patients (aged <65 years), although ME average was higher in both drinker and nondrinker elderly patients (Fig. 2). The morning systolic BP level was significantly higher in elderly patients than in younger patients in both drinkers and nondrinkers (elderly versus younger patients: 141.2 v 136.9 mm Hg in nondrinkers, P < .001; 142.0 v 137.2 mm Hg in drinkers, P < .01).

Smokers

Smokers had a reduced risk for ME difference in this study. Morning systolic BP (139.7 ± 14.9 v 139.9 ± 12.4 mm Hg, P = .93) and evening systolic BP (131.7 ± 14.2 v 133.0 ± 14.3 mm Hg, P = .34) were not significantly different between nonsmokers and smokers.

Determinants of Morning BP

The patients in the highest quartile of morning systolic BP level (>150 mm Hg) were significantly older (68.9 ± 10.0 v 65.7 ± 10.2 years, P < .001), more used antihypertensive drug classes (1.9 ± 0.9 v 1.7 ± 0.9, P = .01), had a higher prevalence of ACEI use (32.6% v 25.6%, P < .05) and α-blocker use (15.3% v 9.1%, P < .01), had higher clinic systolic BP level (147.6 ± 1.0 v

Table 1. Patient characteristics

	ME Difference		
	The Lower 3 Quartiles (n = 727) (−37.3–14.7 mm Hg)	The Highest Quartile (n = 242) (15–53.3 mm Hg)	P
Age (y)	66.2 ± 10.3	68.0 ± 9.8	.01
Gender (% male)	39.9	48.3	.02
Body mass index (kg/m^2)	24.3 ± 5.0	23.9 ± 3.3	n.s.
Smoker (%)	13.1	9.5	n.s.
Regular alcohol drinker (%)	26.0	34.7	.01
Hyperlipidemia (%)	40.3	42.6	n.s.
Diabetes or IGT (%)	16.6	13.6	n.s.
Chronic renal disease (%)	5.0	5.0	n.s.
Stroke (%)	7.4	7.4	n.s.
Angina pectoris (%)	8.4	7.9	n.s.
Myocardial infarction (%)	5.1	7.0	n.s.
Types of drugs	1.7	1.8	n.s.
Calcium channel blockers (%)	71.0	71.9	n.s.
Short—Intermediate	13.2	9.5	n.s.
Long acting	59.8	62.8	n.s.
β-Blockers (%)	19.9	26.9	.03
ACE Inhibitors (%)	27.5	26.9	n.s.
ARBs (%)	31.2	32.6	n.s.
α-Blockers (%)	10.0	12.4	n.s.
Diuretics (%)	12.5	12.8	n.s.
Nitrates (%)	1.5	1.2	n.s.

Nonpaired t test.
n.s. = not significant ($P \geq .05$); IGT = impaired glucose tolerance;
ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blocker.

141.5 ± 0.6 mm Hg, $P < .001$) and higher evening systolic BP level (144.6 ± 13.6 v 127.6 ± 11.7 mm Hg, $P < .001$). In multiple logistic regression analysis, the significant determinants for the highest quartile of morning systolic BP level was older age (10-year increase: OR 1.27, 95% CI 1.06–1.52, $P = .01$) and evening systolic BP level (OR: 1.12, 95% CI 1.10–1.14, $P < .001$) after adjustment by use of antihypertensive drug classes, ACEI use, α-blocker use, and clinic systolic BP level.

Discussion

Self-measured BP data were obtained in 969 consecutive hypertensive patients using antihypertensive medication. The

Table 2. Blood pressure and pulse rate

	ME Difference		
	The Lower 3 Quartiles (n = 727) (−37.3–14.7 mm Hg)	The Highest Quartile (n = 242) (15.0–53.3 mm Hg)	P
Clinic SBP (mm Hg)	143.1 ± 15.8	142.8 ± 15.2	n.s.
Clinic DBP (mm Hg)	80.8 ± 10.2	80.3 ± 9.6	n.s.
Clinic PR (/min)	72.9 ± 10.8	71.5 ± 10.0	n.s.
Morning SBP (mm Hg)	136.8 ± 13.2	148.8 ± 15.0	<.001
Morning DBP (mm Hg)	80.6 ± 10.0	85.3 ± 9.3	<.001
Morning PR (/min)	65.6 ± 9.0	64.6 ± 9.2	n.s.
Evening SBP (mm Hg)	133.9 ± 13.6	125.7 ± 14.3	<.001
Evening DBP (mm Hg)	77.0 ± 9.7	72.5 ± 9.1	<.001
Evening PR (/min)	68.4 ± 9.1	69.3 ± 9.6	n.s.
ME Average SBP (mm Hg)	135.3 ± 12.9	137.2 ± 14.2	n.s.
ME Average DBP (mm Hg)	78.8 ± 9.5	78.9 ± 8.7	n.s.
ME Average PR (/min)	67.0 ± 8.6	66.9 ± 8.8	n.s.

