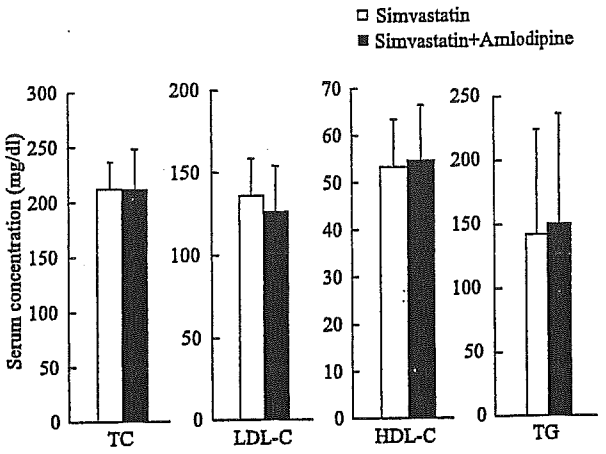

Fig. 2. *Mean levels of serum lipid parameters on the last day of 4 weeks of treatment with simvastatin (5 mg/day) or combined treatment with simvastatin (5 mg/day) and amlodipine (5 mg/day). TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglycerides. Each column represents the mean±SD.*

believed to be relevant to the systemic adverse effects for this class of agents (*35*).

The pharmacokinetics of simvastatin has been shown to be affected by potent CYP3A4 inhibitors (*13–15*, *18*). Amlodipine, which is metabolized by CYP3A4, has been reported to show inhibitory effects on CYP3A4 *in vitro* (*31*). However, the influence of amlodipine on the substrate drugs of CYP3A4 has not been clarified yet. In this study, amlodipine significantly increases the AUC of HMG-CoA reductase inhibitors after co-administration of simvastatin by 30%. It has been reported that the AUC of HMG-CoA reductase inhibitors was increased 4-fold with itraconazole (*13*), which is known to be a potent inhibitor of CYP3A4. Some studies have show adverse effects, including rhabdomyolysis, in patients treated with simvastatin and CYP3A4 inhibitors such as itraconazole and ketoconazole (*8*). These reports suggested that the co-administration of simvastatin with these inhibitors enhanced the risk of adverse effects, because of the dose-dependent toxicity of HMG-CoA reductase inhibitors. In our previous study, diltiazem increased the AUC of HMG-CoA reductase inhibitors 2-fold (*18*). On the other hand, amlodipine increased the AUC of HMG-CoA reductase inhibitors by only 30% in this study. In addition, it has been reported that the CYP3A4 inhibitory effect of diltiazem was higher than that of amlodipine after therapeutic doses (*36*). Therefore, the difference of the impact on the plasma concentrations of HMG-CoA reductase inhibitors may depend on the difference of the CYP3A4 inhibitory potency between amlodipine and diltiazem.

It has been reported that an increase in the plasma concentrations of HMG-CoA reductase inhibitors following co-administration of simvastatin and diltiazem resulted in a reduction of TC and LDL-C levels (*18*). However, we did not observe such a reduction of TC and LDL-C levels, despite the fact that amlodipine increased the plasma concentrations of HMG-CoA reductase inhibitors. The pharmacokinetic interactions observed in the present study, such as the 30% increase in the AUC of HMG-CoA reductase inhibitors, may not have been sufficient to alter the pharmacodynamic response. Moreover, we cannot exclude the possibility that the number of patients was not sufficient to detect the pharmacodynamic differences. Further investigations will be needed to clarify the pharmacodynamic impact of simvastatin with amlodipine on TC and LDL-C.

Table 3. Systolic BP and Diastolic BP during Pretrial Control Period with Enalapril, Simvastatin Monotherapy and Combined Treatment with Simvastatin and Amlodipine

	Systolic BP (mmHg)	Diastolic BP (mmHg)
Simvastatin+enalapril (pretrial control period)	135±19	78±13
Simvastatin	152±22*	89±13*
Simvastatin+amlodipine	140±17	81±11

Values are mean±SD. BP, blood pressure. *$p<0.05$ *vs.* simvastatin+enalapril.

In conclusion, this study is the first report of the drug interaction between simvastatin and amlodipine after a long-term treatment. Although amlodipine increases the plasma concentrations of HMG-CoA reductase inhibitors, the impact of amlodipine on simvastatin is smaller than that of diltiazem. Since these drugs are often used concomitantly for patients with hypertension and hypercholesterolemia, amlodipine could be used more safely with simvastatin than diltiazem.

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A Randomized Clinical Study of Tea Catechin Inhalation Effects on Methicillin-Resistant *Staphylococcus aureus* in Disabled Elderly Patients

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Objectives: To evaluate the effects of tea catechin inhalation on methicillin-resistant *Staphylococcus aureus* (MRSA) in disabled elderly patients.

Design: Seven days, randomized, prospective study.

Setting: Three hospitals in Japan.

Participants: Seventy-two patients aged 78 ± 11 years (mean age ± standard deviation) with cerebrovascular diseases, classified as disabled according to the activity of daily living and were either bedridden or required assistance for standing, and showing presence of MRSA in sputum.

Interventions: Inhalation of 2 mL tea catechin extract solution along with saline (3.7 mg/mL catechins, 43% of catechins are composed of epigallocatechin gallate), or saline alone, 3 times daily using a handheld nebulizer for 7 days.

Measurements: The endpoint of efficacy was the reduction rates of MRSA in sputum. The safety measure was the adverse events observed during the 7 days of inhalation.

Results: The reduction rates calculated as the summation of decrease and disappearance of MRSA in sputum at 7 days were 47% (17 of 36 patients) in the catechin group and 15% (5 of 33 patients) in the control group; the difference in the reduction rates between the 2 groups was statistically significant ($P = .014$). The disappearance rate of MRSA in sputum was higher in the catechin group (31%; 11 patients) when compared with the control group (12%; 4 patients), however the difference in the disappearance rate between the 2 groups was not statistically significant ($P = .091$). No adverse events, such as respiratory tract obstruction, allergic bronchial spasm, or skin eruption, including laboratory changes, were observed during the study.

Conclusion: The catechin inhalation appeared to reduce the MRSA count in sputum. However, the application of tea catechin inhalation as a supplementary treatment for controlling MRSA infection remains controversial. **(J Am Med Dir Assoc 2006; 7: 79–83)**

Keywords: Methicillin-resistant Staphylococcus aureus *(MRSA); catechin; elderly; disabled*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a multidrug-resistant pathogen and is often responsible for serious nosocomial infections associated with significant mortality and morbidity. MRSA often causes life-threatening infections, such as pneumonia or sepsis, in some susceptible patients using immunosuppressant drugs or in the disabled elderly.[1,2] Patients who are colonized or infected with MRSA can cause serious social implications such as hospital-acquired infections or prolonged hospitalization. Moreover, patients with MRSA, particularly elderly patients, are usually isolated, depressed, and suffer from anxiety, which in turn decreases their quality of life.[3] Therefore, control of MRSA is essential for social benefits as well as for the improvement in the health and quality of life of the elderly patients.

Catechins are the major components of tea flavonoids and

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are reported to possess antioxidative, anticancer, hypolipidemic, hypoglycemic, hypotensive, antiviral, and antibacterial effects.[4–6] Recent in vitro experimental studies have revealed that tea catechin extracts induce bactericidal effects as well as demonstrate synergistic effects with antibiotics against MRSA.[7–15] However, thus far, a limited number of studies have been conducted on the clinical effects of tea catechin against MRSA.[16–18] In our previous clinical pilot studies, catechin inhalation showed a temporary effect on the elimination of MRSA in sputum, and this effect was observed in a dose-dependent manner.[17,18] Based on these results, we designed a prospective randomized controlled study to evaluate the effects of tea catechin inhalation on MRSA in disabled elderly patients.

METHODS

A total of 72 inpatients who attended the Department of Neurology at Seirei Hamamatsu General Hospital, Department of Internal Medicine at National Hospital Organization Fukuoka Higashi Medical Center, and Kasaoka Daiichi Hospital, and showed presence of MRSA in their sputum samples were studied between February 2002 and April 2004. The mean age of all patients was 78 ± 11 years, and the patients were randomized prior to receiving inhalation treatment. All study patients had a history of cerebrovascular diseases and were classified as disabled according to the activity of daily living; these patients were either bedridden or required assistance for standing. Cerebrovascular diseases in the patients were diagnosed using magnetic resonance imaging or computerized tomography of the brain. The study was approved by the ethics committee at each study site and was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients or their guardians before participation in the study.

