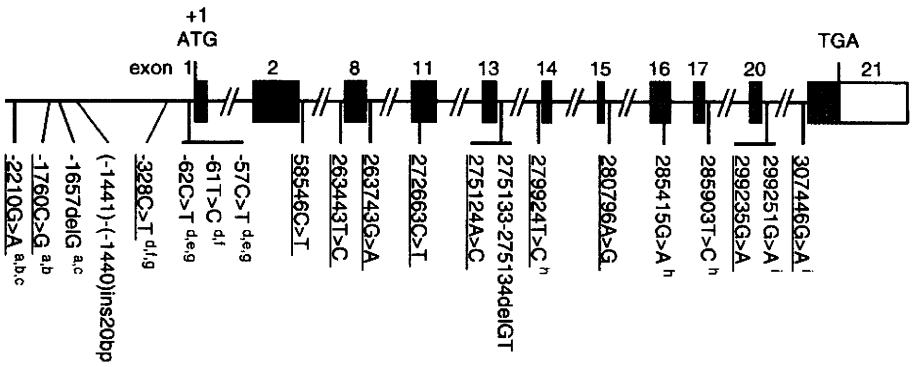

Figure 1 The locations of identified and genotyped single nucleotide polymorphisms (SNPs) in the insulin-like growth factor (IGF)-1 receptor gene. Only SNPs of which allele frequency is >5% are shown. ATG represents the initiation codon, and TGA represents the stop codon. a, b, c, d, e, f, g, h and i indicate haplotype blocks with tight linkage disequilibrium ($r^2>0.5$), and the underlined 12 SNPs were genotyped in this study. In all, 11 of 12 genotyped SNPs had been recorded in public databases (dbSNPs); that is, −2210G>A was identical to dbSNP ID rs8034564, −328C>T to rs13379905, 58546C>T to rs7174918, 263443T>C to rs2272037, 263743G>A to rs951715, 272663C>T to rs3743262, 275124A>C to rs1464430, 279924T>C to rs4486868, 280796A>G to rs2229765, 299235G>A to rs2684789 and 307446G>A to rs2593053.

logistic regression analysis was performed to identify the independent relation of some SNPs to LV hypertrophy and geometric change. Hardy–Weinberg equilibrium was calculated using a χ^2 test. A value of $P<0.05$ was accepted as statistically significant. All analyses were performed using StatView Version 5 Software (Abacus Concepts Inc., Berkeley, CA, USA). Linkage disequilibrium was evaluated by obtaining an r^2 value between polymorphisms using the SNP Alyze ver. 2.0 software (DYNACOM Co, Ltd, Shigehara, Japan).

Results

Locations of identified and genotyped SNPs in the IGF-1 receptor gene were shown in Figure 1. In total, 12 SNPs (three SNPs in promoter, one in exon and eight in intron regions) in the IGF-1 receptor gene were genotyped in the present study. In all, 11 of 12 SNPs had been recorded in public databases (dbSNPs, http://www.ncbi.nlm.nih.gov/SNP/), and the remaining one SNP (−1760C>G) was novel. The genotype distribution of all analysed SNPs did not significantly deviate from the Hardy–Weinberg expectation.

The association of SNP genotypes in the IGF-1 receptor gene with LV structural change was assessed by χ^2 analysis (Table 1). Among 12 SNPs, genotype frequencies of promoter −328C>T and intron-13 275124A>C polymorphisms were significantly associated with LV hypertrophy ($\chi^2=7.513$ and $P=0.023$) and LV concentric change ($\chi^2=7.949$ and $P=0.019$), respectively. Similarly, in allele frequencies, the C allele of −328C>T had a significant relation to LV hypertrophy (odds ratio 1.78, $\chi^2=6.828$ and $P=0.009$), and the A allele of 275124A>C was related to LV concentric change (odds ratio 1.38, $\chi^2=7.259$ and $P=0.007$).

Next, we compared clinical characteristics and echocardiographic parameters between different genotype groups of IGF-1 receptor −328C>T and 275124A>C polymorphisms. There were no significant differences in clinical parameters, such as age, sex, body mass index, hypertension duration, blood pressure and the use of antihypertensive agents between the two subject groups with CC ($n=702$) and CT+TT ($n=92$) of −328C>T (Table 2). However, LV wall thickness, LVMI, and the prevalence of LV hypertrophy were significantly increased in the group with CC genotype. LV systolic and diastolic function (fractional shortening, *E*/*A* ratio and deceleration time) did not differ between the two groups. As for 275124A>C polymorphism, similarly, no significant differences were found in clinical characteristics between the two-genotype groups with AA ($n=470$) and AC+CC ($n=321$) (Table 3).

Table 1 Association of SNP genotypes in the IGF-1 receptor gene with LV structural change

SNP	*Region*	*LV hypertrophy (LVMI≥ 125 g m^{-2})*		*LV concentric change (RWT≥0.44)*	
		χ^2	P	χ^2	P
−2210G>A	Promoter	1.561	0.458	1.550	0.461
−1760C>G	Promoter	0.480	0.787	0.403	0.818
−328C>T	Promoter	7.513	0.023	2.186	0.335
58546C>T	Intron 2	1.691	0.429	1.921	0.383
263443T>C	Intron 7	2.160	0.340	1.494	0.474
263743G>A	Intron 8	1.313	0.519	2.475	0.290
272663C>T	Exon 11	0.582	0.747	3.879	0.144
275124A>C	Intron 13	4.897	0.086	7.949	0.019
279924T>C	Intron 13	2.032	0.362	1.946	0.378
280796A>G	Intron 15	1.597	0.450	3.205	0.201
299235G>A	Intron 20	4.565	0.102	0.164	0.922
307446G>A	Intron 20	3.274	0.195	0.062	0.969

Abbreviations: IGF, insulin-like growth factor; LV, left ventricular; LVMI, left ventricular mass index; RWT, relative wall thickness; SNP, single nucleotide polymorphism.

Table 2 Comparison of clinical characteristics and echocardiographic parameters between the two groups with CC and CT+TT of IGF-1 receptor −328C>T polymorphism

	CC (n = *702*)	*CT+TT* (n = *92*)	P
Age, years	65 ± 10	63 ± 11	0.070
Sex (male), %	55	54	0.867
Body mass index, kg m^{-2}	24.4 ± 3.4	24.0 ± 3.3	0.278
Duration of hypertension, years	18 ± 11	17 ± 11	0.154
Diabetes mellitus, %	23	26	0.522
Fasting plasma glucose, mg per 100 ml	104 ± 20	102 ± 24	0.393
Haemoglobin A1c, %	5.7 ± 0.8	5.7 ± 0.8	0.908
Total cholesterol, mg per 100 ml	202 ± 32	207 ± 37	0.164
Triglycerides, mg per 100 ml	136 ± 118	149 ± 110	0.318
Serum creatinine, mg per 100 ml	1.0 ± 1.1	1.0 ± 1.1	0.999
Systolic blood pressure, mmHg	146 ± 19	143 ± 19	0.185
Diastolic blood pressure, mmHg	85 ± 13	83 ± 14	0.264
Heart rate, b.p.m.	70 ± 11	69 ± 8	0.702
Antihypertensive treatment			
Ca channel blockers, %	72	72	0.900
RAS inhibitors, %	52	48	0.500
β-blockers, %	36	36	0.996
Diuretics, %	24	16	0.121
Others, %	15	12	0.507
Total number of classes	2.0 ± 1.1	1.8 ± 1.1	0.264
Echocardiographic parameters			
IVSTd, mm	10.9 ± 2.0	10.4 ± 1.8	0.020
PWTd, mm	10.7 ± 1.8	10.3 ± 1.5	0.046
LVDd, mm	46.6 ± 5.0	45.8 ± 4.8	0.125
LVDs, mm	28.4 ± 5.4	28.1 ± 5.3	0.598
Fractional shortening	0.39 ± 0.08	0.39 ± 0.07	0.566
LVMI, g m^{-2}	130 ± 39	119 ± 28	0.007
RWT	0.47 ± 0.09	0.46 ± 0.08	0.323
Prevalence of LV hypertrophy, %	48	35	0.019
Prevalence of LV concentric change, %	58	52	0.256
E/A ratio	0.87 ± 0.32	0.88 ± 0.30	0.812
Deceleration time, ms	215 ± 48	217 ± 48	0.673

Abbreviations: IGF, insulin-like growth factor; IVSTd, interventricular septal thickness at end-diastole; LV, left ventricular; LVDd, left ventricular diameter at end-diastole; LVDs, left ventricular diameter at end-systole; LVMI, left ventricular mass index; PWTd, posterior wall thickness at end-diastole; RAS, renin–angiotensin system; RWT, relative wall thickness.
RAS inhibitors represent angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors. Values are mean ± s.d. or percentage.

Table 3 Comparison of clinical characteristics and echocardiographic parameters between the two groups with AA and AC+CC of IGF-1 receptor 275124A>C polymorphism

	AA (n = *470*)	*AC+CC* (n = *321*)	P
Age, years	66 ± 10	65 ± 11	0.209
Sex (male), %	55	56	0.831
Body mass index, kg m^{-2}	24.4 ± 3.3	24.4 ± 3.5	0.859
Duration of hypertension, years	18 ± 11	18 ± 11	0.758
Diabetes mellitus, %	24	23	0.672
Fasting plasma glucose, mg per 100 ml	104 ± 21	104 ± 21	0.820
Haemoglobin A1c, %	5.7 ± 0.8	5.7 ± 0.9	0.926
Total cholesterol, mg per 100 ml	202 ± 32	202 ± 34	0.887
Triglycerides, mg per 100 ml	138 ± 128	137 ± 100	0.902
Serum creatinine, mg per 100 ml	1.0 ± 1.0	1.0 ± 1.3	0.542
Systolic blood pressure, mmHg	145 ± 18	146 ± 20	0.428
Diastolic blood pressure, mmHg	84 ± 13	86 ± 14	0.113
Heart rate, b.p.m.	70 ± 10	70 ± 10	0.554
Antihypertensive treatment			
Ca channel blockers, %	73	71	0.548
RAS inhibitors, %	52	50	0.628
β-blockers, %	37	34	0.346
Diuretics, %	25	20	0.072
Others, %	14	15	0.516
Total number of classes	2.0 ± 1.1	1.9 ± 1.2	0.191
Echocardiographic parameters			
IVSTd, mm	10.9 ± 1.9	10.6 ± 2.0	0.036
PWTd, mm	10.7 ± 1.6	10.5 ± 1.9	0.334
LVDd, mm	46.5 ± 4.9	46.7 ± 5.1	0.500
LVDs, mm	28.3 ± 5.2	28.5 ± 5.7	0.587
Fractional shortening	0.39 ± 0.07	0.39 ± 0.08	0.981
LVMI, g m^{-2}	130 ± 37	127 ± 40	0.276
RWT	0.47 ± 0.08	0.45 ± 0.09	0.047
Prevalence of LV hypertrophy, %	49	42	0.069
Prevalence of LV concentric change, %	61	53	0.027
E/A ratio	0.86 ± 0.32	0.89 ± 0.31	0.389
Deceleration time, msec	214 ± 45	216 ± 51	0.652

Abbreviations: IGF, insulin-like growth factor; IVSTd, interventricular septal thickness at end-diastole; LV, left ventricular; LVDd, left ventricular diameter at end-diastole; LVDs, left ventricular diameter at end-systole; LVMI, left ventricular mass index; PWTd, posterior wall thickness at end-diastole; RAS, renin–angiotensin system; RWT, relative wall thickness.
RAS inhibitors represent angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors. Values are mean ± s.d. or percentage.