Data are shown by mean ± standard deviation.
SBP = systolic blood pressure; DBP = diastolic blood pressure; PR = pulse rate; ME Average = average of morning and evening.

Table 3. Logistic regression analysis for the highest quartile of ME difference

	Model		
	OR	P	95% CI
Age (10 y)	1.21	.01	1.04–1.42
Male gender	1.35	.11	0.93–1.97
β-blocker use	1.52	.02	1.06–2.12
Smoking	0.52	.01	0.31–0.87
Regular alcohol drinker	1.51	.04	1.01–2.26
ME Average SBP (10 mm Hg)	1.09	.12	0.98–1.22

OR = odds ratio; ME average SBP = average of morning SBP and evening SBP.
Other abbreviations as in Table 2

ME average in self-measured BP was well controlled (<135 mm Hg) in 49.3% of all patients. The independent determinants for the exaggerated ME difference (>15 mm Hg, 23.1% of the total sample) were older age, regular alcohol drinking, and β-blocker use.

Regular Alcohol Drinking

The prevalence of regular alcohol drinkers was significantly higher in the exaggerated ME difference group (Q4) than in the other ME difference groups (Q1 to Q3). Kawano et al[12] reported that regular alcohol drinking had a biphasic effect on the self-measured BP profile at home (morning BP increase and evening BP decrease). The mechanism by which hypertension is induced by alcohol consumption is unclear; however, possible mechanisms have been reported to include an imbalance of the central nervous system, impairment of the baroreceptors, an increase in sympathetic activity, stimulation of the renin-angiotensin-aldosterone system, an increase in cortisol levels, an increase in intracellular calcium levels with a subsequent increase in vascular reactivity, stimulation of the endothelium to release endothelin or inhibition of endothelium-dependent nitric oxide production, and chronic subclinical withdrawal.[13] Evening alcohol intake at dinner may have contributed to the lower evening BP, and to increased sympathetic activity, which was expressed as increased evening pulse rate. The increased sympathetic activity could contribute to exaggerated morning BP surge, and thus to increase a ME difference.

Some prospective studies[14,15] showed that regular alcohol drinking increases the risk of cerebral bleeding. Heavy alcohol drinking may lead to hypertension with exaggerated morning BP surge and this may be a trigger for cerebral bleeding. On the other hand, regular alcohol drinking increased the risk for exaggerated ME difference in this study, although many prospective studies[16–19] have shown that mild-to-moderate alcohol drinking reduces the risk of cardiovascular disease. The beneficial effect of regular alcohol consumption may be partly explained by factors such as anti-inflammatory and anticoaglatory effects. The C-reactive protein (CRP), a marker of inflammation, is related to atherosclerosis[20] and higher a CRP level is a risk factor for cardiovascular events.[21] In regular alcohol drinkers, the CRP level had been reported to be decreased[22] and alcohol might have some anti-inflammatory effects in the pathogenesis of atherosclerosis. In addition, Mukamal et al[23] reported that mild-to-moderate alcohol consumption was associated with lower coagulability.

β-Blocker Use

In this study, the β-blocker use was found to be a determinant of exaggerated ME difference. Most of the patients were taking antihypertensive drugs once daily in the morning, but the dosage and timing of taking drugs were different among the patients. Antihypertensive drugs changed the effects on the morning BP[24–26] and Morgan and Anderson[27] compared the difference in the time-dependent effects during the day among placebo, felodipine (CCB), hydrochlorothiazide (diuretic), atenolol (β-blocker), and perindopril (ACEI) users. Atenolol did not reduce BP during sleep, and it caused a significantly smaller reduction of morning BP than the other three drugs.

Morning BP is affected by circadian variation of the autonomic nerves. Panza et al[28] measured forearm vascular resistance at three different times of day (7 AM, 2 PM, and 9 PM) and found that the basal forearm vascular resistance was significantly higher and the blood flow was significantly lower in the morning than in the afternoon and evening. The vasodilator effect of phentolamine (an α-adrenergic antagonist) was also most significant in the morning, indicating that there was a α-sympathetic nerve dominant BP increase in the morning. Pickering et al[29] evaluated the effect of a single daily dose of doxazosin (an α-blocker) given at night and found that the greatest reduction of BP occurred in the morning hours. We recently found that the predominant BP reduction in the morning due to doxazosin was associated with the pro-