The patients were recruited sequentially and were randomized in a single-blind manner. Randomized allocation was performed independently at the Hamamatsu University School of Medicine, and the requisite information was provided to investigative staff at each site. The study patients and guardians were not informed of the type of material in the nebulizer. To estimate the effectiveness of tea catechin inhalation on patients' clinical outcomes, sputum samples were tested at each site by a laboratory technician who had no prior information regarding which of the patients were allocated to the control group or to the catechin group. The patients included in the catechin group received inhalation of 2 mL tea catechin extract solution in saline, and the control group received inhalation of saline alone. The concentration of the catechin solution in saline was equivalent to 3.7 mg/mL catechins; these catechins were composed of 1.6 mg epigallocatechin gallate (EGCG). Using a handheld nebulizer, the catechin solution was inhaled 3 times daily for a period of 7 days. Catechins were in the form of polyphenon 60A (Mitsui Norin Co, Ltd, Tokyo, Japan), and total catechin content was 73.0%, including 31% (−)-EGCG, 21% (−)-epigallocatechin, 8.6% (−)-epicatechin, 8.6% (−)-epicatechin gallate, 2.9% (−)-gallocatechin gallate, and 0.8% (−)-catechin gallate.

Staphylococcus aureus isolated from the sputum was defined as MRSA when it showed a minimum inhibitory concentration (MIC) of more than 4 μg/mL for oxacillin in a disk diffusion method of the National Committee for Clinical Laboratory Standards (NCCLS). All the strains were identified by polymerase chain reaction (PCR) analysis of *mecA* gene expression.[19] If the patients faced difficulties in expectorating sputum themselves, they were assisted by registered nurses. The microbiology laboratory at each hospital evaluated the quality of sputum. The samples of sputum that showed resistance to oxacillin in the disk diffusion test were evaluated for MRSA colony formation units (CFU) using routine laboratory tests; the count of MRSA as CFU was graded based on a semiquantitive scale of 0, 1+, 2+, or 3+. The enrolled patients were confirmed to show an MRSA count of 2+ or 3+ on the CFU scale in their sputum samples at least twice a week prior to their allocation. If a patient was observed to have an MRSA infection, the antibiotic therapy was continued and was not changed during the study. Infected patients were defined as those who exhibited the clinical symptoms of infection, such as bronchopneumonia, along with the presence of MRSA in their sputum samples. On the other hand, colonized patients were defined as those who did not exhibit clinical symptoms of infection, but showed presence of MRSA in their sputum samples. Patients were excluded from participation in the study if they had a history of bronchial asthma; hypersensitivity to tea ingestion; or severe cardiac, renal, or hepatic dysfunction.

For the estimation of patients' clinical outcomes, the reduction rates calculated as the summation of decrease and disappearance of MRSA in sputum between the 2 groups were compared at the beginning and at the end of the inhalation. A decrease in MRSA count was defined as a 2-scale improvement from 3+ to 1+, and the disappearance of MRSA was defined as the change in the count to scale 0. MRSA in sputum was confirmed twice at the end of inhalation, and the higher score was selected for analysis. For the safety evaluations, laboratory data were measured before and after 1 week of inhalation, and the adverse events such as respiratory tract obstruction, allergic bronchial spasm, or skin eruption were also checked at each inhalation time during the study.

All statistical analyses were performed using SPSS for Windows, version 11.0 (SPSS, Inc, Chicago, IL). Data of continuous variables are expressed as means ± SD. The differences in the quantitative data between the groups were assessed by the Student *t* test. The chi-square test was used to compare categorical variables with variables divided in quartiles. Statistical differences in the reduction or disappearance of MRSA between the catechin group and the control group were evaluated by the multivariate logistic regression analysis. A *P* value less than .05 was considered to be statistically significant.

RESULTS

Sixty-nine patients completed the study; 3 patients dropped out because of their refusal to provide consent since they were transferred to a nursing home (Figure 1). The clinical profiles of the subjects who participated in the study are summarized in Table 1. MRSA infection was diagnosed in 16 patients, whereas 53 patients were observed to be colonized with MRSA. During the study, the infected patients were administered a glycopep-

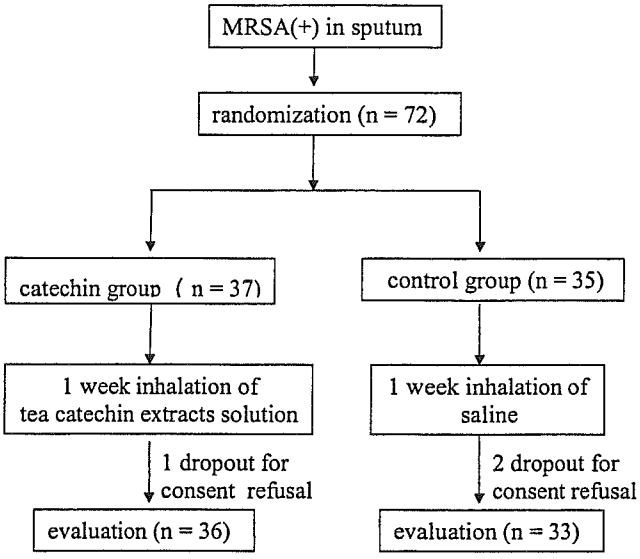

Fig. 1. *Flow of the study protocol.*

tide or aminoglycoside antibiotic, such as vancomycin, teicoplanin, or arbekacin, in combination with other antibiotics. On the other hand, no antibiotics were administered to the colonized patients. Forty-one patients were catheterized with a nasogastric, tracheal, or urethral tube. No significant differences were observed between the catechin group and the control group with respect to age, sex, MRSA infection or colonization status, degree of activity of daily living, existence of decubitus ulcers, catheterization, and laboratory data for indications of anemia, nutritional status, inflammation, or hepatic or renal dysfunction.

After 1 week of inhalation, the reduction rates calculated as the summations of decrease and disappearance of MRSA in sputum were 47% (17 of 36 patients) in the catechin group and 15% (5 of 33 patients) in the control group; the difference in the reduction rates between the 2 groups was observed to be statistically significant (P = .014). The disappearance rate of MRSA in sputum was higher in the catechin group (31%; 11 patients) when compared with that in the control group (12%; 4 patients); however, the difference in the disappearance rate between the 2 groups was not statistically significant (P = .091) (Table 2).

In the subgroup analysis of 53 patients colonized with MRSA, the reduction rates of MRSA were 50% (13 of 26 patients) in the catechin group and 19% (5 of 27 patients) in the control group; the difference in the reduction rate between the 2 groups was observed to be statistically significant (P = .027). The disappearance rate of MRSA in sputum was higher in the catechin group (31%; 8 patients) when compared with that in the control group (15%; 4 patients); however, the difference in the disappearance rate between the 2 groups was not statistically significant. Of 16 patients infected with MRSA, the reduction in MRSA count was observed in 4 patients in the catechin group, whereas none of the patients in the control group showed a reduction in MRSA count. Among the 16 infected patients, 4 patients were administered vancomycin; 5, teicoplanin; and 1, arbekacin in the catechin group, whereas in the control group, 3 patients were administered vancomycin; 1, teicoplanin; and 2, arbekacin, in combination with imipenem, panipenem, or ceftazidime. Among the infected patients who showed reduction in MRSA count, one patient was administered vancomycin, whereas 3 patients were administered teicoplanin, in combina-

Table 1. *Clinical Profiles of the Catechin Inhalation Group and the Control Group*

	Catechin Group n = 36	Control Group n = 33	*P* Value
Patient age, y*	78 ± 9.5	78 ± 13	.97
Men/women	19/17	18/15	.88
MRSA infected/colonized	10/26	6/27	.89
Activity of daily living			.13
Bedridden	27	19	
Standing with assistance	9	14	
Decubitus ulcers (+)	9	4	.17
Catheterization (+)	23	18	.43
Nasogastric tube	17	12	.36
Tracheal tube	3	2	.72
Urethral tube	13	8	.28
WBC count, cells/mL*	9000 ± 3400	8600 ± 4500	.65
Hemoglobin, g/dL*	11.6 ± 1.8	11.1 ± 1.9	.28
CRP, mg/dL*	4.2 ± 4.8	4.8 ± 5.9	.63
Total protein, g/dL*	6.5 ± 0.7	6.8 ± 0.8	.14
AST, IU/L*	28 ± 17	24 ± 9.7	.20
ALT, IU/L*	23 ± 18	20 ± 17	.52
BUN, mg/dL*	22 ± 10	23 ± 13	.60
Cr, mg/dL*	0.9 ± 0.6	0.7 ± 0.4	.10

WBC, white blood cell; CRP, C-reactive protein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; BUN, blood urea nitrogen; Cr, creatinine.