Among echocardiographic parameters, interventricular septal thickness, RWT and the prevalence of LV concentric change were significantly increased in the group with AA genotype, although LV dimension, LVMI, and LV systolic and diastolic function did not differ between the two groups.

To confirm whether the influence of these specific SNP genotypes in the IGF-1 receptor gene on LV structural changes was independent of various clinical parameters, we analysed possible predictive factors using a multiple logistic regression analysis in all subjects. As shown in Table 4, the presence of CC genotype of −328C>T was a significant predictor of LV hypertrophy, independent of age, sex, body mass index, hypertension duration, complication of diabetes mellitus, and systolic and diastolic blood pressure (odds ratio 1.67 vs CT + TT and $P = 0.033$). In addition, AA genotype of 275124A>C was found to be an independent determinant for LV concentric change (odds ratio 1.37 vs AC + CC and $P = 0.039$).

Finally, the combined effect of CC of −328C>T and AA of 275124A>C genotypes on LV geometric patterns was analysed. The patient group with both CC genotype of −328C>T and AA genotype of 275124A>C (group 2, $n = 424$) had a significantly higher rate of LV concentric hypertrophy compared with the other subjects (group 1, $n = 366$) (Figure 2). In contrast, the rate of patients with normal geometry was significantly lower in group 2 than in group 1. There were no differences in basal

Table 4 Independent relation of two IGF-1 receptor SNPs to LV hypertrophy and concentric change by multiple logistic regression analysis

	LV hypertrophy (LVMI⩾125 g m^{-2})		*LV concentric change (RWT⩾0.44)*	
	OR (95% CI)	P	*OR (95% CI)*	P
Age, 10 years	1.14 (0.97–1.34)	0.101	1.13 (0.97–1.33)	0.121
Sex, male	1.97 (1.44–2.68)	<0.001	1.38 (1.01–1.87)	0.040
Body mass index, 1 kg m^{-2}	1.06 (1.01–1.10)	0.020	1.09 (1.04–1.14)	<0.001
Hypertension duration, 1 year	1.01 (0.99–1.03)	0.168	1.01 (1.00–1.03)	0.052
Diabetes mellitus, yes	1.21 (0.84–1.73)	0.304	1.25 (0.86–1.82)	0.235
Systolic blood pressure, 10 mm Hg	1.14 (1.03–1.27)	0.015	1.06 (0.95–1.18)	0.328
Diastolic blood pressure, 10 mm Hg	0.84 (0.72–0.97)	0.019	0.86 (0.74–1.00)	0.051
IGF-1 receptor SNP genotype				
CC of −328C>T	1.67 (1.03–2.69)	0.033	1.19 (0.76–1.89)	0.449
AA of 275124A>C	1.22 (0.91–1.65)	0.187	1.37 (1.01–1.85)	0.039

Abbreviations: CI, confidence interval; IGF, insulin-like growth factor; LV, left ventricular; LVMI, left ventricular mass index; OR, odds ratio; RWT, relative wall thickness; SNP, single nucleotide polymorphism.

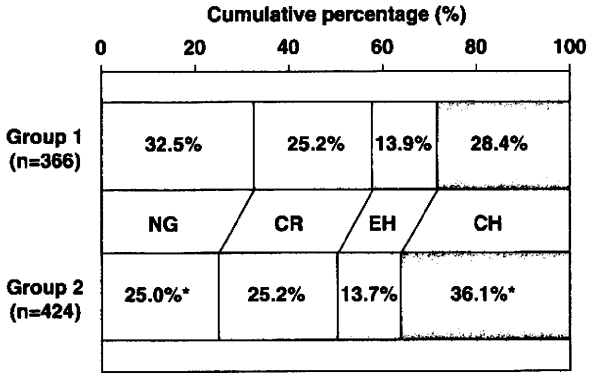

Figure 2 The combined effect of CC of −328C>T and AA of 275124A>C genotypes in the insulin-like growth factor (IGF)-1 receptor gene on left ventricular (LV) geometric patterns. Group 2 indicates the subjects with both CC genotype of −328C>T and AA genotype of 275124A>C, and the other subjects (that is, with CT+TT of −328C>T and/or AC+CC of 275124A>C) belong to group 1. CR, concentric remodelling (normal LVMI and increased RWT); CH, concentric hypertrophy (increased LVMI and RWT); EH, eccentric hypertrophy (increased LVMI and normal RWT); NG, normal geometry (normal LVMI and RWT). *$P<0.05$ compared with group 1.

clinical characteristics and antihypertensive treatment between the two groups (data not shown).

Discussion

Earlier studies have revealed that a promoter polymorphism in the IGF-1 gene is related to several cardiovascular complications, such as myocardial infarction, heart failure and cardiac hypertrophy.[20–23] On the other hand, the genetic variations in the IGF-1 receptor have been shown to be associated with non-cardiovascular human disorders, such as growth retardation, cancer, dementia and osteoporosis.[24–27] However, because IGF-1 receptor expression is also observed in the cardiovascular system, especially abundantly in the heart, the possible association of IGF-1 receptor gene polymorphisms with cardiac structure and function should be elucidated. In this study, we showed that two SNPs of the IGF-1 receptor gene, promoter −328C>T and intron-13 275124A>C, were significantly associated with LV hypertrophy in hypertensive patients. Thus, this is the first study that reported the significant influence of the IGF-1 receptor gene variation on cardiac hypertrophic change in human hypertension.

This study also showed that the combination of IGF-1 receptor −328C>T and 275124A>C polymorphisms was related to specific patterns of LV geometry. The patients with both CC genotype of −328C>T and AA genotype of 275124A>C had a significantly higher rate of LV concentric hypertrophy compared with the other subjects. These findings suggest the possibility that blood pressure level-independent diversity of LV structure in hypertensives is partially attributable to the genetic variation of the IGF-1 receptor. In addition, as for the association between LV geometry and cardiovascular prognosis, hypertensive patients with concentric hypertrophy among four LV geometric patterns have the highest incidence of cardiovascular events and death.[2,28] Therefore, having both CC genotype of −328C>T and AA genotype of 275124A>C may be a risk marker for poor cardiovascular prognosis in hypertensive subjects.

Our earlier *in vitro* study showed that IGF-1 promotes not only hypertrophy of cardiomyocytes but also collagen production by cardiac fibroblasts.[29] As cardiac fibrosis induces the deterioration of LV function, we examined whether −328C>T and 275124A>C polymorphisms were associated with LV systolic and diastolic dysfunction as well as LV hypertrophy. However, neither systolic function (fractional shortening) nor diastolic function (*E*/*A* ratio and deceleration time) had a significant association with these SNPs. Therefore, it is unlikely that LV function, apart from LV hypertrophy, may be influenced by IGF-receptor gene polymorphisms.

This study has not provided specific information regarding the mechanism by which the observed SNPs of the IGF-1 receptor gene influence LV mass and geometry. That is, it remains unclear whether these SNPs are functional or just risk markers. As −328C>T polymorphism is present in the promoter region, this SNP may affect the expression level of the IGF-1 receptor gene. On the other hand, 275124A>C polymorphism in intron-13 does not functionally affect the expression of the IGF-1 receptor protein. Further studies will be necessary to clarify the function of these polymorphisms or to identify the causative polymorphisms that are in linkage disequilibrium with these polymorphisms.

In addition, it remains to be elucidated whether the significant association of IGF-1 receptor gene polymorphisms with LV hypertrophy observed in hypertensive patients is also seen in other cardiac diseases, such as hypertrophic cardiomyopathy, valvular heart disease and old myocardial infarction.

In conclusion, this study showed that two SNPs of the IGF-1 receptor gene, promoter −328C>T and intron-13 275124A>C, were significantly associated with LV hypertrophy, and that the combination of these two polymorphisms was related to specific patterns of LV geometry in patients with essential hypertension. Thus, the genetic variation of the IGF-1 receptor may be involved in the diversity of LV structure in hypertensives, particularly in the progression of LV concentric hypertrophy.

What is known about this topic

- Blood pressure explains only 10–25% of the variation in left ventricular mass, suggesting that non-haemodynamic factors are involved in the cardiac growth in human hypertension.
- Insulin-like growth factor (IGF)-1 is a strong promoter of cardiomyocyte growth through its receptors abundantly expressed in myocardium.
- Circulating levels of IGF-1 are associated with left ventricular hypertrophy and geometric change in hypertensive subjects.