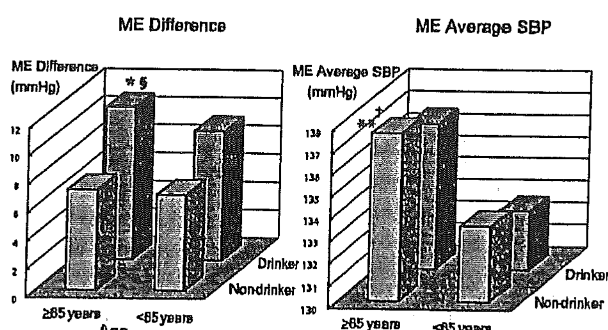

FIG. 2. Impact of age and drinking on ME difference. ME difference = morning systolic blood pressure (SBP) − evening SBP; ME Average SBP = average of morning SBP and evening SBP. *$P < .01$, **$P < .001$ v age <65 years and nondrinker group; §$P < .01$ v age ≥65 years and nondrinker group; †$P < .01$ v age <65 years and drinker group.

gression of silent hypertensive cerebral disease.[30] Thus, the association between the exaggerated ME difference and β-blocker may be due to the predominant α-sympathetic activation due to β-sympathetic blockade. However, because of the limitations of this study, further prospective evaluations will be needed to evaluate the relationship between β-blockers and exaggerated ME difference.

Age

Older age was also a risk factor for exaggerated ME difference in this study. Morning BP is influenced by α-sympathetic nerve activation.[31] Autonomic nerve function is altered with aging. In the elderly, muscle sympathetic nerve activity has been reported to be increased.[32] Dinenno et al[33] reported that human aging is associated with a reduction in forearm postjunctional α_1-adrenergic responsiveness to endogenous norepinephrine release. In addition, autonomic support of BP changes with aging are due to decreased cardiac vagal inhibition of heart rate and cardiac output and basal sympathetic activity.[34] These imbalances of α_1-sympathetic nerve and β-sympathetic nerve effects may cause increased variability of BP in the elderly.

Baroreceptor sensitivity, a regulator of BP, plays an important role in the regulation of BP and has been reported to be decreased in the elderly.[35] Jones et al[36] showed that aging in men was associated with a marked reduction in baroreceptor buffering of BP and that this was related to increases in basal sympathetic nerve activity and a reduction in systemic α_1-adrenergic vascular responsiveness. In a study of hypertensive patients using direct BP and electrocardiogram monitoring for a 24-h period, the baroreflex sensitivity index (BRI) measured on the basis of the ratio delta RR/delta Ps (delta Ps = spontaneous decrease in systolic BP, delta RR = change in RR) was minimal early in the morning.[37] These data show that impaired baroreceptor sensitivity is a key physiological mechanism of exaggerated ME difference in relation with the predominant α-sympathetic activity in the elderly. Actually in this study, the effect of alcohol on the ME difference was greater in elderly hypertensives than in younger hypertensives.

Smoking

Smoking is a risk factor for cerebrovascular disease. Smoking increases BP and heart rate during the smoking period,[38] although the relationship between smoking and sustained hypertension is controversial.[39] Mann et al[40] reported that smoking is associated with increased daytime BP without causing a change in night-time BP. We expected that smoking would be associated with an increase in ME difference; however, the obtained result was the opposite.

Study Limitations

There is no data that show the prognostic significance of morning minus evening systolic BP difference (ME difference) by self-home BP measurements. In our preliminary analysis, which showed that the ME difference was an independent predictor for stroke, the ME difference was defined by the ambulatory BP data.[8] Because ME difference measured using morning and evening self-home measurements (awake, seated home measurements) may have different prognostic significance from ME difference based on the ambulatory BP data, further studies will be necessary to evaluate the clinical significance of ME difference measured by self-home BP measurements.

The self-measured BP level can be a risk for target organ damage and cerebrovascular events. We showed the determinant of ME difference and how to evaluate self-home BP in treated hypertensive patients.

In addition, ME difference include two types. One is the exaggerated morning BP elevation, and the other is the large evening BP reduction. We were unable to exclude the effect of evening BP reduction (such as regular alcohol drinkers) in exaggerated ME difference. Moreover, the determinants of absolute value of ME difference was almost the same as that of ME difference (morning minus evening BP value).

In conclusion, older age, regular alcohol drinking, and β-blocker use were independent determinants of the risk of exaggerated ME difference in medicated hypertensive patients. Morning BP levels should be monitored in medicated hypertensive patients having these conditions, even if their clinic BP is well controlled.

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Regular Article

Incidence of heparin-PF4 complex antibody formation and heparin-induced thrombocytopenia in acute coronary syndrome

Takefumi Matsuo[a,*], Takanobu Tomaru[b], Kazuomi Kario[c], Takeshi Hirokawa[d]
on behalf of HIT Research Group of Japan

[a]*Hyogo Prefectural Awaji Hospital, Sumoto, 656-0013, Japan*
[b]*Clinical Physiology, Toho University Sakura Hospital, Tokyo, Japan*
[c]*Cardiology, Jichi Medical School, Tochigi, Japan*
[d]*Clinical Research Projects, Mitsubishi Pharma Corporation, Tokyo, Japan*

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Percutaneous coronary intervention