* Values are expressed as mean ± standard deviation.

Table 2. *Comparison of the Reduction and Disappearance Rates of Methicillin-Resistant* Staphylococcus aureus *in Sputum Between the Catechin Group and the Control Group*

	Numbers of Patients		
	Catechin Group	Control Group	*P* Value
Total patients (n = 69)	n = 36	n = 33	
Reduction†	17 (47%)	5 (15%)	.014*
Disappearance	11(31%)	4 (12%)	.091
Colonized patients (n = 53)	n = 26	n = 27	
Reduction	13 (50%)	5 (19%)	.027*
Disappearance	8 (31%)	4 (15%)	.40
Infected patients (n = 16)	n = 10	n = 6	
Reduction	4 (40%)	0 (0%)	.12
Disappearance	3 (30%)	0 (0%)	.89

* $P < .05$

† Reduction: the summation of decrease and disappearance of methicillin-resistant *Staphylococcus aureus*.

tion with imipenem, panipenem, or ceftazidime. No adverse events, such as respiratory tract obstruction, allergic bronchial spasm, or skin eruption, including laboratory changes, were observed in all patients during the study.

DISCUSSION

The present study demonstrating the effects of tea catechin inhalation on MRSA in a prospective randomized controlled manner is the first to be reported in the literature. The results showed that tea catechin inhalation for 1 week appeared to be effective in reducing the MRSA count when compared with saline inhalation alone. The results are consistent with those of our previous pilot study on the effects of a 4-week inhalation period of tea catechin on MRSA as compared to saline/bromhexine inhalation.[17] Furthermore, the tendency of reduction in MRSA counts was also observed in the colonized patients who were not administered any antibiotics. This tendency was also observed in the infected patients, however this was not significant probably due to the small sample size.

Despite a significant decrease in MRSA counts, the effect of tea catechin on MRSA was not sufficiently strong as to induce a complete eradication of MRSA from sputum. In our previous pilot study, we had observed that the effect of tea catechin inhalation on MRSA was greatest at 1 week of inhalation, however this effect was transient.[17] Therefore, the inhalation method has limited application as a supplementary treatment in combination with the standard therapy for the control of MRSA. Additionally, we should consider some of the limitations of the present study. First, the study design was not completely blinded. Although none of the patients participating in the study or their guardians were informed of the type of material used in the nebulizer, they could identify the material based on their knowledge of the color of tea catechin solution as transparent yellow and that of saline as colorless. Second, tea catechin is not an approved drug; therefore thorough informed consent is essential prior to participation in the study. Additionally, to ensure quality, the solution should be carefully prepared in a hospital clean room under sterile conditions.

The precise mechanism of action of tea catechin against MRSA has not yet been fully elucidated. Some natural products, such as vegetables and fruits, are reported to exhibit inhibitory effects on microorganisms.[20] Among them, tea catechins, a group of natural-occurring polyphenols, possess strong antioxidative activity, and the production of hydrogen peroxide is reported to be involved in the bactericidal activity against several bacterial strains, including MRSA.[21] Recent experimental studies have revealed that EGCG, the major low-molecular-weight polyphenol in green tea leaf extracts, is the main causative component of antibacterial activity and induces synergistic effects with antibiotics against MRSA.[7–15] EGCG can reverse methicillin resistance in MRSA in vitro. This phenomenon can be explained by the prevention of penicillin-binding protein 2' (PBP2') synthesis and inhibition of beta-lactamase secretion.[7] MIC of EGCG against MRSA was reported to be 100 μg/mL or less, and EGCG concentration less than the MIC value reversed the high level resistance of MRSA to beta-lactams.[9] Combinations of EGCG along with some non-beta-lactam antibiotics were also reported to show additive effects.[11,12] We also observed that tea catechins showed antimicrobial activity and induction of synergistic effects with some antibiotics, such as oxacillin, ceftazidime, imipenem, or vancomycin (data not shown in text). The result that tea catechins have the ability to restore the activity of antibiotics that have lost their potency against MRSA is of clinical importance since the overuse of antibiotics has led to development of antibiotic-resistant strains.

Natural chemical products, such as acetic acid and hypertonic saline as well as tea catechins, are known to possess antimicrobial activity.[22–24] With regard to a possible mechanism of inhalation effect of these agents on bacteria, it has been speculated that the hyperosmolarity of the nebulized solution may play an important role in the prevention of bacterial infections of the respiratory tract along with the improvement in mucociliary transport and removal from submucosal and adventitial edema.[23,24]

Precise information on recommended dosage, therapeutic window of tea catechin against MRSA, or concomitant drug interaction has not yet been obtained. In the pharmacokinetic study of tea catechin, low systemic bioavailability has been reported in the literature.[25] Therefore, inhalation might be suitable for reaching the site of action in the respiratory tract, and this therapy is speculated to cause less systemic adverse effects with effective dosage.

Tea catechins have been reported to be well tolerated, except in tea-factory workers with occupational asthma induced by the inhalation of green tea dust.[26,27] Moreover, the serum aspartate aminotransferase and creatinine levels are not altered following the consumption of tea catechin at concentrations up to 1000 mg/d for 3 months in normal volunteers.[28] The study also confirmed that no harmful side effects were observed in the elderly patients during 7 days of inhalation at a concentration of 22.2 mg/d using a handheld nebulizer. Although the results should be carefully interpreted because the sample size was small, catechin inhalation might be a safe supplementary treat-

ment in clinical practice. Further large-scale studies are required for confirming the safety of catechin inhalation.

CONCLUSION

The catechin inhalation appeared to reduce the MRSA count in sputum. However, the application of catechin inhalation as a supplementary treatment for controlling MRSA infection remains controversial. Further studies are required for the evaluation of catechin inhalation effects on MRSA.

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HMG-CoA還元酵素阻害薬Pravastatin服用患者におけるリスクファクターと血清脂質値に関する調査

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Risk Factors and Serum Cholesterol Concentrations in the Patients Given HMG-CoA Reductase Inhibitor, Pravastatin

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Purpose : HMG-CoA reductase inhibitors (statins) have been widely used in the treatment of hypercholesteremia in Japan as well as in Western countries. Although statins have been shown to be effective in the prevention of coronary heart disease (CHD) in high-risk patients, the potential benefit of statins on the overall mortality has not been proven in subjects at lower risk for CHD. In this study, we investigated the risk factors and serum cholesterol concentrations in patients given pravastatin.

Methods : Patients who were given pravastatin during the period from June 2002 until May 2003 in the Hamamatsu University Hospital were studied. Data for height, body weight, age, gender, smoking and history of diabetes mellitus, hypertension and CHD in the patients were collected from their case records. Serum cholesterol concentrations were determined before and after the treatment with pravastatin. The ethics committee in the Hamamatsu University approved this study.

Results : There were 213 male (37.4%) and 356 female (62.6%) patients given pravastatin. The mean age of the patients was 63.9 yrs, and % of the patients aged under 50 yrs was 10.7%. Seventy-seven % of the patients had no history of CHD. Female patients without smoking, diabetes mellitus, hypertension and CHD constituted 17% of all patients. Total and LDL cholesterol levels in all groups were significantly decreased by 17.6% and 25.5%, respectively, after the administration of pravastatin. Treatment with pravastatin was started at the lower total cholesterol levels in male patients or patients with CHD than in female patients or patients without CHD.

Conclusion : Our results suggest that significant numbers of patients with a low risk for CHD were prescribed the statins, and that placebo-controlled large-scale trials should be conducted to demonstrate the benefit and safety of statin treatment on overall mortality in Japan.