What this study adds

- Two single nucleotide polymorphisms of the IGF-1 receptor gene, promoter −328C>T and intron-13 275124A>C, were associated with left ventricular hypertrophy in patients with essential hypertension.
- The genetic variation of the IGF-1 receptor may be involved in the diversity of left ventricular structure in hypertensives.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

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Blood Pressure and Medication During Long-Term Antihypertensive Therapy Based on Morning Home SBP in Hypertensive Patients: Hypertension Control Based On Home Systolic Pressure (HOSP) Substudy

Yuhei Kawano, Takeshi Horio, Kei Kamide, Yoshio Iwashima, Fumiki Yoshihara, Satoko Nakamura

Division of Hypertension and Nephrology, National Cardiovascular Center, Osaka, Japan

ABSTRACT

We examined blood pressure (BP) and medication over 5 years in 80 hypertensive patients who participated in the Hypertension Control Based on home systolic pressure (HOSP) study that compares effects of strict and mild control of morning home systolic blood pressure (SBP) as well as amlodipine- and losartan-based regimens. Average morning home SBP after 5 years was 126 mmHg in the strict control group and 135 mmHg in the mild control group. The strict control group and the losartan group required more combination therapy than the other groups. These results show that long-term strict control of morning BP is feasible. Amlodipine appears to be more effective in controlling morning BP than losartan when the medication is administered alone in the morning.

KEYWORDS: hypertension; home blood pressure (BP); antihypertensive therapy; amlodipine; losartan

INTRODUCTION

It has been shown that home blood pressure (BP) is superior to office BP in the prediction of hypertension-related organ damage and prognosis (1–4). It is also known that systolic BP (SBP) is more closely associated with cardiovascular prognosis than diastolic BP (DBP) in the middle-aged and elderly population (5,6). In addition, BP shows circadian variation with morning rise, and cardiovascular events such as stroke and myocardial infarction occur most frequently in the morning (7,8).

We have been conducting a clinical trial based on morning home SBP named HOSP (Hypertension Control Based on Home Systolic Pressure) study (9,10). The HOSP study is a multicenter, randomized trial with two target levels of morning home SBP and two initial drugs in middle-aged and elderly patients with essential hypertension. The HOSP pilot study started in 2000 (9), and the main study was launched in 2003 (10). There are also several substudies that examine target organ damage and other clinical parameters at the National Cardiovascular Center (11).

Recent guidelines recommend strict BP control in the management of hypertension (12,13), although the control of hypertension in treated patients is still suboptimal (14,15). Self-measurement of BP at home is now popular in Japan, and the Japanese guidelines appreciate its usefulness in the diagnosis and management of hypertension (13). However, there are few studies that have assessed the feasibility of long-term strict control of home BP in hypertensive patients. In the present study, we examined the level of home BP and antihypertensive medication in patients who participated in the HOSP pilot study at our institute to assess the feasibility of strict control of home BP for 5 years.

METHODS

Subjects

The study subjects were 80 hypertensive patients who participated in the HOSP pilot study at the National Cardiovascular Center. The inclusion criteria of the HOSP pilot study were patients with essential hypertension, aged 40–79 years old, without cardiovascular complications, and with office and morning home SBP

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Correspondence: Yuhei Kawano, MD, PhD, Division of Hypertension and Nephrology, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan. E-mail: ykawano@hsp.ncvc.go.jp

TABLE 1 Characteristics and baseline data of study participants

Patient characteristics	
Number, sex	N = 80 (men 39, women 41)
Age (years)	64 ± 9
Dyslipidemia (%)	67
Diabetes mellitus (%)	7
Hyperuricemia (%)	9
Drinking habits (%)	42
Smoking habits (%)	5
ECG-LVH (%)	26
Microalbuminuria (%)	38
Baseline data	
Office blood pressure (mmHg)	159 ± 12 / 95 ± 9
Morning home blood pressure (mmHg)	150 ± 10 / 91 ± 9*
Evening home blood pressure (mmHg)	143 ± 15 / 87 ± 10 **,+

Abbreviation: ECG-LVH - left ventricular hypertrophy on electrocardiogram.
*P < 0.05; **P < 0.01 vs. office blood pressure;+P < 0.05 vs. morning home blood pressure.

of 140–199 mmHg during a drug-free period (9). Characteristics of the study participants a shown in Table 1. Sixty-six patients (82%) were previously treated and the remaining 14 patients (18%) were never treated. Written informed consent was obtained for all participants prior to the study.

Protocol

The study protocol was approved by the Institutional Review Board and the Ethical Committee of the National Cardiovascular Center. The HOSP pilot study is an open, randomized study with 2 × 2 factorial design. Patients were treated according to prespecified target levels of morning home SBP, and either a calcium channel blocker (CCB) amlodipine or an angiotensin receptor blocker (ARB) losartan was used as an initial agent. After a 4 week drug-free period, subjects were randomly assigned to strict control (target morning home BP <130 mmHg) or mild control (130–139 mmHg) group, and to amlodipine (2.5–5 mg once daily in the morning) or losartan (25–50 mg once daily in the morning) group. Several other classes of antihypertensive drugs (diuretics, beta-blockers, and alpha-blockers) were added if morning home BP was not controlled after a 3-month monotherapy period. If home BP did not reach the target level despite multiple combination therapies, the daily dose of amlodipine and losartan was increased to 10 mg and 100 mg, respectively. The treatment period was 5 years if possible.

Measurements

Office BP was measured twice in the sitting position by a physician using a mercury sphygmomanometer at every visit (2 weeks–2 months interval). Home BP was measured three times in the early morning (before breakfast) and late evening (before going to bed) in the sitting position almost everyday by patients using an automated validated device (Omron HEM-757, Omron Corp., Kyoto, Japan). Serum biochemical parameters and urinary albumin excretion were measured at the baseline period and during the treatment period.

Statistical Analysis

Office and home BPs at the baseline period (drug-free period) and at 3 months, 1 year, 3 years, and 5 years of the treatment period were used for statistical analysis. Office BP records at two visits (four records) were averaged, and home BP records for 3 days before the two visits (18 morning records and 18 evening records) were averaged. All data were expressed as mean ± SD. A student's *t*-test, a chi-square test, and an analysis of variance were applied where appropriate. The data were analyzed using the StatView software (version 5.0, Abacus Concept Inc., Berkeley, CA). P values less than 0.05 were considered statistically significant.

RESULTS

Average baseline office BP, morning home BP, and evening home BP are shown in Table 1. Office BP was significantly higher than home BPs, and morning home BP was significantly higher than evening home BPs. Follow-up data were obtained in 78 patients (98%) at year 1 and in 59 patients (74%) at year 5. The main reason of loss of follow-up was relocation of the subjects.

Figure 1 shows the time course of morning home SBP in strict and mild control groups. There were significant differences in home SBP between the two groups throughout the 5 years of treatment period. Although the average SBP level did not reach the target level in either strict control group (134 ± 11 mmHg) or mild control group (141 ± 12 mmHg) at month 3 (end of monotherapy period), morning home SBP achieved the target level in both groups from year 1 to year 5. The average level of morning home SBP was 126 mmHg in

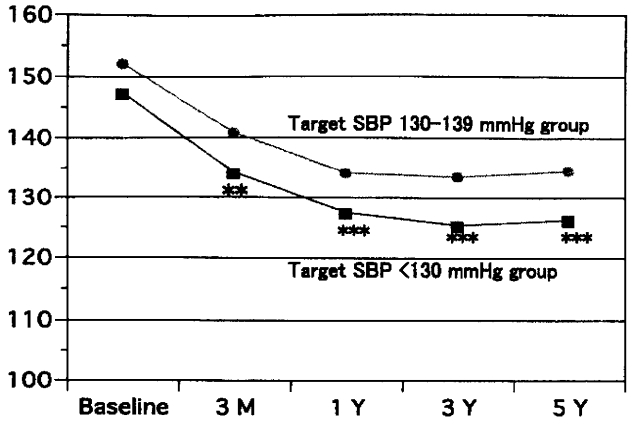

FIGURE 1 Time course of morning home systolic blood pressure (SBP) in strict and mild control groups. **P < 0.01; ***P < 0.001 between groups.

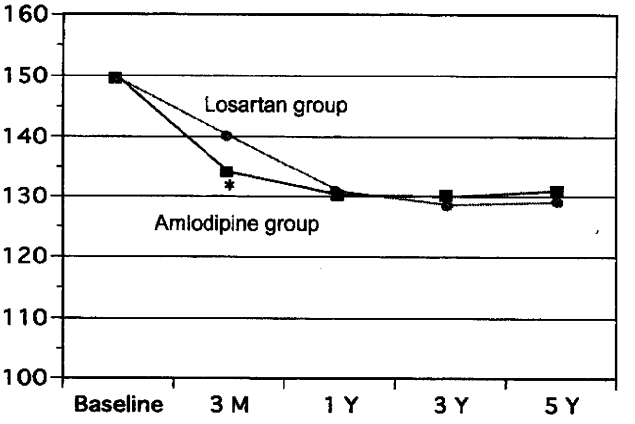

FIGURE 2 Time course of morning home SBP in amlodipine and losartan groups. *P < 0.05 between groups.

the strict control group and 135 mmHg in the mild control group at year 5 (Table 2). Office SBP and evening home SBP were also significantly lower in the strict control group than in the mild control group at the end of the treatment period.

Figure 2 shows the time course of morning home SBP in amlodipine and losartan groups. Morning home SBP was significantly lower in the amlodipine group (134 ± 8 mmHg) than in the losartan group (140 ± 13 mmHg) at the end of the monotherapy period (month 3); however, the difference disappeared from year 1 to year 5. At the end of the treatment period, there were no significant differences in office BP and home BPs between the two groups (Table 2).

The rate of target BP achievement is shown in Table 3. The achievement rate was about 80% in a whole group and in each subgroup. About half of the patients required combination therapy, and the average number of antihypertensive drugs in all subjects was 1.7 (Table 3). Beta blockers were most frequently used in the amlodipine group, while diuretics were the most common concomitant drugs in the losartan group. The rate of combination therapy and the number of antihypertensive drugs were significantly higher in the strict control group than in the mild control group. The losartan group also needed more combination therapy and antihypertensive drugs than the amlodipine group.

DISCUSSION

In the present study, morning home SBP achieved target levels for 5 years in the strict control group as well as in the mild control group with antihypertensive therapy using amlodipine or losartan as an initial agent. This result indicates that long-term control of home SBP to less than 130 mmHg is feasible in the treatment of hypertension.