Abstract A multicenter prospective study on the rate of seroconversion of antibodies to heparin-PF4 complexes (heparin-induced thrombocytopenia [HIT] antibodies) during and after heparin treatment for 4 weeks was carried out in Japanese patients with acute coronary syndrome (ACS). A total of 254 ACS patients treated with heparin were enrolled consecutively from 12 facilities of cardiology. Two patients with preexisting HIT antibodies were excluded from the analysis. The total seroconversion rate for four weeks during and after heparin treatment was 8.7% (n=22, 95% confidence interval [CI]: 5.9—13.1), including values of 3.2% (n=8) at the end of heparin infusion and 5.5% (n=14) at 4 weeks. Among 22 seroconverted patients, four developed HIT and two of the four had the complication of thrombosis. The incidence of HIT was 1.6% (n=4, 95% CI: 0.04—3.1). The risk for thromboembolic development was higher in the seroconverted patients (odds ratio, 17.4, 95% CI: 5.2—58.4, p<0.0001) than nonconverted patients. An analysis of factors affecting the seroconversion rate was carried out. The seroconversion rate for ACS patients who underwent percutaneous coronary intervention (PCI; n=163) was 12.3%, significantly higher than the 2.3% in patients who did not undergo PCI (n=89), leading to an odds ratio of 6.1 (95% CI: 1.4—26.7, p=0.009). A significant odds ratio was obtained for each factor affecting the seroconversion: 3.5 (95% CI: 1.3—9.9, p=0.014) for more than 5 days of heparin infusion, 3.0 (95% CI: 1.2—7.6, p=0.035) for

* Corresponding author. Tel.: +81 799 22 1200; fax: +81 799 24 5704.
E-mail address: matsuo@awaji-hosp.sumoto.hyogo.jp (T. Matsuo).

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a thrombotic history and 2.7 (95% CI: 1.1–6.8, $p=0.039$) for hyperlipidemia. No other factor, including age or diabetes mellitus, contributed to the seroconversion. Therefore, PCI, duration of heparin treatment and thrombotic history facilitated the seroconversion in ACS patients. PCI patients treated for more than 5 days with heparin showed a maximal seroconversion rate of 18.3% (95% CI: 13.8–22.2). This high rate in PCI patients did not interact with age, type of underlying disease of unstable angina or myocardial infarction or thrombotic history.

In conclusion, ACS patients demonstrating seroconversion are at risk of thromboembolic development due to the likelihood of immunomediated endothelial dysfunction. The increase in the rate of seroconversion in ACS patients would be affected by factors such as PCI with mechanical stress, longer duration of heparin treatment, thrombotic history and presence of hyperlipidemia. If PCI is undertaken with heparin anticoagulation for more than 5 days, seroconversion would easily occur, and the seroconverted patients could subsequently suffer from HIT.

Introduction

Heparin treatment has been in use in many years, and there is now concern over heparin-induced thrombocytopenia (HIT), an immune-mediated thrombocytopenic disorder and a potentially life-threatening adverse reaction to therapy with heparin. HIT associated with thrombosis (HITT) is frequently complicated by venous and arterial thrombosis and can potentially lead to limb amputation and death in patients with HIT.

The formation of antibodies to heparin-PF4 complexes (HIT antibodies) is thought to be the main causative factor in the development of HIT. Namely, macromolecular immune-complexes of heparin-PF4 and HIT antibodies bind to platelets via their FcγIIA receptor leading to the aggregation of platelets, release of microparticles and hence to the production of thrombin [1]. The incidence of seroconversion in 109 medical patients treated with unfractionated heparin (heparin) was reported to be 17%, and one patient (0.9%) with thrombocytopenia, having a >50% decrease from baseline, was diagnosed with HIT. A follow-up assay of HIT antibodies has been a useful tool for the detection of heparin-treated patients at risk of developing HITT [2]. The rate of seroconversion of HIT-antibodies was higher in patients who underwent cardiopulmonary bypass surgery without developing HIT than those who actually developed HIT. This concept has been depicted in the iceberg model of HIT [3]. The difference in the seroconversion rate between the sources of heparin was studied in 207 patients undergoing cardiac surgery. The rates were 44.4% and 30.6% for bovine and porcine heparin, respectively. It has been suggested that bovine heparin induces seroconversion more than porcine heparin [4]. A follow-up study after the onset of HIT showed that HIT antibodies fell to undetectable levels at a median of 50 to 85 days and did not recover with subsequent reexposure to heparin [5]. In the clinical setting, several subtypes of HIT, including rapid [5], delayed [6] and early-onset [7], have been reported besides the typical onset, often associated with acute systemic reaction and/or thrombotic complications. However, the location of a thrombus has thought to be influenced by atherosclerosis, postoperative state and the mechanical stress of catheter manipulations, and to be superimposed by HIT antibody-induced endothelial damage. Procoagulant activity derived from the damaged endothelium may also contribute to thrombosis [8].