Key words : HMG-CoA reductase inhibitors, statins, pravastatin, hypercholesteremia, risk factor

緒　論

近年，わが国においてもライフスタイルの欧米化などにより動脈硬化性疾患が増加し，死因統計で癌と並ぶ大きな位置を占めるようになった．国内外の多くの研究から血清コレステロール値が上昇するに従い，男

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Table 1 Demographic characteristics of the patients treated with pravastatin at the point of the survey

	Male	Female	Total
Number of patients	213 (37.4%)	356 (62.6%)	569 (100%)
Age [years]	63.2±11.6	64.2±12.2	63.9±12.0
Height [cm]	164.3±6.2	151.8±6.1	156.5±6.1
Weight [kg]	63.2±10.0	52.0±8.9	56.2±9.3
Periods for the treatment with pravastatin [month]	48.9±40.4	59.5±46.5	55.5±44.6
Smoking	63 (11.1%)	31 (5.4%)	94 (16.5%)
Risk factors			
Coronary heart disease	80 (14.1%)	52 (9.1%)	132 (23.2%)
Diabetes mellitus	73 (12.8%)	126 (22.1%)	199 (34.9%)
Hypertension	141 (24.8%)	206 (36.2%)	347 (61.0%)

Values are numbers of patients (% of all patients (n=569)), or mean ± SD.

女を問わず虚血性心疾患発症リスクは増加することが示され[1~3]，高コレステロール血症治療の重要性がますます高まっている．高コレステロール血症に対する薬物療法の選択肢はいくつかあるが，なかでも3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) 還元酵素阻害薬（スタチン）は強力なLDLコレステロール（LDL-C）低下作用を有することから，現在第一選択薬として用いられている．欧米諸国を中心に行われた多くの大規模臨床試験では，虚血性心疾患患者を対象とした二次予防試験だけでなく，虚血性心疾患既往歴のない一次予防の場合においても，スタチンによるLDL-Cの低下が心血管イベントの発生率や虚血性心疾患死亡率，さらに総死亡率を低下させることが示されている[4~6]．

一方，わが国では虚血性心疾患の発生率が欧米諸国の1/4から1/10と低いことが知られている[7]．さらに遺伝的素因やライフスタイルも欧米諸国のそれらと異なることから，欧米諸国における大規模試験の結果を日本人にそのまま適応できるかどうか疑問視する意見もある[8]．

わが国においては1989年にpravastatinが発売されて以来，数種のスタチンが臨床適用され，多くの患者に投与されている．しかしわが国においてスタチンがどのような背景を持つ患者に使用されているかを実態調査した報告はほとんどない．スタチンの適正使用を推進するためにも，スタチン使用の実態を把握することは重要である．本研究では，浜松医科大学附属病院においてpravastatinを投与されている患者を対象とし，リスクファクター（年齢，性，喫煙習慣，糖尿病，高血圧，虚血性心疾患の既往）およびpravastatin服用前後の血清脂質値を調査した．

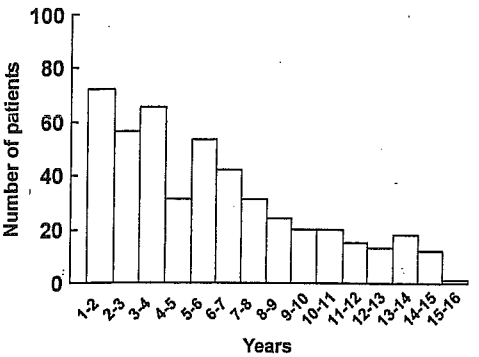

Fig. 1 Periods treated with pravastatin

方　法

浜松医科大学附属病院において2002年6月から2003年5月の間にpravastatin（メバロチン®）を投与された全患者(581例)中，カルテおよび病院オーダリングシステムを調査しえた569例を対象とした．調査期間（2003年6月～2003年8月）中のpravastatin最終投与日における対象患者の身長，体重，年齢と喫煙歴ならびに虚血性心疾患，糖尿病および高血圧の既往の有無について調査した．さらにpravastatin服用前と調査時における血清脂質値が調査可能であった478例において総コレステロール（TC），HDLコレステロール（HDL-C），LDLコレステロール（LDL-C）およびトリグリセリド（TG）を調査した．Pravastatin服用前かつ調査時の臨床検査値をカルテないしオーダリングシステム上から調査することが可能であった症例においては，アスパラギンアミノトランスフェラーゼ（AST），アラニンアミノトランスフェラーゼ（ALT），クレアチンキナーゼ（CK），血清クレアチニン（s-Cre），血液尿素窒素（BUN），随時血

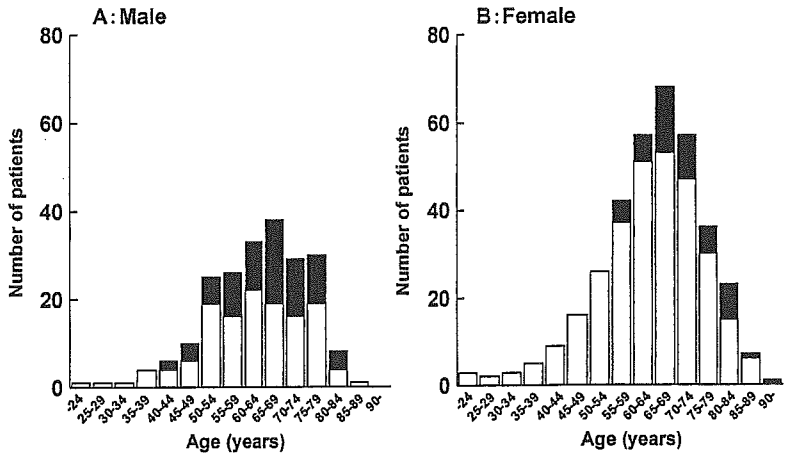

Fig. 2 Number of male (A) and female (B) patients with coronary heart disease (CHD, ■) or without CHD (□)

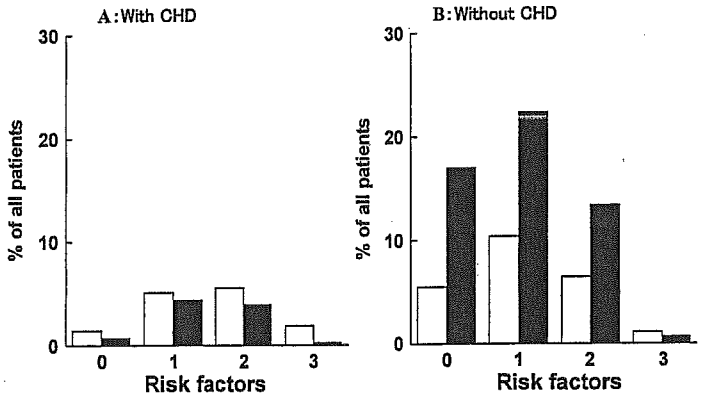

Fig. 3 Number of risk factors (smoking, diabetes mellitus and hypertension) in the patients with (A) and without (B) coronary heart disease (CHD)

Data are % of all patients (n=569).
□: Male, ■: Female

糖（BS）およびヘモグロビン A_{1C}（HbA_{1C}）についても調査した．データは平均値±標準偏差で表示した．統計学的解析は Student's t-test を用い，危険率5%未満を有意差ありと判定した．本研究は浜松医科大学倫理委員会の承認の下に施行した．

成　績

調査した患者のうち男性は213例（37.4%），女性は356例（62.6%）であり，女性患者が男性患者の1.7倍を占めた．対象患者の年齢は63.9±12.0歳であり男女間に有意な差異は認められなかった（Table 1）．対象患者における pravastatin 服用期間は1年以内の頻度が最も高く経時的に減少する傾向が認められた（Fig. 1）．また平均服用期間は55.5±44.6月であった．対象患者の既往歴では高血圧が最も多く全体の61.0%であった．次いで糖尿病が34.9%，虚血性心疾患が23.2%，喫煙が16.5%であった（Table 1）．対象患者の年齢分布では男女ともに65歳から69歳にピークが認められ，49歳以下の患者は全体の10.7%であった．虚血性心疾患の既往のある患者は男性では40歳から認められたのに対し，女性では55歳からであった（Fig. 2）．

虚血性心疾患の既往の有無について調べたところ，男性患者の37.6%（全体の14.1%）と女性患者の14.6%（全体の9.1%）では虚血性心疾患の既往を有していた．すなわち全対象患者の23.2%が二次予防目的のスタチン使用であった（Table 1）．一方，全対象患者のうち22%においては，虚血性心疾患の既往がなく，かつ喫煙歴，糖尿病，高血圧のいずれも有していなかった．その中で女性患者は97例（17.1%）を占めた（Fig. 3）．