It is well known that hypertension is an important risk factor for cardiovascular disease (5,6,16), and antihypertensive treatment is effective in improving the cardiovascular prognosis in hypertensive patients (17). The control of hypertension in treated patients,

TABLE 2 Office and home blood pressures after 5 years

	All subjects (n = 59)	Mild control group (n = 28)	Strict control group (n = 31)	Amlodipine group (n = 31)	Losartan group (n = 28)
Office SBP (mmHg)	138 ± 13	142 ± 12	135 ± 13*	137 ± 13	140 ± 13
Morning home SBP (mmHg)	130 ± 7	135 ± 6	126 ± 5***	131 ± 7	129 ± 7
Evening home SBP (mmHg)	127 ± 10	131 ± 10	123 ± 7***	130 ± 8	124 ± 11
Office DBP (mmHg)	81 ± 8	81 ± 8	80 ± 7	81 ± 8	80 ± 8
Morning home DBP (mmHg)	81 ± 7	82 ± 7	80 ± 7	82 ± 7	80 ± 8
Evening home DBP (mmHg)	77 ± 8	77 ± 8	77 ± 8	79 ± 7	75 ± 8

Abbreviations: SBP - systolic blood pressure; DBP - diastolic blood pressure.
*P < 0.05; ***P < 0.001 vs. mild control group.

TABLE 3 Rate of target blood pressure achievement and combination therapy and number of antihypertensive drugs after 5 years

	All subjects	Mild control group	Strict control group	Amlodipine group	Losartan group
Target BP achievement (%)	79	81	77	82	75
Combination therapy (%)	46	29	61*	32	61^{+}
Number of antihypertensive drugs	1.7 ± 0.8	1.4 ± 0.7	1.9 ± 0.9*	1.5 ± 0.7	1.9 ± 0.9^{+}
Concomitant antihypertensive drugs					
Diuretics (n)	17	6	11	4	13
Beta blockers (n)	17	4	13	8	9
Alpha blockers (n)	5	1	4	2	3

*$P < 0.05$ vs. mild control group; $^{+}P < 0.05$ vs. amlodipine group.

however, is still not satisfactory despite wide distribution of many antihypertensive drugs (14,15) and a recommendation of strict BP control by guidelines (12,13). The level of home BP is generally lower than that of office BP, and recent guidelines adopted 135/85 mmHg as a criterion for hypertension by home BP (12,13). However, the control of home BP in treated patients also appears to be suboptimal in general practice. In the Japan Home Versus Office Blood Pressure Measurement Evaluation (J-HOME) study, the average level of home BP in treated patients was 140/82 mmHg and 60% of patients were not adequately controlled (18). Our results suggest that a stepped care approach based on home BP is effective in the control of home and office BPs.

It has been shown that home BP is more closely related to target organ damage and cardiovascular prognosis than office BP (1–4,19). However, antihypertensive trials have been based on office BP measured during daytime. Although there are several studies that examined BP control by home BP monitoring (20,21), there are few trials that assess the effects of different target levels of home BP on cardiovascular prognosis in hypertensive patients. Ongoing HOSP main study (10) and hypertension objective treatment based on measurement by electrical devices of blood pressure (HOMED-BP) study (22) will provide valuable information about the optimal level of home BP in the management of hypertension.

In the present study, morning home SBP was significantly lower in the amlodipine group than in the losartan group at the end of the monotherapy period, and the amoldipine group needed less combination therapy and antihypertensive drugs than the losartan group at the end of follow-up. However, the effect of both drugs on evening home BP at the monotherapy period was comparable. These results indicate that morning administration of amlodipine is more effective in reducing morning BP than morning administration of losartan.

The different effects of amlodipine and losartan on morning BP appear to be related to their pharmacokinetic profiles rather than the mechanisms of antihypertensive action. The plasma half-life of losartan after oral administration is 1.5–2 h although that of active metabolite E-3174 is longer (4–5 h) (23). On the other hand, the plasma half-life of amlodipine is 33–50 h, and is much longer than that of losartan (24). It has been shown that the effect of losartan on morning home BP was smaller than other ARBs, and its morning/evening ratio is about 0.5 (25). We and others observed sustained the BP-lowering effect of amlodipine for 24 h with the trough/peak ratio of 0.85–0.9 by ambulatory BP monitoring (24,26). Therefore, amlodipine is superior to losartan in 24-hour BP control with once daily administration.

However, losartan appears to be superior to amlodipine regarding the renal protective effect. In a HOSP substudy, the reduction in urinary albumin excretion was significant in the losartan group but not in the amlodipine group (11). A similar renoprotective effect of losartan in comparison to amlodipine was also shown in patients with chronic kidney disease and hypertension in a Japan Losarton Therapy intended for the global renal protection in hypertensive patients (JLIGHT) study (27).

In conclusion, long-term control of morning home SBP to less than 130 mmHg is feasible in most hypertensive patients. Amlodipine appears to be more effective than losartan in lowering morning BP with once daily administration in patients with hypertension when the medication is administered alone in the morning.

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Chronic kidney disease as an independent risk factor for new-onset atrial fibrillation in hypertensive patients

Takeshi Horio[a], Yoshio Iwashima[a], Kei Kamide[a], Takeshi Tokudome[b], Fumiki Yoshihara[a], Satoko Nakamura[a] and Yuhei Kawano[a]

Objective **Chronic kidney disease (CKD) has recently been recognized to be a powerful predictor of cardiovascular morbidity and mortality. Atrial fibrillation (AF), which is a common arrhythmia in hypertensives, is associated with increased risks of cardiovascular events and death. However, the association between CKD and the onset of AF has not been fully elucidated. The present study assessed the hypothesis that CKD may influence the onset of AF in hypertensives.**

Methods **A total of 1118 hypertensive patients (mean age, 63 years) without previous paroxysmal AF, heart failure, myocardial infarction, or valvular disease were enrolled. CKD was defined as decreased glomerular filtration rate (<60 ml/min per 1.73 m^2) and/or the presence of proteinuria (≥1+).**

Results **During follow-up periods (mean, 4.5 years), 57 cases of new-onset AF were found (1.1% per year). Kaplan–Meier curves revealed that the cumulative AF event-free rate was decreased in the CKD group (log-rank test $P<0.001$). By univariate Cox regression analysis, age, smoking, left atrial dimension, left ventricular mass index, and the presence of CKD were significantly associated with the occurrence of AF. Among these possible predictors, CKD (hazard ratio 2.18, $P=0.009$) was an independent determinant for the onset of AF in multivariate analysis. Advanced stages of CKD (stages 4 and 5) were strongly related to the increased occurrence of AF.**

Conclusion **The present study demonstrated that the complication of CKD, especially progressed renal dysfunction, was a powerful predictor of new-onset AF in hypertensive patients, independently of left ventricular hypertrophy and left atrial dilatation.** ***J Hypertens*** **28:1738–1744 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: atrial fibrillation, hypertension, kidney, proteinuria, renal function

Abbreviations: AF, atrial fibrillation; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; IVST, interventricular septal thickness; LA, left atrial; LV, left ventricular; LVDd, left ventricular diameter at end-diastole; LVD, left ventricular diameter at end-systole; PWT, posterior wall thickness; RAS, renin–angiotensin system

[a]Division of Hypertension and Nephrology, Department of Medicine, National Cardiovascular Center, Suita, Japan and [b]Cardiovascular Center Research Institute, Suita, Japan

Correspondence to Dr Takeshi Horio, MD, Division of Hypertension and Nephrology, Department of Medicine, National Cardiovascular Center, 5-7-1, Fujishirodai, Suita, Osaka 565-8565, Japan
Tel: +81 6 6833 5012; fax: +81 6 6872 7486; e-mail: thorio@ri.ncvc.go.jp

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Introduction

Atrial fibrillation (AF) is the most common clinically significant arrhythmia in patients with hypertension, even in the absence of antecedent valvular heart disease or coronary artery disease. AF is a significant risk factor for ischemic stroke and heart failure events, and is also associated with increased risks of total and cardiovascular death, especially due to stroke [1]. Therefore, the occurrence of AF in hypertensive patients not only decreases their quality of life but also has a considerable influence on their prognosis and survival. Older age, blood pressure levels, especially ambulatory systolic blood pressure, increased left ventricular (LV) mass, and increased left atrial (LA) size have been known to be risk factors for the onset of AF in hypertensive patients [2–5]. In particular, a previous study showed that age and LV mass were independent determinants of AF incidence in initially untreated patients with essential hypertension [3].

Renal impairment is a powerful predictor of cardiovascular prognosis. Decreased estimated glomerular filtration rate (eGFR) is clearly associated with the increase in future cardiovascular events [6]. Proteinuria, even microalbuminuria, also increases the risk of cardiovascular events and death [7]. Thus, the involvement of renal impairment in the development of cardiovascular disease has recently been noticed. However, no study has shown the association between the onset of AF and renal impairment in hypertensive patients. To assess the hypothesis that chronic kidney disease (CKD) may affect the incidence of AF, the present study investigated the influence of renal impairment and CKD on the new onset of AF in hypertensives.

Methods

Study participants

From 1263 consecutive hypertensive patients who underwent echocardiography at the Division of Hypertension and Nephrology of our hospital between February 1997 and October 2003, 1118 patients (580 men and 538 women; mean age, 63 years) with normal sinus rhythm

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who had had no history of previous paroxysmal AF and in whom biochemical and urinary data were simultaneously obtained were enrolled in the present study. Patients with various cardiac disorders such congestive heart failure, myocardial infarction, myocardial disease, pericardial disease, valvular heart disease, LV asynergy, or LV systolic dysfunction (fractional shortening <0.25) were excluded from this study. Individuals after permanent pacemaker implantation or patients receiving dialysis were also excluded. Hypertension was defined as a systolic blood pressure of 140 mmHg or more and/or a diastolic blood pressure of 90 mmHg or more by repeated measurements or when medication was taken for treatment of hypertension. Diabetes mellitus was diagnosed according to the American Diabetes Association criteria, such as a fasting plasma glucose of 7.0 mmol/l or more and/or a plasma glucose level at 2 h after a 75-g oral glucose load of 11.1 mmol/l or more, or when medication was taken for treatment of hyperglycemia.

All procedures of the present study were carried out in accordance with institutional and national ethical guidelines for human studies. All participants enrolled in this study were Japanese, and all gave informed consent to participate in this study.