To estimate the incidence of seroconversion in hospitalized patients with acute coronary syndrome (ACS) requiring heparin anticoagulation, a prospective cohort study was conducted. Also, some of the factors facilitating seroconversion in patients with ACS were studied. Percutaneous coronary intervention (PCI) is usually chosen to achieve the revascularization of a narrowed or occluded coronary artery in the clinical setting. This study was also carried out to clarify whether or not PCI promotes seroconversion because PCI induces plaque disruption and platelet activation through mechanical stress and is prone to cause thrombogenesis.

Materials and methods

A total of 254 patients with ACS who had received heparin (unfractionated porcine heparin, Japan pharmacopoeia) at 12 medical facilities of cardiology were enrolled consecutively between May 2001 and May 2002. Informed consent was obtained from each patient. The diagnosis of

ACS, consisting of unstable angina and acute myocardial infarction with or without ST-segment elevation, was based on clinical, electrocardiographic and angiographic presentations and cardiac enzyme tests, correlating with the duration and extent of coronary occlusion as a consequence of plaque rupture.

There were 109 patients with unstable angina and 145 patients with acute myocardial infarction. Blood samples withdrawn before heparin treatment as the baseline, 24 h later, at the end of heparin treatment and at 4 weeks, respectively, were used for tests of the platelet count and HIT antibodies in addition to routine chemical laboratory tests. PCI was performed in 165 patients, with a success rate of potency of 98%. Heparin was administered intravenously to achieve optimal anticoagulation under the activated partial thromboplastin time (APTT), targeting a value of 1.5–2.5 times the baseline. The combined use of antiplatelet drugs was employed in 96.0% of 254 patients. Aspirin was used with ticlopidine and/or cilostazol in an estimated 71.7% of patients (Table 1).

Hematological analysis, including the platelet count, was performed at the same time as the blood sampling. Serum samples were frozen immediately and stored at −70 °C until completion of the HIT antibody test. The HIT antibody titer was measured with a sandwich-type ELISA (Asserachrom, HPIA, Stago, France), according to the instructions of a kit that detected HIT antibodies including IgG, IgA and IgM isotypes. While the results of the ELISA test merely confirm the presence of HIT antibody, HIT was defined as a >50% reduction in the platelet count or an absolute platelet count of $<100\times10^9$/L during and after heparin treatment with no other cause of thrombocytopenia and a positive result in the HIT-antibody test corresponding to thrombocytopenia. As the ELISA carried a high rate of false-positive results, a positive patient without HIT-related thrombocytopenia was not suspected of having HIT [9]. When a patient with HIT showed thromboembolic development, it was evaluated whether the development was compatible with HIT-associated thrombosis or not.

Table 1 Clinical characteristics of 254 patients

Age (mean±S.D., year old)	66.4±11.4
Sex, *n* (%)	
Male	205 (80.7%)
Female	49 (19.3%)
Details of ACS, *n* (%)	
Unstable angina pectoris	109 (42.9%)
Acute myocardial infarction	145 (57.1%)
PCI performed, *n* (%)	
Performed	165 (65.0%)
Not performed	89 (35.0%)
Heparin application (mean±S.D.)	
A bolus dose ($\times10^4$ IU)	0.9±0.2
Maintenance dose (×IU/kg/h)	8.1±2.4
Days of infusion	5.0±3.0
Mean APTT prolongation(times baseline)	2.3±1.6
Previous heparin history, *n* (%)	63 (24.8%)
Combined use of drugs, *n* (%)	
Antiplatelet drug:	244 (96.0%)
Aspirin	61 (24.0%)
Aspirin+ticlopidine or cilostazol	123 (48.4%)
Aspirin+ticlopidine+cilostazol	52 (20.5%)
Others (dipiridamole or sarpogrelate)	13 (5.1%)
Tissue plasminogen activator	30 (11.8%)
Warfarin	21 (8.3%)

ACS: acute coronary syndrome.
APTT: activated partial thromboplastin time.
PCI: percutaneous coronary intervention.

Clinical outcomes, including death and thromboembolic developments, were studied. Thromboembolic development associated with or without HIT was evaluated by clinical and echoradiographic imaging, and was classified into venous, arterial and cardiac thrombosis. Cardiac thrombosis was subclassified into subacute coronary thrombosis, new or recurrent myocardial infarction and intracardiac thrombosis. Bleeding episodes from arterial or venous puncture sites and intracranial, gastrointestinal and genitourinary tracts were recorded for 4 weeks. A statistical analysis was conducted. Paired continuous variables were compared using the paired *t* test. Categorical variables were compared using the chi square test, and odds ratios with confidence intervals (CI) are presented. All statistical tests were two-sided, and significance was defined as $p<0.05$.