Pravastatin 服用開始前後における血清脂質値の調

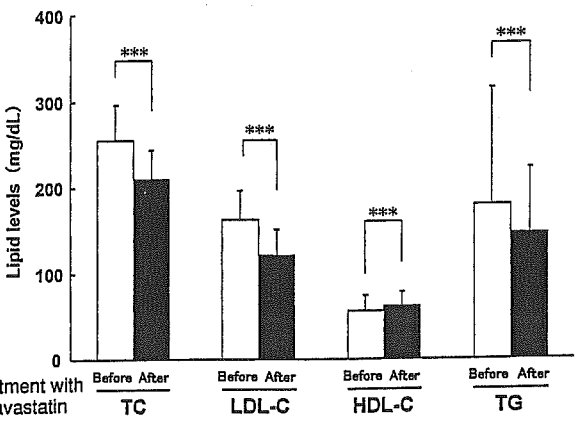

Fig. 4 Lipid profiles in the patients before (□) and after (■) the treatment with pravastatin
TC : Total cholesterol, LDL-C : LDL cholesterol, HDL-C : HDL cholesterol, TG : triglyceride, $^{***}p<0.001$

査によると，対象患者の TC は 255±41 mg/dL から 210±34 mg/dL へと 17.6%有意に低下した．同様に LDL-C および TG はそれぞれ 25.5%および 18.7%有意に減少した．一方 HDL-C は 8.7%有意に増加した (Fig. 4)．さらに対象患者を性別および虚血性心疾患の既往によって層別化したところ，TC，LDL-C および TG は性別および虚血性心疾患の既往にかかわらずいずれの群においても有意に低下した (Fig. 5)．また HDL-C は女性で心血管疾患の既往がある群を除き有意に増加した．さらに男女ともに虚血性心疾患の既往がある群では既往なし群に比べ，また虚血性心疾患の既往にかかわらず，女性に比べ男性においてより低い TC レベルから pravastatin の投与が開始されていた (Fig. 5 A)．

Table 2 に pravastatin 服用患者における服用開始前および服用後の臨床検査値を，糖尿病既往あり群となし群に分けて示した．糖尿病既往なし群では，いずれの検査値においても服用前後で有意な差は認められなかった．一方，糖尿病既往あり群では pravastatin 服用後では，BUN および s-Cre は有意に高値を，HbA_{1c} は有意に低値を示した．

考　察

本研究では，わが国においてスタチンがどのような背景を持つ患者に使用されているかを推測する目的で，浜松医科大学附属病院において pravastatin を投与されている患者の背景を調査し，さらに本薬剤が血清脂質値に及ぼす影響について検討した．

今回は pravastatin 服用患者の 569 症例の背景について調査した．この症例数は浜松医科大学附属病院における pravastatin 処方数の 98%にあたる．今回の対象患者において虚血性心疾患既往歴のある患者は全体の 23%のみであった．現在までに行われている大規模臨床試験から，虚血性心疾患の二次予防におけるスタチン投与の有用性は明確に示されているが，一次予防の場合には二次予防の場合に比べその有用性が低くなることが知られている[4]．今回の調査から，わが国におけるスタチン投与患者の多くが，比較的有用性の低いと考えられる一次予防であると推察された．また女性で虚血性心疾患，糖尿病，高血圧の既往および喫煙歴のない患者が全体の 17%占めていた．虚血性心疾患に対するスタチン投与の有用性は，患者のベースラインリスクに依存することが明らかにされており[9]，虚血性心疾患の絶対リスクが欧米諸国に比べ低いわが国において一次予防，とくに高コレステロール血症のみを有する女性患者など，低リスク群に対するスタチンの有用性は十分に証明されているとは言えない．今後 EBM の観点からも医療経済的な視点からも，日本人におけるスタチン投与の有用性の検証が必要であると思われる．

今回の対象患者のうち 478 症例 (全症例の 84%) において，pravastatin 開始および調査時の血清脂質値が調査可能であった．Pravastatin 開始時の TC および LDL-C はそれぞれ 255 mg/dL および 162 mg/dL であった．この値は欧米および日本で行われた大規模臨床試験でのスタチン開始時での値とほぼ同値かやや低い値である[4~6,10~12]．今回，pravastatin の投与によって TC は 18%，LDL-C は 26%有意に低下した．Pravastatin を用いた大規模臨床試験における TC および LDL-C の低下率はそれぞれ 20%および 25%程度であることから[5,6,11,12]，それらの試験同様，本研究結果は pravastatin の良好なコレステロール低

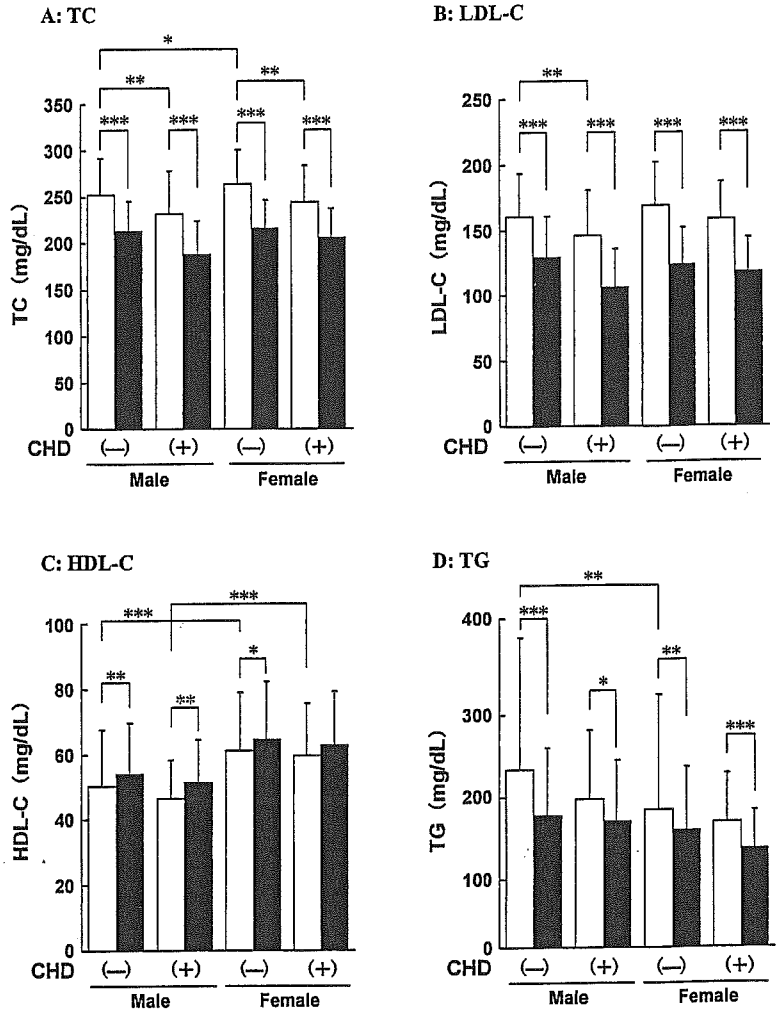

Fig. 5 Lipid profiles before (□) and after (■) the treatment with pravastatin in male and female patients with or without coronary heart disease (CHD)

TC : Total cholesterol, LDL-C : LDL cholesterol, HDL-C : HDL cholesterol, TG : triglyceride, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

Table 2 Laboratory data before and after the treatment with pravastatin in the patients with or without diabetes mellitus

Laboratory data	Diabetes mellitus	Number of patients	Before pravastatin	After pravastatin
AST	−	220	24.3±12.7	23.0±10.0
	+	132	22.8±9.2	22.8±13.9
ALT	−	221	23.0±10.0	21.7±16.8
	+	129	22.8±12.4	22.2±20.6
CPK	−	192	106±83	108±58
	+	116	99.8±93.3	115±106
BUN	−	208	16.4±5.2	16.9±6.2
	+	130	16.8±7.0	18.4±9.1**
s-Cre	−	199	0.819±0.300	0.838±0.336
	+	131	0.770±0.367	0.909±0.615***
BS	−	107	104±19	105±23
	+	118	163±74	153±84
HbA_{1c}	−	53	5.57±0.49	5.59±0.54
	+	104	7.64±1.74	7.37±1.61*

Values are mean ± SD, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$

AST : L-asparate aminotransferase, ALT : L-alanine aminotransferase, CK : creatine kinase, s-Cre : serum creatinine, BUN : blood urea nitrogen, BS : blood glucose, HbA_{1c} : hemoglobin A_{1c}

Table 3 Demographic characteristics of the patients in the quartile treatment periods with pravastatin

	Periods with pravastatin [month]			
	0.9—22.1	22.1—50.8	50.9—87.2	87.5—174.5
Number of patinets	118	119	119	118
Male	56 (48%)	40 (34%)	49 (41%)	33 (28%)
Age* [years]	59.4±13.2	64.2±11.7	64.6±11.9	65.4±11.0
Smoking*	23 (19%)	21 (18%)	18 (15%)	16 (14%)
Risk factors				
Coronary heart disease	36 (31%)	30 (25%)	23 (19%)	21 (18%)
Diabetes mellitus	39 (33%)	34 (29%)	41 (34%)	48 (41%)
Hypertension	63 (53%)	79 (66%)	72 (61%)	76 (64%)

Values are number of patients or mean±SD.
( ) : % of numbers in the quartile treatment periods with pravastatin.
*Data at the point of the survey are presented.