Echocardiography

A comprehensive two-dimensional echocardiography was performed using a cardiac ultrasound unit (Sonos 5500; Philips Medical Systems, Andover, Massachusetts, USA) as previously described [8]. Echocardiographic parameters were measured by the consensus of two experienced investigators who were blinded to the clinical data of the patients. Measurements included interventricular septal thickness (IVST), posterior wall thickness (PWT), LV diameter at end-diastole (LVDd), LV diameter at end-systole (LVDs), and LA diameter. LV fractional shortening was calculated as (LVDd-LVDs)/LVDd. LV mass was estimated using the formula validated by Devereux and Reichek [9]: LV mass $(g) = 1.04 \times \{(IVST + PWT + LVDd)^3 - LVDd^3\} - 13.6$. LV mass was normalized for body surface area and expressed as the LV mass index.

Clinical parameters

At the time of the echocardiographic examination, blood pressure, heart rate, and body mass were determined. Blood pressure was measured by a physician in a hospital outpatient clinic with the patient in a sitting position after over 10 min of rest, using an appropriate-size arm cuff and mercury sphygmomanometer. The first and fifth Korotkoff sounds were used to identify systolic and diastolic values, respectively, and measurements were taken to the nearest 2 mmHg.

Peripheral blood and urine samples were obtained in the morning after an overnight fast. The serum creatinine level was determined by the enzymatic method and eGFR was calculated by the formula of the Modification of Diet in Renal Disease Study with a modified equation for Japanese [10]: eGFR (ml/min per $1.73\,m^2$) = $194 \times age^{-0.287} \times serum\ creatinin^{-1.094} \times 0.739$ (if woman). Urinary protein excretion was assessed by the dipstick test from spot urine samples.

CKD was defined as decreased eGFR less than 60 ml/min per $1.73\,m^2$ and/or the presence of proteinuria (≥1+). The classification of CKD stages was performed according to the guidelines of the National Kidney Foundation classification of CKD [11] as follows; eGFR 90 ml/min per $1.73\,m^2$ or more with proteinuria (stage 1), eGFR 60–89 ml/min per $1.73\,m^2$ with proteinuria (stage 2), and stages 3, 4, and 5 were classified by the levels of eGFR (30–59, 15–29, and <15 ml/min per $1.73\,m^2$, respectively), regardless of the presence of proteinuria.

Follow-up

After the initial assessment, all patients visited our hospital periodically (every 1–2 months) for the treatment of hypertension and concurrent diseases. The pulse and heart beat were checked at every examination. Individuals with irregular pulse or cardiac rhythm and/or patients with complaint of palpitation or chest discomfort received 12-lead electrocardiogram and 24-h Holter recordings. In addition, all patients received standard 12-lead electrocardiogram at least once a year. AF was defined as absence of P waves before each QRS complex, irregular atrial electrical activity with fibrillatory waves varying in size, shape and timing, and completely irregular RR intervals. New-onset AF as the study endpoint was defined as the first presentation of AF during follow-up. Transient postoperative AF, occurring as an isolated episode within one month after surgery, was not counted as an outcome event. Because newly documented AF, not the duration or persistence of the arrhythmia, was the outcome event of interest, no distinction was made between paroxysmal and persistent AF. For patients without any AF event, the date of censor was that of the last contact with the patient.

Statistical analysis

Statistical analysis was performed using StatView Version 5 Software (Abacus Concepts Inc., Berkeley, California, USA). Values were expressed as mean ± SD. An unpaired Student's *t*-test was used for comparison between the two groups. AF event-free curves were derived by means of the Kaplan–Meier method and were compared by log-rank test. Possible predictors of new-onset AF were tested by univariate Cox proportional hazards regression analysis. Then, a multivariate analysis was applied to identify independent predictors and their predictive power. Independent predictors of AF incidence were also evaluated by using a stepwise regression analysis. A value of $P < 0.05$ was accepted as statistically significant.

Table 1 **Clinical characteristics of total participants ($n = 1118$)**

Variable	
Age (years)	63 ± 11
Sex (men) (%)	52
Body mass index (kg/m^2)	24.3 ± 3.4
Duration of hypertension (years)	16 ± 11
Diabetes mellitus (%)	24
Smokers (current or past) (%)	48
Systolic blood pressure (mmHg)	146 ± 16
Diastolic blood pressure (mmHg)	82 ± 11
Heart rate (bpm)	67 ± 8
eGFR (ml/min per 1.73 m^2)	68 ± 32
Urinary protein	
(−) to (±) (%)	74
(1+) to (2+) (%)	16
≥(3+) (%)	10
Antihypertensive treatment	
Ca channel blockers (%)	69
RAS inhibitors (%)	35
β-blockers (%)	29
Diuretics (%)	17
Others (%)	13
Statin use (%)	29

Values are mean ± SD or percentage. eGFR, estimated glomerular filtration rate; RAS, rennin–angiotensin system.

Results

Patient characteristics

The clinical characteristics of all patients are summarized in Table 1. The average duration of hypertension of the patients was 16 ± 11 years, and they had had history of antihypertensive treatment of 11 ± 9 years as average. At baseline, 83% of the study patients were receiving antihypertensive drugs, and 17% were treated with diet and/or exercise therapy only. Ca channel blockers, rennin–angiotensin system (RAS) inhibitors (i.e., angiotensin II receptor blockers and angiotensin-converting enzyme inhibitors), β-blockers, diuretics, and other classes of agents were used alone or in various combinations in 69, 35, 29, 17, and 13% of the study patients, respectively.

The mean duration of follow-up was 4.5 years (range, 0.1–9.1 years), for a total of 5079 patient-years of observation. During follow-up, 57 cases of new-onset AF were found, indicating the incidence was 1.1% per year. Of these 57 AF cases, 39 (68%) were symptomatic and the other 18 (32%) were asymptomatic at the time of the first documented event.

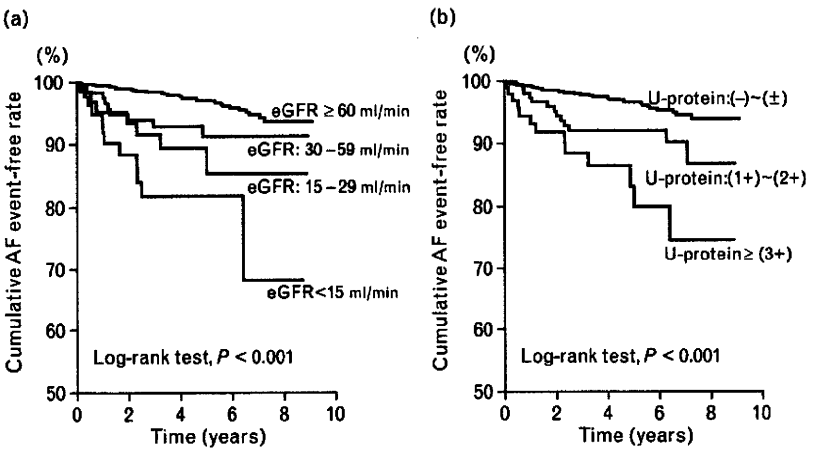
Relations of estimated glomerular filtration rate and proteinuria to the incidence of atrial fibrillation

The effect of eGFR and proteinuria on the incidence of new-onset AF was evaluated. The cumulative AF event-free rate was significantly decreased according to the reduction of basal eGFR (Fig. 1a). Likewise, AF event-free rate was clearly decreased according to the increase in urinary protein levels (Fig. 1b). In the Cox regression analysis, both eGFR (hazard ratio 0.82 per 10 ml/min per 1.73 m^2, $P < 0.001$) and proteinuria [(1+) to (2+): hazard ratio 2.31, $P = 0.012$; ≥(3+): hazard ratio 5.07, $P < 0.001$ vs. (−) to (±)] were significantly related to the incidence of AF.

Effect of chronic kidney disease on the incidence of atrial fibrillation

We divided the present patients into two groups by the absence or presence of CKD, which was defined as decreased eGFR less than 60 ml/min per 1.73 m^2 and/or the presence of proteinuria (≥1+). The participant group with CKD was associated with older age, higher

Fig. 1



Atrial fibrillation (AF) event-free curves obtained with the Kaplan–Meier method in the respective groups divided by basal estimated glomerular filtration rate (eGFR, a) or urinary protein levels (U-protein, b). (a) All participants were divided into four groups according to basal eGFR levels. Cumulative AF event-free rates in the groups with basal eGFR of ≥60 ($n = 818$), 30–59 ($n = 128$), 15–29 ($n = 73$), and <15 ml/min per 1.73 m^2 ($n = 99$) were 93.6, 91.2, 85.3, and 68.2%, respectively (log-rank test, $P < 0.001$). (b) All participants were divided into three groups according to basal U-protein levels. Cumulative AF event-free rates in the groups with basal levels of U-protein of (−) to (±) ($n = 827$), (1+) to (2+) ($n = 183$), and ≥ (3+) ($n = 108$) were 93.9, 86.7, and 74.7, respectively (log-rank test, $P < 0.001$).

Table 2 Comparison of basal characteristics between the two groups without and with CKD

	CKD (−) ($n=732$)	CKD (+) ($n=386$)
Age (years)	62 ± 11	65 ± 11*
Sex (men) (%)	47	61*
Body mass index (kg/m^2)	24.5 ± 3.4	23.8 ± 3.4*
Duration of hypertension (years)	15 ± 10	18 ± 11*
Diabetes mellitus (%)	18	35*
Smokers (current or past) (%)	44	55*
Systolic blood pressure (mmHg)	144 ± 15	150 ± 17*
Diastolic blood pressure (mmHg)	82 ± 11	81 ± 11*
Heart rate (beats/min)	67 ± 8	67 ± 8
eGFR (ml/min per 1.73 m^2)	83 ± 20	40 ± 30*
Urinary protein		
(−) to (±) (%)	100	25*
(1+) to (2+) (%)	0	47*
≥(3+) (%)	0	28*
Antihypertensive treatment		
Ca channel blockers (%)	61	83*
RAS inhibitors (%)	32	41*
β-Blockers (%)	26	35*
Diuretics (%)	10	30*
Statin use (%)	26	33*
LA diameter (mm)	36 ± 5	37 ± 5*
LV mass index (g/m^2)	121 ± 31	145 ± 44*
LV fractional shortening	0.42 ± 0.07	0.40 ± 0.07*

Values are mean ± SD or percentage. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; LA, left atrial; LV, left ventricular; RAS, rennin–angiotensin system. *$P<0.05$ compared with CKD (−).

proportion of men, smaller body mass index, and higher rate of diabetes mellitus and smokers (Table 2). In addition, the patients with CKD had longer duration of hypertension, higher systolic blood pressure, and more use of antihypertensive drugs. As for echocardiographic parameters, LA diameter and LV mass index were significantly greater, and LV fractional shortening was slightly lower in patients with CKD than in those without CKD.