Results

There was no correlation between platelet counts and the titers of HIT antibodies, and there was no significant decrease in platelet counts associated with the seroconversion during the study term. Two patients already had a positive HIT titer before heparin treatment and were excluded from the analysis. At the end of the infusion period (5.0±3.4 days, mean±S.D.), eight patients had become positive. While off heparin, 14 patients were newly found to be positive. Thus, a total of 22 patients (8.7%, 95% CI: 5.9–13.1) seroconverted during the study term. The median optical density (OD) of HIT antibodies determined by ELISA in the seroconverted patients had also increased at the end of heparin infusion and further 4 weeks later (Fig. 1).

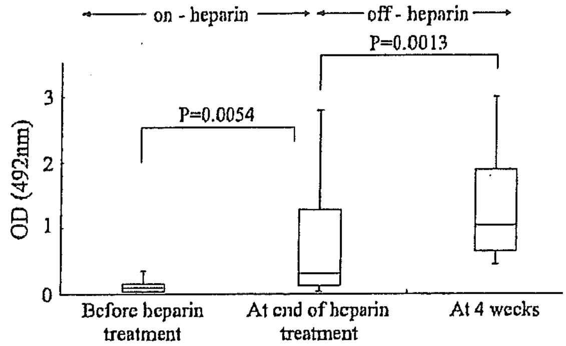

Figure 1 Median optical density (OD) of heparin-PF4 complex antibodies in patients on and off heparin infusion. n=22, excluding two patients having preexisting HIT antibodies. Box plots indicate the median, interquartile range (25–75%) and outliers (min, max).

It was examined whether there was a new or exacerbating thromboembolism between seroconverted and nonconverted patients. Thromboembolic developments were found significantly more often in the 22 seroconverted rather than the 230 nonconverted patients. The odds ratio of risk for thrombotic developments during the study term rose to 17.4 (95% CI: 5.2–58.4) in seroconverted patients compared with nonconverted patients (Table 2). Thromboembolic developments in seven seroconverted patients occurred during the period of heparin infusion rather than cessation of heparin treatment (25.0 vs. 0.9%, $p<0.001$). Among the 22 patients who showed seroconversion, four developed thrombocytopenia with a 50% reduction in platelet counts from the baseline as a clear sigh of HIT. Two of these four patients who developed thrombotic events (one, coronary stent thrombosis; the other, myocardial infarction) were diagnosed as having HIT with thrombosis. Of the other five seroconverted patients with thromboembolic developments, two suffered from thromboembolic events (one, subacute coronary stent thrombosis; the other, recurrent myocardial infarction) on the first day in the hospital, and another two patients developed recurrent myocardial infarction on the third day after the heparin infusion. However, they did not show any drop in the platelet count, and their HIT antibodies remained negative at the time of these events. One seroconverted patient developed an ischemic stroke without thrombocytopenia 13 days after the heparin treatment ceased. These five patients could be excluded as having definite HIT because of neither HIT-compatible thrombocytopenia nor seroconversion. Of six nonconverted patients who showed no drop in the platelet count comparable to HIT, five were treated with PCI and subsequently developed thromboembolism (two, recurrent myocardial infarction; one, stent thrombosis, intracardiac thrombosis, ischemic stroke, respectively). The other patient who received no PCI developed myocardial infarction from an unstable angina.

Odds ratios were calculated to identify factors affecting the seroconversion in ACS (Table 3). Aging itself did not facilitate the seroconversion. The highest odds ratio, 6.1 (95% CI: 1.4–26.7), was obtained in ACS patients treated with PCI. The infusion of heparin for over 5 days (odds ratio, 3.5,

Table 3 Factors affecting the rate of seroconversion in 252 ACS patients

Factors	N	Seroconversion rate (%)	Odds ratio (95% CI)	p-value
PCI				
Not performed	89	2.3	6.1 (1.4–26.7)	0.009
Performed	163	12.3*		
Underlying disease				
Unstable angina	107	3.7	3.6 (1.2–11.1)	0.022
Myocardial infarction	145	12.4*		
Period of heparin treatment				
<5 days	122	4.1	3.5 (1.3–9.9)	0.014
≧5 days	130	13.1*		
Thrombotic history				
No thrombosis	207	6.8	3.0 (1.2–7.6)	0.035
Thrombosis	45	17.8*		
Complications				
Normolipidemia	187	6.4	2.7 (1.1–6.5)	0.039
Hyperlipidemia	65	15.4*		
Nondiabetic	181	6.6	2.3 (1.0–5.6)	0.081
Diabetes mellitus	71	14.1		
Age				
<70 years	136	8.8	1.0 (0.4–2.4)	1.000
≧70 years	116	8.9		

* $p<0.05$.

Table 2 Comparison of thromboembolic development rate between seroconverted and nonconverted patients

	Seroconverted n (%): 22 (100)	Nonconverted n (%): 230 (100)	Odds ratio (95% CI)	p-value
Thromboembolic development	7[a] (31.8)	6 (2.6)	17.4 (5.2–58.4)	<0.0001

[a] Includes two patients having HIT with thrombosis.