下作用を示すものである．

今回興味深いことに，男女ともに虚血性心疾患の既往がある群では既往なし群に比べ，pravastatin はより低値の TC レベルから処方が開始されていることが明らかとなった．また虚血性心疾患の既往にかかわらず，女性に比べ男性でより低い TC から pravastatin の処方が開始されていた．このことは，処方者が虚血性心疾患発症リスクを考慮し，男性や虚血性心疾患の既往のある患者に対して，より低い TC から投与を開始したものと考えられる．

スタチン投与による臨床検査値の変動は，糖尿病の既往なし群では認められなかった．糖尿病を有する患者で pravastatin 服用後において HbA_{1c}が有意に低下していた．本研究では糖尿病の治療開始時期などの調査は行っていないため，HbA_{1c}が低下した理由は明らかではないが，pravastatin 服用期間中に糖尿病の治療が開始されたのではないかと思われる．さらに糖尿病を有する患者において腎機能検査値（s-Cre, BUN）の有意な上昇を認めた．このメカニズムは明らかではないが，糖尿病の合併症として腎機能障害の頻度は高く，非糖尿病患者群では pravastatin 投与によっても s-Cre と BUN の有意な変化は認められないことから，糖尿病の自然経過を反映するものかもしれない．

今回の調査は浜松医科大学附属病院の pravastatin 服用患者を対象とした．本研究結果は大学病院のような特定機能病院のものであり，直接わが国全体の処方動向と一致するものではないかもしれない．一般病院や診療所などにおける同様な調査の結果と併せて考慮する必要があるだろう．

さらに本研究では 2002 年 6 月から 1 年間の期間に pravastatin を投与されているほぼ全患者について調査し，2002 年 6 月からさかのぼって平均 4.5 年間の投与期間について調査した．したがって調査対象には，長期間投与されている患者と比較的最近投与が開始されている患者が混在している（Fig. 1）．このうちとくに長期間にわたって投与されている患者についてのデータの解釈には慎重でなければならない．すなわち数年前に投与が開始され，2002 年の 6 月から 1 年間の期間のいずれかの時点でも引き続き，pravastatin が投与されている患者は，数年前に投与開始となった患者の一部分と考えられ，死亡例，当該医療機関への来院を中止したもの，来院は続けているとしても副作用や十分な効果がみられないために投与を中止または変更したもの，または逆に血清脂質の正常化などの理由で治療を中止したものなどは，本研究の調査対象には含まれていない．これらの理由で調査対象に含まれていない患者の背景と，調査対象に含まれている長期にわたって投与が続けられている患者の背景が相違する可能性は否定できない．Pravastatin 服用期間に対して対象患者の背景因子を検討したところ，年齢および虚血性心疾患の既往率以外の因子に関しては明らかな傾向は認められなかった（Table 3）．平均年齢は服用期間が長くなるほど高い傾向が認められた．さらに虚血性心疾患の既往患者の割合は服用期間が短いほど増加する傾向が認められた．この理由として長期投与患者では虚血性心疾患発症にともなう他剤への変更または患者の死亡や転院が潜在する可能性が考えられる．したがって，今回の調査結果では pravastatin 服用患者の虚血性心疾患既往率を低く見積もっている可能性は否定できない．一方でこの結果は，最近になって pravastatin は一次予防に比べ二次

予防に対し積極的に用いられるようになったことを示しているのかもしれない．

結　論

本研究の対象患者においてpravastatinは血清コレステロール値を有意に低下しており，本剤の高脂血症治療における臨床的有用性が確認された．さらに処方者は心血管疾患発症リスクを考慮し，男性や虚血性心疾患の既往のある患者に対して，より低いTC値から投与を開始していることが明らかとなった．

一方，本研究では比較的虚血性心疾患発症リスクが低いと考えられる患者に対してpravastatin処方頻度が高いことが明らかとなった．虚血性心疾患の既往がない女性など低リスク患者に対するスタチン使用の有用性についてはいまだ十分に証明されているとは言えず，今後このような患者群に対するスタチン投与のエビデンス構築が必要と考えられる．

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3P-058

イソニアジドによる肝機能障害と *NAT2* 遺伝子多型との関連

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【目的】抗結核薬の重篤な副作用として，肝機能障害がよく知られており，その起因薬としてイソニアジド（INH）が主たるものといわれている．これまで，肝機能障害の発現頻度は，INH の主代謝酵素である *NAT2* の遺伝子多型の slow acetylator（SA）では高頻度で起こると報告されていた．しかし，近年 intermediate acetylator（IA）での肝機能障害発現も報告され，この *NAT2* と INH による肝機能障害発現に関しては未だ明らかではない．そこで本研究の目的は，INH を含む抗結核薬で治療されている日本人肺結核患者における肝機能障害の発現に及ぼす *NAT2* の遺伝子多型と INH の薬物動態の関係を明らかにすることにある．

【方法】国立病院機構福岡東医療センターに入院中の肺結核患者で，遺伝子倫理委員会並びに臨床研究倫理委員会の承認のもとで文書による説明と同意の得られた 46 名（平均年齢 52.2 歳，平均体重 52.9 kg）を対象に行った．*NAT2* の遺伝子型は Invader Assay により判定した．INH およびその代謝物（AcINH）の血中および尿中濃度は，HPLC 法により測定した．

【結果・考察】肝機能障害を発現した患者は 6 名で，そのうち *NAT2* の RA（rapid acetylator）28 名中 2 名（7%）で遺伝子型は野生型のホモ，*NAT2* の IA 15 名中 2 名（13%）で遺伝子型は野生型と変異型のヘテロであった．残りは SA 3 名中 2 名（67%）で変異型のホモであった．

RA 26 名，IA 13 名，SA 3 名での INH の薬物動態と遺伝子型の関係では，尿中 AcINH/INH 濃度比において SA（平均値：1.10）は RA（平均値：6.77）に比し低値を示した．INH の AUC においては SA（平均値：123.0）は RA（平均値：33.9）に比し高値を示した．また，服薬後 0.5，1，2，4，7 時間の血中 INH 濃度と AUC の相関では，4 時間後の血中 INH 濃度と AUC が最も相関が良好であった．今回の研究において，肝機能障害発現頻度は従来報告されているように SA で発現頻度が高くなる結果を得た．このことから，Invader Assay による *NAT2* の遺伝子型解析は，INH 服用による肝障害発現に関する情報を迅速に臨床の現場に提供できるものと思われる．また，INH 服用後 4 時間の血中 INH 濃度と AUC の相関が高いことから，INH の投与設計においては血中 INH 濃度の 4 時間値が利用できることが示唆された．

ORIGINAL RESEARCH ARTICLE

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The Effect of Aging on the Relationship between the Cytochrome P450 2C19 Genotype and Omeprazole Pharmacokinetics

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Abstract

Background and objective: The metabolic activity of cytochrome P450 (CYP) 2C19 is genetically determined, and the pharmacokinetics of omeprazole, a substrate for CYP2C19, are dependent on the CYP2C19 genotype. However, a discrepancy between the CYP2C19 genotype and omeprazole pharmacokinetics was reported in patients with liver disease or advanced cancer. The objective of the present study was to evaluate the effect of aging on the relationship between the CYP2C19 genotype and its phenotype.

Methods: Twenty-eight elderly and 23 young Japanese volunteers were enrolled after being genotyped. Each subject received a single intravenous dose of omeprazole (10mg and 20mg for the elderly and the young groups, respectively) and blood samples were obtained up to 6 hours after dose administration to determine the plasma concentrations of omeprazole and its metabolites, 5-hydroxyomeprazole and omeprazole sulfone. Pharmacokinetic parameters were obtained by noncompartmental analysis. Linear regression models were used to examine the joint effects of covariates such as genotype, age, etc., on the pharmacokinetic parameters, and the pharmacokinetic parameters showing statistical significance were compared by ANOVA.