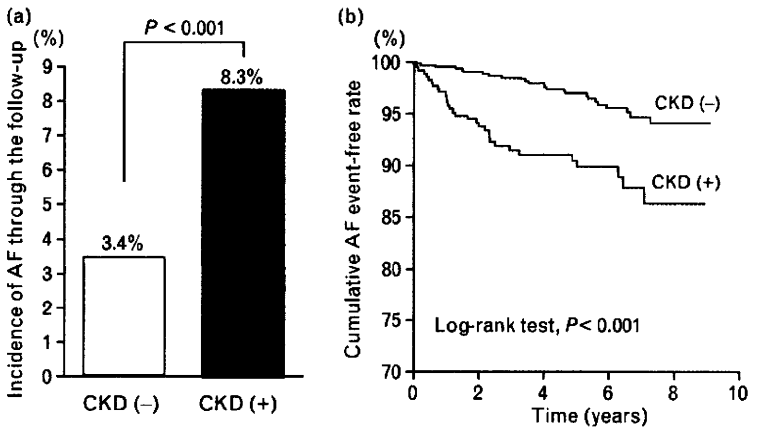
When comparing the incidence of new-onset AF between the two groups, the total incidence of AF through the follow-up periods was markedly higher in the patient group with CKD, compared to that without CKD (Fig. 2a). The cumulative AF event-free rate was also significantly decreased in the CKD group, compared to the non-CKD group (Fig. 2b).

As several confounding factors might be involved in the association between CKD and the incidence of AF in the present participants, we examined the independent predictors of new-onset AF by Cox regression analysis. In the univariate analysis, age, smoking, use of diuretic, LA diameter, LV mass index, and the presence of CKD were significantly related to the incidence of AF (Table 3). Among these possible predictive factors, age, smoking, and the presence of CKD were independent predictors of new-onset AF by the multivariate analysis. The adjusted hazard ratio of having CKD for new-onset AF during follow-up was 2.18 (95% confidence interval 1.21–3.90, $P=0.009$). Independent predictors of AF incidence were re-examined by stepwise regression analysis including all clinical and echocardiographic variables as possible independent factors. The presence of CKD as well as age, smoking, and LA diameter was an independent predictor of new-onset AF (age, hazard ratio 1.48 per 10 years, $P=0.008$; smoking, hazard ratio 1.92, $P=0.037$; LA diameter, hazard ratio 1.43 per 5 mm, $P=0.015$; CKD, hazard ratio 2.36, $P=0.004$).

Chronic kidney disease stages and the incidence of atrial fibrillation

The association of CKD stages with the incidence of AF was finally examined. In the univariate Cox analysis, the occurrence of new-onset AF was significantly increased in

Fig. 2



(a) Incidence of atrial fibrillation (AF) through the follow-up periods in the two groups without and with chronic kidney disease (CKD). The total rates of new-onset AF in the patients without and with CKD were 3.4% (0.7% per year) and 8.3% (2.1% per year), respectively ($P<0.001$). (b) AF event-free Kaplan–Meier curves in the two groups without and with CKD. Cumulative AF event-free rates in the non-CKD group and CKD group were 94.1 and 86.3%, respectively (log–rank test, $P<0.001$).

Table 3 Predictors of new-onset AF by univariate and multivariate Cox regression analysis

	Hazard ratio (95% CI)	P
Univariate analysis		
Age, 10 years	1.65 (1.24–2.19)	<0.001
Sex, men	1.51 (0.89–2.55)	0.128
Body mass index, 1 kg/m^2	1.01 (0.93–1.09)	0.839
Duration of hypertension, 1 year	1.02 (1.00–1.05)	0.100
Diabetes mellitus, yes	1.34 (0.75–2.40)	0.318
Smoking (current or past), yes	2.23 (1.29–3.84)	0.004
Systolic blood pressure, 10 mmHg	1.06 (0.90–1.25)	0.480
Diastolic blood pressure, 10 mmHg	0.88 (0.69–1.13)	0.316
Heart rate, 1 bpm	0.98 (0.94–1.01)	0.165
Ca channel blocker, yes	1.56 (0.84–2.89)	0.162
RAS inhibitor, yes	0.82 (0.47–1.44)	0.492
β-Blocker, yes	1.38 (0.81–2.35)	0.236
Diuretic, yes	2.23 (1.23–4.03)	0.008
Statin, yes	1.00 (0.57–1.76)	0.990
LA diameter, 5 mm	1.43 (1.10–1.87)	0.008
LV mass index, 10 g/m^2	1.09 (1.03–1.15)	0.004
LV fractional shortening, 0.01	0.98 (0.94–1.02)	0.250
CKD, yes	2.99 (1.77–5.05)	<0.001
Multivariate analysis		
Age, 10 years	1.54 (1.16–2.04)	0.003
Smoking (current or past), yes	1.78 (1.01–3.15)	0.047
Diuretic, yes	1.23 (0.65–2.32)	0.533
LA diameter, 5 mm	1.26 (0.94–1.68)	0.118
LV mass index, 10 g/m^2	1.03 (0.96–1.10)	0.457
CKD, yes	2.18 (1.21–3.90)	0.009

In the multivariate analysis, all variables that had a significant association in the univariate analysis were included as possible independent factors. AF, atrial fibrillation; CI, confidence interval; CKD, chronic kidney disease; LA, left atrial; LV, left ventricular; RAS, rennin–angiotensin system.

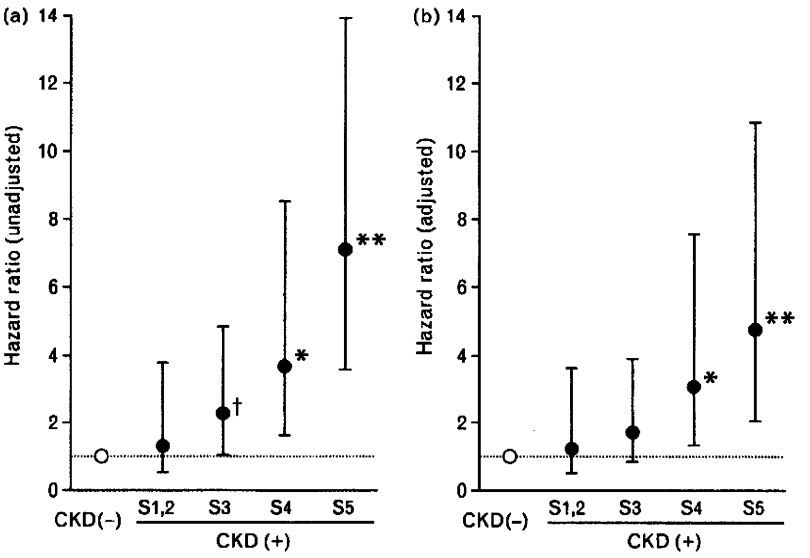
the participant groups with CKD stage 3 and more advanced stages (Fig. 3a). After adjustment for confounding factors (i.e., age, smoking, use of diuretic, LA diameter, and LV mass index) by the multivariate analysis, CKD stages 4 and 5 were still significantly associated with the increased incidence of AF (Fig. 3b).

Discussion

The present study has shown that CKD defined as decreased eGFR and/or the presence of proteinuria is longitudinally associated with the incidence of new-onset AF in hypertensive patients. Our results indicate that antecedent existing CKD has a significant influence on new-onset AF in hypertensives.

Several clinical and population-based studies showed that the prevalence of AF was independently associated with decreased eGFR and increased levels of urinary albumin [12–14], although these cross-sectional investigations did not elucidate whether antecedent renal dysfunction affects the incidence of AF. Prospective observational studies examining postoperative AF showed that renal impairment (decreased eGFR or renal failure) was associated with an increased risk of AF after cardiac surgery [15,16]. A recent study reported that decreased baseline eGFR was associated with an increased risk of subsequent new onset AF in a large scale of community-based cohort [17]. The findings of our study are fundamentally consistent with these observations. However, previous

Fig. 3



Relation of chronic kidney disease (CKD) stages to the incidence of atrial fibrillation (AF) evaluated by univariate (a) and multivariate (b) Cox regression analysis. Respective data present hazard ratios (open or solid circles) and the 95% confidence intervals (vertical lines) in the groups without CKD ($n = 732$) and with CKD stages 1–2 (S1,2, $n = 86$), 3 (S3, $n = 128$), 4 (S4, $n = 73$), and 5 (S5, $n = 99$). In the multivariate analysis, all variables that had a significant association in the univariate analysis (i.e., age, smoking, use of diuretic, left atrial diameter, and left ventricular mass index) were included as confounding factors. $^{\dagger}P < 0.05$, $^{*}P < 0.01$, $^{**}P < 0.001$ vs. CKD (–).

studies have shown that many factors are involved in the development of AF in a general population and patients with cardiovascular disorders [18–20]. As for hypertensive patients, it has been revealed that age, systolic blood pressure, LV mass, and LA size are related to the incidence of AF [2–5,21]. Thus, there was the possibility that these factors might mediate the association between CKD and AF incidence observed in the present and other studies, because GFR generally decreases with age, and pressure and volume load augmented by renal dysfunction directly increases LV mass and LA size. In fact, the present patients with CKD had older age, higher systolic blood pressure, and greater LV mass index and LA diameter, compared with those without CKD. In addition, age, LV mass index, and LA diameter as well as CKD were relating factors to the incidence of AF in the univariate Cox regression analysis of this study. By the multivariate analysis, however, the association of CKD with new-onset AF was warranted to be still significant independently of these confounders, although the adjusted hazard ratio of CKD for AF incidence was diminished compared to the crude risk ratio before adjustment. Therefore, the present study has demonstrated for the first time that the existence of CKD in hypertensive patients is an independent predictor of new-onset AF, apart from the effects of aging, LV hypertrophy, and LA dilatation.