95% CI: 1.3–9.9), myocardial infarction as an underlying disease (odds ratio, 3.6, 95% CI: 1.2–11.1), hyperlipidemia (odds ratio, 2.7, 95% CI: 1.1–6.5) and diabetic complications (odds ratio 2.3, 95% CI: 1.0–5.6) all facilitated the seroconversion. In 45 patients having a thrombotic history, including cerebrovascular (n=9), cardiovascular (n=33) and peripheral vascular diseases (n=3), the odds ratio was estimated at 3.0 (95% CI: 1.4–26.7).

In a multiple-logistic regression analysis of factors affecting the seroconversion rate, significantly adjusted odds ratios were obtained for PCI (adjusted odds ratio; 6.5, 95% CI: 1.5–29.1, p=0.014) and over 5 days heparin infusion (4.3, CI: 1.5–12.4, p=0.008), respectively. Therefore, both the PCI procedure and over 5 days heparin infusion were important factors contributing to the seroconversion.

Seroconversion rates in 163 ACS patients who underwent PCI increased significantly to 18.3% when the patients received heparin for over 5 days (p=0.030). In the PCI patients who had a thrombotic history, hyperlipidemia, diabetes mellitus or either underlying disease, each factor contributing to the seroconversion increased compared with that in PCI patients associated with none of these factors (Table 4). The increased risk of seroconversion in the group who underwent PCI was characteristic when ACS patients having one of these factors were treated with the PCI procedure. Overall outcome was estimated with three deaths due to cardiogenic shock in 230 nonseroconverted patients. Six bleeding episodes from four gastrointestinal tracts and two arterial puncture sites were also found, including two patients on heparin and four patients off heparin, among the non-seroconverted patients.

Table 4 Factors affecting the rate of seroconversion on heparin treatment in 163 patients with PCI

Factors	N	Seroconversion rate (%)	Odds ratio (95% CI)	p-value
Period of heparin treatment				
<5 days	81	6.2	3.4 (1.2–9.9)	0.030
≧5 days	82	18.3*		
Thrombotic history				
No thrombosis	131	9.9	2.5 (0.9–7.0)	0.075
Thrombosis	32	21.9		
Complications				
Normolipidemia	115	9.6	2.2 (0.8–5.7)	0.120
Hyperlipidemia	48	18.8		
Nondiabetic	109	10.1	1.9 (0.7–4.6)	0.310
Diabetes mellitus	54	16.7		
Underlying disease				
Unstable angina	44	6.8	2.3 (0.6–8.1)	0.284
Myocardial infarction	119	14.3		

* p<0.05.

Discussion

The seroconversion rate measured by ELISA and the clinical characteristics of 254 ACS patients treated with heparin were studied. Two subjects having preexisting HIT antibodies were excluded from the analysis. The seroconversion rate in the remaining ACS patients was estimated at 8.7% 4 weeks after the start of heparin treatment. Twenty-two patients became positive, and four patients, including two with thrombosis, developed HIT during the study term. The incidence of thromboembolic development was significantly higher in seroconverted patients than nonconverted patients. HIT antibodies have a characteristic binding affinity for PF4 in a complex with heparin-like molecules on the endothelium and generate the expression of tissue factor. And the occlusion of the coronary artery after PCI could be brought about by interaction with tissue factor in atheroma and platelets leading to the generation of prothombinase and thrombin [10]. It is possible that HIT antibodies in seroconverted patients induce the production of thrombin through the expression of tissue factor, and the HIT antibodies themselves might be a cause of thrombophilia.

Seroconversion increases the chance of a complex forming with PF4 under optimal conditions with heparin as a source of the antigen. As HIT antibodies were found in 17% of patients treated with heparin for more than 7 days, the presence of HIT antibodies in patients with heparin therapy has been concluded to predict the risk of developing HIT unless the antibodies become negative [11]. Therefore, a longer infusion of heparin may precipitate the chance of seroconversion. The incidence of seroconversion in patients with deep vein thrombosis treated with heparin increased from 9.1% on day 5–7 to 20.1% on day 21. Also, positive seroconversion increased in accordance with the duration of treatment with low molecular weight heparin [12]. Although seroconversion rates of 27–50% were reported among patients undergoing cardiac surgery [13–15], the incidence of HIT appeared to be less than 2% [16]. In the present study, the incidence of HIT was 1.6%, a figure similar to that for cardiac surgery, although the rate of seroconversion following cardiac surgery is much higher than 8.7% in ACS patients. The incidence of serologically confirmed HIT in 358 heparin-treated patients, including those with heart or cerebrovascular diseases, was very low (0.3%), and there were 30 seroconverted patients with no thrombocytopenia, a rate of 8.4% [17].