Results: There were significant differences between genotypes in the area under the plasma concentration-time curve of the young group and the elderly group. The number of mutation alleles and age were significant covariates for systemic clearance (CL), but age was the only significant covariate for volume of distribution at steady state (V_{ss}). There were significant age- and genotype-related differences and a significant age × genotype interaction in CL (20.6 ± 11.0/12.7 ± 4.0/3.2 ± 1.0 and 5.4 ± 4.0/3.7 ± 1.4/2.1 ± 0.7 L/h for homozygous extensive metabolisers [EMs]/heterozygous EMs/poor metabolisers [PMs] of the young and the elderly groups, respectively). In V_{ss}, a significant difference was found between the young and the elderly groups (219 ± 115 and 107 ± 44.5 mL/kg, respectively), but not between three genotypes (178 ± 142, 173 ± 79 and 110 ± 51 mL/kg for homozygous EMs, heterozygous EMs and PMs, respectively).

Conclusion: The elderly EMs showed wide variance in the *in vivo* CYP2C19 activity and were phenotypically closer to the elderly PMs than the young EMs were to the young PMs. Some of the elderly homozygous EMs, as well as heterozygous EMs, have a metabolic activity similar to PMs, and the CYP2C19 genotype may therefore not be as useful as phenotyping in the elderly.

Background

The metabolic activity of cytochrome P450 (CYP) 2C19 is genetically determined and the pharmacokinetics of many drugs metabolised by CYP2C19 are dependent on the CYP2C19 genotype.[1-3] Omeprazole, a proton pump inhibitor, is a therapeutic agent that is widely used in hyperacidity-related disorders or disorders caused by *Helicobacter pylori* infection. The CYP2C19 genotype influences the pharmacokinetics and therapeutic effects of omeprazole,[4-8] however, the discrepancy in the relationship between the CYP2C19 genotype and its phenotype is noted in some situations. Patients with advanced metastatic cancer[9] or those with liver disease[10] were reported to have a poor metaboliser (PM) phenotype of CYP2C19, even though they were genotyped as extensive metabolisers (EMs). A previous study reported a few subjects >65 years of age who showed a discrepancy between the CYP2C19 genotype and its phenotype in a Japanese population.[11]

The aging process is characterised by a progressive loss of physiological functions of many organs; this age-related alteration is also found in drug metabolism.[12,13] The age-associated changes involve the microsomal mixed-function oxidative system. Omeprazole disposition is also altered with age – the elimination rate is decreased and the plasma half-life is prolonged in the elderly.[14] These findings suggest that the relationship in the elderly between the CYP2C19 genotype and its phenotype may differ from that in young adults because of the age-associated decline in the metabolic activity of CYP2C19; however, little is known about the influence of the aging process on this relationship. In the elderly, if the relationship between the CYP2C19 genotype and its phenotype differs from that in young adults, the CYP2C19 genotype may not be a good predictor of the metabolic activity in the elderly. We therefore examined the relationship between the CYP2C19 genotype and its phenotype, as measured by omeprazole pharmacokinetics in the elderly, with three different CYP2C19 genotypes, and compared the relationship to that in the young adults.

Methods

Subjects and Study Design

Twenty-eight elderly (age range 66–85 years) and 23 young (age range 21–36 years) Japanese volunteers were enrolled in the study after they provided written informed consent. The study protocol was approved by the Ethics Committee of Hirosaki University School of Medicine, Hirosaki, Japan. The baseline demographic characteristics of the elderly and the young groups are summarised in table I. Subjects in the elderly group were recruited from ambulatory patients of Kawauchi Clinic, Kawauchi, Aomori, Japan. Each subject was genotyped for CYP2C19 before the study and considered eligible for the study on the basis of physical examination and routine laboratory tests. No subject had any signs or symptoms suggesting cardiac, renal or hepatic disorder, and they did not take any medication known or suspected to affect omeprazole pharmacokinetics[2,15] within 1 week before the study. Subjects in the young group were members of a healthy volunteer panel in the department genotyped for CYP2C19 in advance. These subjects did not take any medication for at least 7 days before the study.

Genotyping

For CYP2C19 genotyping, venous blood (10mL) was obtained and genomic DNA was extracted from the peripheral lymphocytes using an extraction kit

(QIAamp DNA Blood Maxi Kit, QIAGEN GmbH, Hilden, Germany). The mutated alleles for *CYP2C19*, *CYP2C19**3 and *CYP2C19**2 had been identified using the allele-specific primers described by de Morais et al.[16] A subject with one or two mutation alleles is categorised as a heterozygous EM or PM, while a subject without mutation alleles is categorised as a homozygous EM.

Phenotyping and Analytic Methods

For CYP2C19 phenotyping, each subject received omeprazole (10mg for the elderly group and 20mg for the young group), with a single intravenous bolus administration over 30 seconds after an overnight fast. Since plasma omeprazole concentration was expected to be higher in the elderly group after intravenous administration compared with the young group, the elderly subjects received half the dose administered to the young group. No meals were allowed until 3 hours after drug administration. Blood samples (10mL) were obtained through an indwelling catheter placed in an antecubital vein of the contralateral arm of each subject before and at 5 minutes and 0.5, 1, 2, 3, 4 and 6 hours after drug administration. Plasma was separated immediately and kept at −20°C until analysis. Plasma concentrations of omeprazole and its metabolites (5-hydroxyomeprazole and omeprazole sulfone) were determined by the high-performance liquid chromatography method of Kobayashi et al.[17] The method was validated for the concentration range 10–10 000 ng/mL. Intra- and interday relative standard deviations (SD) were <8.9% at the 10 ng/mL concentration. The lower limit of quantification was 5 ng/mL for each compound.

Pharmacokinetic Analysis

Pharmacokinetic parameters were obtained by noncompartmental analysis. In the calculation of the global moments of the plasma concentration-time course, the concentration at time zero was estimated using the initial two points (5 and 30 minutes), and the terminal elimination rate constant (k_e) was estimated using regression analysis of the log-linear part of the concentration-time curve. The area under the plasma concentration-time curve (AUC) and the mean residence time (MRT) were calculated by the trapezoidal rule from time zero to the last quantifiable plasma omeprazole concentration, and then extrapolated to infinity using k_e. The systemic clearance (CL) was calculated as the dose divided by

Table I. Baseline demographics of all subjects[a]

Genotype	No. of subjects (M/F)	Age [y] (M/F)	Bodyweight [kg] (M/F)	Body mass index [kg/m^2] (M/F)
Young group				
Homozygous EMs	8 (5/3)	25.0 ± 4.0 (24.8 ± 4.0/25.3 ± 4.9)	60.8 ± 14.3 (67.4 ± 13.4/49.7 ± 7.6)	21.4 ± 3.8 (23.1 ± 3.8/18.7 ± 1.5)
Heterozygous EMs	9 (4/5)	25.3 ± 4.5 (27.5 ± 6.2/23.6 ± 1.9)	55.0 ± 17.6 (69.3 ± 17.8/43.6 ± 4.2)	20.9 ± 5.5 (24.3 ± 7.1/18.1 ± 1.1)
PMs	6 (4/2)	28.0 ± 5.3 (28.3 ± 5.7/23, 32)	66.8 ± 16.0 (76.0 ± 7.9/42, 55)	23.5 ± 5.0 (25.9 ± 3.9/23, 32)
All	23 (13/10)	25.9 ± 4.5 (26.7 ± 5.1/24.9 ± 3.8)	60.1 ± 16.1 (70.6 ± 13.0/46.4 ± 6.2)	21.8 ± 4.7 (24.3 ± 4.8/18.4 ± 1.6)
Elderly group				
Homozygous EMs	8 (0/8)	76.1 ± 4.5	53.9 ± 7.3	25.8 ± 2.6
Heterozygous EMs	12 (6/6)	78.1 ± 6.4 (76.5 ± 5.5/79.7 ± 7.3)	53.9 ± 8.1 (57.4 ± 9.1/49.7 ± 4.5)	24.2± 2.3 (24.0 ± 2.6/24.4 ± 2.2)
PMs	8 (0/8)	78.5 ± 4.9	55.5 ± 8.5	26.6 ± 4.2
All	28 (6/22)	77.6 ± 5.4 (76.5 ± 5.5/78.0 ± 5.5)	54.4 ± 7.7 (58.0 ± 9.1/53.4 ± 7.2)	25.3 ± 3.1 (24.0 ± 2.6/25.7 ± 3.1)

a Values are expressed as mean ± SD, unless specified otherwise.