Verdecchia *et al.* [3] showed that age and LV mass were the sole independent predictors of new-onset AF in a large cohort of initially untreated patients with essential hypertension. In our patients with chronically treated hypertension, LV mass index was not an independent determinant of the incidence of AF. The exact reason for the discrepant findings is unclear, but there was a possibility that antihypertensive treatment before the enrollment might have modified LV mass in our study.

In the univariate analysis of our study, basal systolic or diastolic blood pressure was not significantly related to the incidence of AF. Previous studies showed that systolic blood pressure and pulse pressure were good predictors of incident AF in large cohorts of the general population [22,23]. In hypertensive patients, however, there have been discrepant findings concerning the significant influence of blood pressure levels on the incident of AF [2–4,21]. Antihypertensive treatment and changes in blood pressure during follow-up might have modified the outcome and have spoiled the possible relation between systolic blood pressure and incident AF in our retrospective observational study. Since the present patients with CKD had a significantly higher systolic blood pressure than those without CKD, there might be a possibility that elevated blood pressure in the CKD group promoted renal dysfunction further, resulting in contribution of new-onset AF partly.

In the present study, the incidence of new-onset AF was clearly associated with the decrease in eGFR. In fact, CKD stages 4 and 5 were a significant predictor of incident AF after adjustment for confounding factors by the multivariate analysis. The incidence of AF was also increased according to the severity of proteinuria. Therefore, our findings suggested that advanced renal dysfunction including massive proteinuria chiefly contributed to the incidence of new-onset AF in the present hypertensive patients.

The causal mechanism by which renal impairment has a great and partly cardiac overload-independent influence on the occurrence of AF in hypertensive patients could not be drawn from our observational study, but there are some possible speculations. The increased risk of developing AF in CKD may be related in part to activation of signaling pathways of inflammation, because previous studies have shown that renal insufficiency is associated with elevations of inflammatory markers such as C-reactive protein [24] and that C-reactive protein predicts increased risk for developing future AF [25]. Possible involvement of oxidative stress and endothelial dysfunction in the development of AF has also been shown [26,27]. Since the patients with chronic renal failure have increased levels of oxidative stress markers and impaired endothelial function [28], oxidative stress and endothelial dysfunction caused by renal impairment may be involved in the increased risk of new-onset AF in patients with CKD. In addition, these mechanisms might be also involved in the association between smoking habit and incident AF observed in the present study, because smoking is known to increase oxidative stress and deteriorate endothelial function.

Limitations

Screening 24-h electrocardiographic recordings were not performed in our study, although standard 12-lead electrocardiograms were periodically done for all the present patients. Therefore, it is possible that asymptomatic cases of AF may have gone undetected. In fact, 68% of 57 cases of newly documented AF were accompanied by some symptom such as palpitation and chest discomfort, and the other 32% were asymptomatic cases in the present study. However, all patients visited our hospital periodically (every 1–2 months) and the pulse and heart beat were checked at every examination. Individuals with irregular pulse or cardiac rhythm received 12-lead electrocardiogram and 24-h Holter recordings, even they had no cardiac symptom. In addition, the incidence of new-onset AF in our study (1.1% per year) was similar to the incidence rates in other studies for patients with essential hypertension (0.5–1.7% per year) [3–5,21] and higher than those in middle-aged and elderly adults from population-based studies (0.2–1.1% per year) [17,18,22,23,29,30]. Thus, it is less likely that there were a considerable number of missed AF cases in the present study. Furthermore, since any misclassification or underdetection of incident AF is

expected to occur at random and independent of renal function, such misclassification would not overestimate the true risk of new-onset AF associated with CKD. The small number of new-onset AF during follow-up, however, must be considered as a limitation of the study, especially in comparing AF incidence rates among more than three groups.

Several studies have revealed that RAS inhibitor treatment and hydroxymethylglutaryl coenzyme A reductase inhibitor (statin) use are associated with reduced incidence of AF in patients with cardiovascular disease [21,31,32]. As another study limitation, therefore, we must consider the possibility that these treatments might bias the outcome of the present study.

Moreover, there was a possibility that the obtained findings in this study might be limited to the Japanese population. Further studies are needed to validate our results in Western and other racial populations.

In conclusion, the present study demonstrated that CKD defined as decreased eGFR and/or the presence of proteinuria was associated with an increased risk of new-onset AF in hypertensive patients, and that the impact of CKD on the incidence of AF was independent of LV hypertrophy and LA dilatation. In particular, advanced stages of CKD were strongly related to the increasing occurrence of AF. In managing hypertensive patients, therefore, it may be important to prevent the progression of renal dysfunction in prevention of the occurrence of new-onset AF.

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There are no conflicts of interest.

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Liver-targeted siRNA delivery by polyethylenimine (PEI)-pullulan carrier

Jeong-Hun Kang [a,†], Yoichi Tachibana [b,†], Wakako Kamata [b,†], Atsushi Mahara [b], Mariko Harada-Shiba [c], Tetsuji Yamaoka [b,d,*]

[a] Laboratory for Advanced Diagnostic Medical Devices and Department of Biomedical Engineering, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan
[b] Department of Biomedical Engineering, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan
[c] Department of Bioscience, Advanced Medical Engineering Center, National Cardiovascular Center Research Institute, 5-7-1 Fujishiro-dai, Suita, Osaka 565-8565, Japan
[d] CREST, Japan Science and Technology Corporation, 5 Sanbancho, Chiyoda-ku, Tokyo 102-0075, Japan

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ABSTRACT

Recently, small interfering RNA (siRNA)-based therapeutics have been used to treat diseases. Efficient and stable siRNA delivery into disease cells is important in the use of this agent for treatment. In the present study, pullulan was introduced into polyethylenimine (PEI) for liver targeting. PEI/siRNA or pullulan-containing PEI/siRNA complexes were delivered into mice through the tail vein either by a hydrodynamics- or non-hydrodynamics-based injection. The incidence of mortality was found to increase with an increase in the nitrogen/phosphorus (N/P) ratio of PEI/siRNA complexes. Moreover, the hydrodynamics-based injection increased mice mortality. Introduction of pullulan into PEI dramatically reduced mouse death after systemic injection. After systemic injection, the PEI/fluorescein-labeled siRNA complex increased the level of fluorescence in the lung and the PEI-pullulan/siRNA complex led to an increased fluorescence level in the liver. These results suggest that the PEI-pullulan polymer may be a useful, low toxic means for efficient delivery of siRNA into the liver.

1. Introduction

Small interfering RNA (siRNA)-based therapeutics, which are now recognized as a medical approach for the treatment of difficult-to-cure diseases such as viral infections and tumors, are attracting considerable attention in recent times.[1,2] However, naked siRNA is unstable in the bloodstream and is rapidly eliminated through the urinary system. Moreover, its negative charge inhibits efficient cellular uptake due to the negative charge of the cell surface. Thus, efficient and stable siRNA delivery into diseased cells is critical in this treatment modality. Many researchers have attempted to induce various chemical modifications into siRNA or to form complexes with several cationic carriers such as cationic polymers, liposomes, peptides, or proteins.[3–5]

Among cationic polymers, polyethylenimine (PEI) is the most popular synthetic polymer and has a high cationic charge density. It has been widely used to deliver siRNAs into cell lines or tissues. Naked siRNAs are unstable and are rapidly degraded, but PEI is able to form stable complexes with siRNAs, leading to the protection of genes from enzymatic degradation. Moreover, PEI shows a strong buffer capacity over a wide range of pH values; this plays an important role in the escape of genes from the endosome after endocytosis. On the other hand, the high cationic density of PEI allows for the formation of highly condensed complex with siRNAs, but complex formation with PEI can lead to cytotoxicity.[6–10] Information on the safety and biodistribution of PEI or PEI/siRNA complexes both in vitro and in vivo would contribute to improving the safety and efficiency of siRNA delivery using PEI.

In the present study, we introduced pullulan into PEI. Pullulan is a water-soluble polysaccharide consisting of three α-1,4-linked glucose polymers with different α-1,6-glucosidic linkages. It is used for liver targeting because of its high affinity for the asialoglycoprotein receptor in the liver.[11–13] We delivered PEI/siRNA or pullulan-containing PEI/siRNA complexes into mice through the tail vein by a hydrodynamics- or non-hydrodynamics-based injection. The incidence of mortality was found to increase with increasing the nitrogen/phosphorus (N/P) ratio of PEI/siRNA complexes. On the other hand, the introduction of pullulan into PEI reduced mouse mortality and increased liver-targeting efficiency.

2. Results and discussion

2.1. Polymers

A linear 22-kDa PEI was used for the synthesis of the siRNA and PEI-pullulan polymer complex (Fig. 1). The amount of pullulan in

* Corresponding author. Tel.: +81 6 6833 5012; fax: +81 6 6835 5476.
E-mail address: yamtet@ri.ncvc.go.jp (T. Yamaoka).
† These authors contributed equally to this work.

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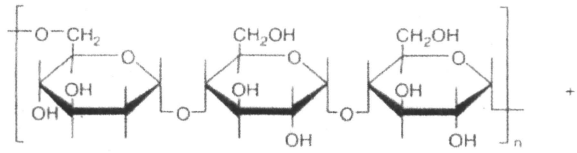

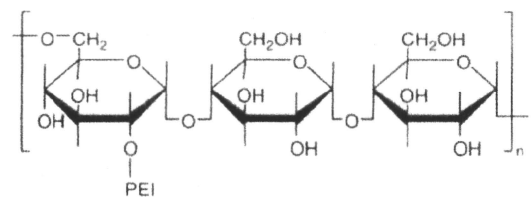

Figure 1. Chemical structure of pullulan and PEI-pullulan. To synthesize the PEI-pullulan polymer, 48.6 mg of pullulan (M_w, 107,000; 0.3 unit mmol) and 24.3 mg of carbonyldiimidazole (CDI; 0.15 mmol) were stirred in 30 mL of anhydrous dimethylsulfoxide (DMSO) at room temperature and then 13.2 mg of linear PEI (M_w, 22 kDa; 0.3 mmol) was added to the mixture.

the polymer was estimated to be 39 mol % and molecular weight of polymer was 2.6×10^5 (see Supplementary data). The zeta potentials of polymer/siRNA complex increased with increasing N/P ratio and showed nearly neutral at N/P ratios of 48 and 96 (see Supplementary data).