In this study, 20 of 22 seroconverted patients underwent PCI with heparin anticoagulation. PCI requires an adequate heparinization to avoid thrombosis caused by endothelial disruption due to catheter manipulation during the procedure. PCI with adequate heparinization leads to a state of hypercoagulability during and after the intervention [18]. In addition to the PCI-induced endothelial disruption, HIT antibodies might evoke tissue factor expression through immunological injury, resulting in platelet to platelet interaction and endothelial hyperplasia. Endothelial hyperplasia is suggested to contribute to the growth of a thrombus in HIT [19]. In the present study, PCI was the main factor facilitating seroconversion. Other affecting factors included heparin treatment for over 5 days, a thrombotic history and hyperlipidemia.

However, an early cessation of heparin treatment after the onset of thrombocytopenia has been shown to be ineffective against morbid events in HIT patients [20]. In the present study, the seroconversion rate and titer were found to increase in patients on and off heparin, and the risk of developing HIT continued after the cessation of treatment. It is unclear whether a seroconverted patient in whom the cause of a sudden drop in the platelet count is unknown should be treated with an alternative anticoagulant. However, during PCI in patients with HIT, argatroban is a reasonable alternative [21]. PCI with heparin could lead to a positive seroconversion in the presence of one or more of these affecting factors, and the duration of treatment with heparin had a significant effect on seroconversion in PCI patients. It is suggested that PCI, duration of heparin treatment, thrombotic history and hyperlipidemia have a booster effect on the heparin-induced seroconversion.

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LETTERS TO THE EDITOR

ANOTHER DATA/RHETORIC MISMATCH ON DONEPEZIL

To the Editor: Lopez et al. report that "cholinesterase inhibitor (CEI) use had a clinically meaningful effect on the natural history of Alzheimer Disease (AD)," slowing disease progression and lowering risk of nursing home admission after 2 years.[1]

The design of the study is worrisome. Of 1,139 patients who enrolled in the AD Research Center over 7 years, 270 were selected; 135 began taking CEIs "immediately after enrollment, and continued to take them throughout the following 12 months," and 135 never took the drug. How these individuals were selected is not otherwise described. They were matched on a few characteristics, such as age, Mini-Mental State Examination score, and education.

This study resembles a study by Dr. Geldmacher et al. that showed that patients who took a CEI faithfully (80% of pills or more) had a significant delay in nursing home placement (NHP).[2] Both of these nonrandomized studies failed to report important baseline characteristics of the groups being studied. In the Geldmacher article, for example, nonadherent patients were far less likely to have a spouse caregiver than faithful users,[3] yet the authors, who claimed it was the donepezil that "resulted in significant delays in NHP," omitted this fact.[2]

Both papers are easily distinguished from AD 2000,[4] a properly randomized, controlled trial with the largest number of placebo-controlled patient-years of any cholinesterase study.[5] In AD 2000, "no significant benefits were seen with donepezil compared with placebo in institutionalization or progression of disability... [or] in behavioral and psychological symptoms, carer psychopathology, formal care costs, unpaid caregiver time, adverse events, or death or between 5 mg and 10 mg of donepezil."[4] In the United Kingdom, the National Institute for Clinical Excellence Appraisal Committee has recently issued the following preliminary recommendation: "Donepezil, rivastigmine and galantamine are not recommended for use in the treatment of mild to moderate Alzheimer's disease (AD)."[6]

Would Dr. Lopez modify the discussion of his paper, where he emphasizes the important benefits of donepezil, in view of the results from AD 2000, a larger, better-designed trial that failed to show any meaningful difference at all?

Thomas E. Finucane, MD
Johns Hopkins Geriatrics Center
Baltimore, MD

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RESPONSE TO DR. FINUCANE

To the Editor: We previously addressed Dr. Finucane's critique of our article.[1,2] We will therefore focus on his comparisons of our article to the work of Dr. Lopez et al.[3] and the AD 2000 trial.[4] Our study and the Lopez et al. study both support a role for cholinesterase inhibitors in the treatment of Alzheimer's disease (AD), specifically as they pertain to delayed nursing home placement. Otherwise, even a cursory review reveals considerable differences in the research paradigm, funding agency, subject ascertainment, and analytical methods between studies.

Dr. Finucane repeats the incorrect assertion that we omitted differences in caregiver characteristics from our report. We explicitly state on p. 940 that caregiver type (spouse vs nonspouse) was included as a covariate in our analysis and that there were no statistically significant differences in the proportion of spousal caregivers across our exposure groups.[2]

We concur with Dr. Finucane that our study and the report by Dr. Lopez et al. are both easily distinguished from the AD 2000 trial, but Dr. Finucane fails to acknowledge major methodological shortcomings of the AD 2000 trial, including the facts that systematic diagnosis of AD was not required for entry into that study, investigators were instructed to enroll only subjects in whom treatment response could not be predicted (excluding those patients who were likely to show a treatment response), and marked underenrollment and excessive attrition rates were likely to have exerted significant biases on the interpretation of the data. Dr. Finucane's uncritical endorsement of the AD 2000 conclusions also does not acknowledge the approximately 70%

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