EMs = extensive metabolisers; **F** = female; **M** = male; **PMs** = poor metabolisers.

AUC, and the volume of distribution at steady state (V_{ss}) was obtained from equation 1:

$$V_{ss} = \frac{CL \times MRT}{bodyweight} \quad \text{(Eq. 1)}$$

The terminal elimination half-life ($t_{1/2}\beta$) was calculated as $0.693/k_e$.

The hydroxylation index (HI) was calculated from equation 2:

$$HI = \frac{\text{5-hydroxyomeprazole } AUC_{last}}{\text{omeprazole } AUC_{last}} \quad \text{(Eq. 2)}$$

where AUC_{last} is the AUC from time zero to the last quantifiable plasma concentration in μmol/L. Two metabolite AUCs (5-hydroxyomeprazole and omeprazole sulfone) were calculated by the trapezoidal rule from time zero to the last quantifiable plasma concentration.

Statistical Analysis

Data are presented as mean ± SD. The AUC was compared between genotypes by the use of one-way ANOVA. Linear regression models were used to examine the joint effect of covariates on the pharmacokinetic parameters, including CL, HI, MRT, $t_{1/2}\beta$ and V_{ss}. The following variables were analysed for inclusion in the model: the number of mutation alleles, age, sex and body mass index (BMI). The BMI was excluded from the variables in the analysis for V_{ss} because the parameter was corrected by bodyweight. In the parameters showing statistical significance, differences were evaluated using one-way or two-way ANOVA, as appropriate. All statistical analyses were performed with the use of SPSS for Windows (version 8.0.1, SPSS Japan Inc., Tokyo, Japan). A p-value of <0.05 was considered statistically significant.

Results

Mean plasma concentration-time curves of omeprazole, 5-hydroxyomeprazole and omeprazole sulfone in the subjects with each genotype are illustrated in figure 1. Mean AUCs of omeprazole and its metabolites are summarised in table II. Significant differences were found in omeprazole AUCs of the young and the elderly groups. In homozygous and heterozygous EMs, 5-hydroxyomeprazole AUC was greater than omeprazole sulfone AUC, but mean AUC ratios of omeprazole metabolites of the elderly EMs were relatively small compared with those of the young EMs. Since the omeprazole dose for the elderly group was different from that for the young group, the plasma concentrations are corrected by dose (figure 2). Mean dose-corrected concentrations in the elimination phase of the elderly PMs were very similar to those of the young PMs, and the dose-corrected concentrations of the elderly heterozygous and homozygous EMs ranged between the young PMs and heterozygous EMs.

Statistical results of the linear regression analysis are summarised in table III. The number of mutation alleles and age were significant covariates for CL, HI, MRT and $t_{1/2}\beta$, but age was the only significant covariate for V_{ss}. The genotype- and age-related differences in CL, HI, MRT and $t_{1/2}\beta$ were compared by using two-way ANOVA, and the age-related difference in V_{ss} was compared by using one-way ANOVA.

Statistical results of the two-way ANOVA are summarised in table IV, and each dataset is illustrated in figure 3. For CL (figure 3a), HI (figure 3b), MRT (figure 3c) and $t_{1/2}\beta$ (figure 3d), similar statistical trends were found: age × genotype interaction ($p = 0.0018$, $p = 0.0028$, $p = 0.0005$ and $p = 0.0162$, respectively); age (all $p < 0.0001$) and genotype ($p < 0.0001$, $p < 0.0001$, $p = 0.0366$ and $p < 0.0001$, respectively). The mean pharmacokinetic parameter values of PMs were significantly different from those of homozygous or heterozygous EMs in both the young and the elderly groups, but the ratios between PMs and homozygous EMs in the elderly group were small compared with those in the young group. Some elderly homozygous EMs as well as heterozygous EMs had these pharmacokinetic parameters similar to young and elderly PMs, and the mean values of these parameters of elderly homozygous EMs ranged between the young PMs and heterozygous EMs.

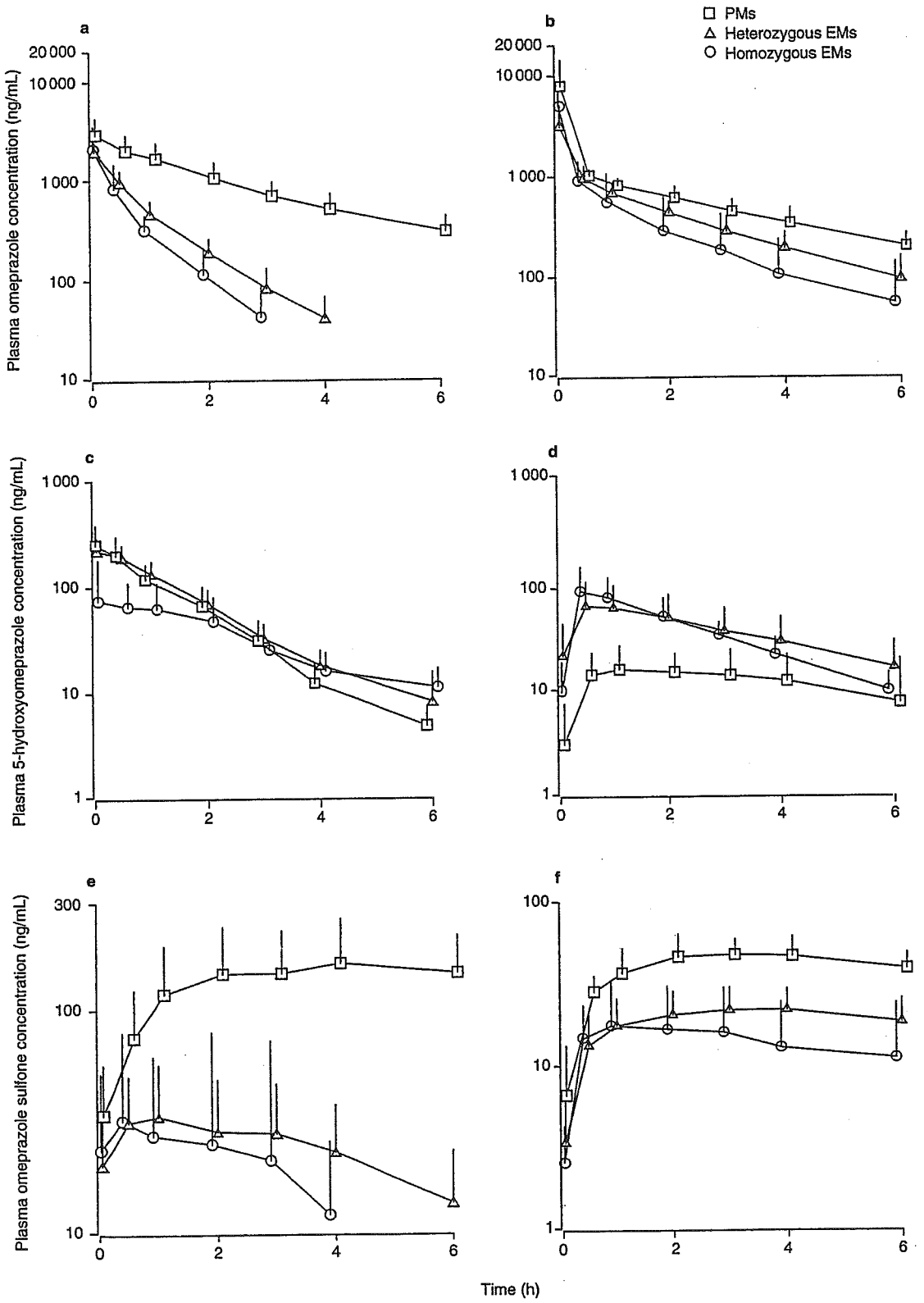

Fig. 1. The time course of plasma omeprazole concentrations (mean + SD) in the young (**a**) and the elderly (**b**), plasma 5-hydroxyomeprazole concentrations in the young (**c**) and the elderly (**d**), and plasma omeprazole sulfone concentrations in the young (**e**) and the elderly (**f**) subjects with each genotype. **EMs** = extensive metabolisers; **PMs** = poor metabolisers.