2.2. Measurements of complex diameters

The complexes of polymer and siRNA were prepared at several N/P ratios (1.5, 3, 6, 12, 24, and 48) and were determined using a Zetasizer. The particle size decreased with increasing N/P ratio. PEI/siRNA complexes showed <200 nm for all N/P ratios, whereas PEI-pullulan/siRNA complexes with ratios of 12 to 48 were <200 nm (Fig. 2).

2.3. Electrophoresis of the polymer/siRNA complex

Polymers were mixed with siRNA at several N/P ratios. The complexes were analyzed by electrophoresis. Bands corresponding

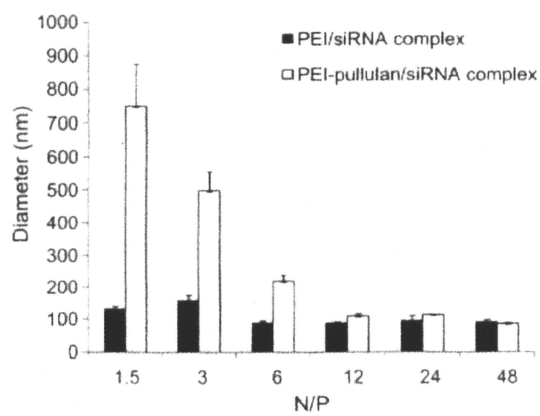

Figure 2. Diameter of the PEI/siRNA or PEI-pullulan/siRNA complexes. Polymer and siRNA complexes were simply prepared by incubating siRNA and polymer in water. The diameters of the complexes were determined using a Zetasizer.

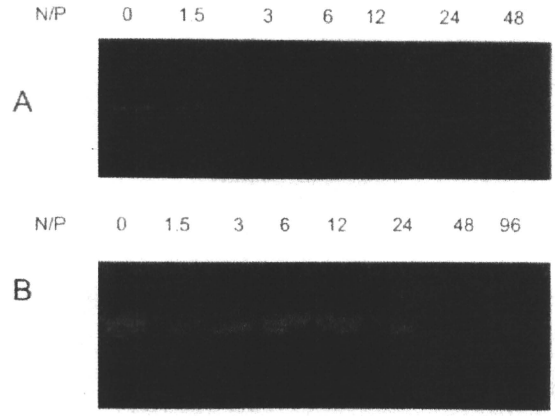

Figure 3. Electrophoresis of (A) PEI/siRNA and (B) PEI-pullulan/siRNA complexes. Various concentrations of the polymer were mixed with the siRNA and analyzed by 19% polyacrylamide gel electrophoresis. A N/P ratio of 0 implies siRNA alone.

to free siRNA in the PEI/siRNA complex were not observed when the polymer was present at N/P ratios of above 3, whereas when the N/P ratios were 1.5 and 3, bands corresponding to free siRNA were observed. In the case of the PEI-pullulan/siRNA complex, no suppression of siRNA was indentified in those complexes with N/P ratios of 1.5 to 24, while siRNA migration in complexes with N/P ratios of ⩾48 was suppressed (Fig. 3). These results show that introduction of pullulan into PEI weakens the polymer and siRNA complex.

2.4. Safety of polymer/siRNA complexes in vivo

PEI alone, the PEI/siRNA complex, and the PEI-pullulan/siRNA complex were injected into mice using a hydrodynamics-based or a non-hydrodynamics-based procedure. PEI alone or the PEI/siRNA complex with high N/P ratios (⩾6.0) increased mice mortality after systemic injection using the non-hydrodynamics-based procedure (Fig. 4); note that all mice died when complexes with N/P ratios of ⩾12 were injected (data not shown). Similarly, previous studies reported that the PEI/DNA complex with a N/P ratio of 6 resulted in the death of 50% of the injected mice.[14,15] However, all mice died when PEI alone or the PEI/siRNA complex with a N/P ratio of 3 was injected using the hydrodynamics-based procedure. Hydrodynamics-based transfection was developed to deliver naked DNA or RNA into the liver by intravenous injection of a large volume of DNA or RNA solution at high velocity. This is an efficient method for liver-specific in vivo gene delivery.[16,17] However, in our study, high mouse mortality was observed when the hydrodynamics-based procedure was used for the in vivo delivery of PEI/siRNA complexes.

All dead mice lapsed into dyspnea less than 30 min after injection and showed hemorrhage-like dark red regions in the lung. There was no difference in mortality between mice injected with PEI alone and those injected with the PEI/siRNA complex, but more severe hemorrhage-like dark red regions were observed in the former (Fig. 4A and B).

Concerning the death of mice after systemic injection, Fahrmeir's group suggested that free PEIs after complex formation with DNA correlate with mouse mortality.[18] Several studies showed that increased gene expression in the lung is associated with lung damage and mouse mortality after intravenous injection of PEI/DNA or modified PEI/DNA.[15,19,20] In the present study, PEI/siRNA showed a similar in vivo toxicity to PEI/DNA.

On the other hand, no mortality was observed in mice injected with PEI-pullulan/siRNA complexes with N/P ratios of 6 to 48 by the hydrodynamics-based procedure mice (Fig. 4B) and the non-hydrodynamics-based procedure (data not shown). These

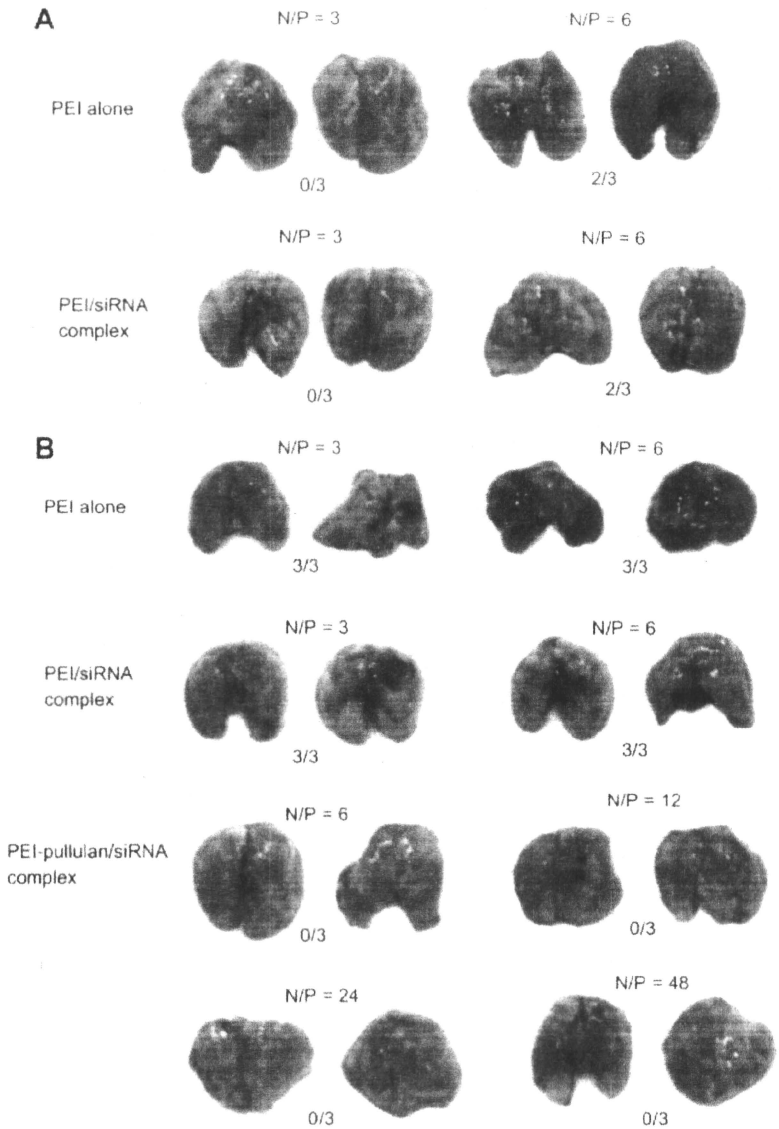

Figure 4. Delivery of PEI alone or polymer/siRNA complexes into mice by using the (A) non-hydrodynamics- or (B) hydrodynamics-based procedure. Numbers of dead mice per total mice are described below

results suggest that intravenous injection with PEI alone or the PEI/siRNA complex at high N/P ratios can increase mortality, but introduction of pullulan into PEI results in low mortality. Moreover, hydrodynamics-based injection can increase the mouse mortality rate, compared to non-hydrodynamics-based injection. High in vivo toxicity or mortality caused by systemic injection of the PEI-based complex is an obstacle to be overcome. Many research efforts such as the introduction of poly(ethylene glycol) (PEG)[15] and removal of free PEIs after complex formation[18] were reported to efficiently reduce in vivo toxicity or mortality. In the present study, introduction of pullulan to PEI dramatically reduced in vivo toxicity and mortality.

2.5. Biodistribution after injection of the polymer/siRNA complex into mice

siRNA formed a complex with PEI at a N/P ratio of 3 and with PEI-pullulan at a N/P ratio of 48. Complexes were injected into the mice via the tail vein using the non-hydrodynamics-based procedure. The fluorescence in each tissue (heart, lung, liver, spleen, and kidney) was detected at 1 or 3 h after the injection. At 1 h after the injection of the PEI/siRNA or PEI-pullulan/siRNA complex, fluorescence was identified mainly in the lung and kidney. At 3 h, fluorescence increased in the livers of the PEI-pullulan/siRNA complex-injected mice, but was barely found in the livers of the PEI/siRNA-injected mice (Fig. 5).

Several studies have reported that linear and branched PEI/gene complexes show different biodistribution and transfection efficiency.[6–9] The linear PEI/gene complex exhibits more efficient transgene expression in the lung when injected intravenously, as compared to the branched PEI/gene complex;[6,7,9,14,21] however the transgene expression of the branched PEI/gene complex may be more efficient in other tissues (e.g., kidney).[9,22] Further, although PEI cytotoxicity depends on molecular weight and N/P ratios, the branched PEI/gene complex is found to have higher toxicity or cause more tissue damage as compared to the linear PEI/gene complex.[8,9,23]

In the present study, we used a linear 22-kDa PEI for complex formation with siRNA and for synthesizing the PEI-pullulan