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-

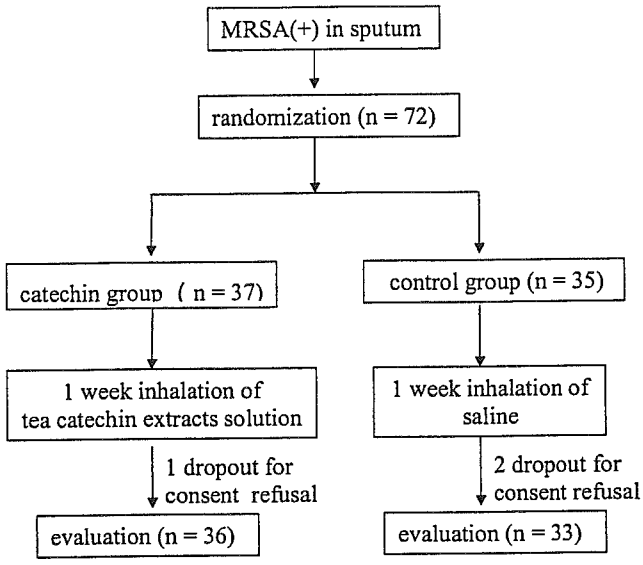

Fig. 1. *Flow of the study protocol.*

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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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．

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Proteasome Function and Pathological Proteins in the Pathogenesis of Parkinson's Disease

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Parkinson's disease (PD) is a multifactorial disease that appears to arise from the effects of both genetic and environmental influences (1). Parkinsonism induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) showed rigidity, akinesia, stooped posture, and response to levodopa therapy; and it was indistinguishable from idiopathic PD. The discovery of MPTP demonstrated that toxic substances could cause PD. Epidemiological studies showed that pesticides and heavy metals are the principle environmental factors that appear to have an impact on the development of PD. The genetic contribution in PD has been debated for over a century. More recently, an increasing number of well-documented multigenerational parkinsonian kindreds have been reported with evidence of autosomal dominant inheritance with variable penetrance. Genes associated with either PD or Parkinson-related disorders include parkin, DJ-1, ubiquitin C-terminal hydrolase isozyme L1 (UCH-L1), nuclear receptor-related factor 1, and α-synuclein. α-Synuclein is particularly notable because it aggregates and is the main component of Lewy bodies (LBs). Because ubiquitin also accumulates in LBs, and parkin and UCH-L1 interact with the ubiquitin proteasomal system, proteasomal dysfunction is thought to contribute to the pathophysiology of PD. However, α-synuclein expression levels by themselves have no significant effect on proteasome peptidase activity, subunit expression, and proteasome complex assembly and function (2). Other mechanisms resulting in synuclein aggregation (not simply expression levels) may be the key to understanding the possible effect of aggregated synuclein on proteasome function. Aggregated α-synuclein binds to the proteasome and inhibits proteasomal activity. When rats were treated with stereotaxic unilateral infusion of lactacystin, a selective proteasome inhibitor, into the substantia nigra pars compacta, the animals became progressively bradykinetic, adopted a stooped posture, and displayed contralateral head tilting. Administration of apomorphine to lactacystin-treated rats reversed behavioral abnormalities and induced contralateral rotations (3). Lactacystin caused dose-dependent degeneration of dopaminergic cell bodies and processes with the cytoplasmic accumulation and aggregation of α-synuclein to form inclusion bodies. When proteasome inhibitors were injected systematically into adult rats over a period of 2 weeks, animals developed progressive parkinsonism with bradykinesia, rigidity, tremor, and an abnormal posture, which improved with apomorphine treatment. These findings support the notion that failure of the ubiquitin-proteasome system to degrade and clear unwanted proteins is an important etiopathogenic factor in PD (4).

On the other hand, Inden et al. found that injection of proteasome inhibitors to the substantia nigra pars compacta of rats did not cause cell loss or dysfunction of dopaminergic cells and protected dopaminergic cells from the toxic effect of 6-hydroxydopamine (6-OHDA) (5). These results showed the proteasome-involved toxic effect of 6-OHDA and inhibition of the proteasome in the animals subjected to 6-OHDA treatment caused inclusion bodies that did not cause cell loss. Accumulation of α-synuclein might protect the dopaminergic cells from the 6-OHDA toxicity as aggresomes. Parkin protein functions as a ubiquitin ligase. Mutations in the parkin gene induce ubiquitin-proteasome dysfunction and cause autosomal recessive juvenile parkinsonism. However, most patients with park2 PD did not exhibit the LBs.

The dose of proteasome inhibitors or the state of proteasome inhibited might cause the different results in

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these reports. More studies would be needed to reveal the function of the proteasome in the neurodegeneration of dopaminergic cells.

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Full Paper

Effect of Clarithromycin on the Pharmacokinetics of Cabergoline in Healthy Controls and in Patients With Parkinson's Disease

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Abstract. Cabergoline is used in the treatment of Parkinson's disease (PD). Clarithromycin is a potent inhibitor of CYP3A4 and P-glycoprotein and is often co-administered with cabergoline in usual clinical practice. We studied the effect of clarithromycin co-administration on the blood concentration of cabergoline in healthy male volunteers and in PD patients. Study 1: Ten healthy male volunteers were enrolled and were randomized to take a single oral dose of cabergoline (1 mg/day) for 6 days or a single oral dose of cabergoline plus clarithromycin (400 mg/day) for 6 days. Study 2: Seven PD patients receiving stable cabergoline doses were enrolled. They were evaluated for the plasma cabergoline concentration before and after the addition of clarithromycin 400 mg/day for 6 days, and again 1 month after discontinuation of clarithromycin. The dose and duration of clarithromycin were decided according to usual clinical practice. In healthy male volunteers, mean C_{max} and $AUC_{0-10\,h}$ of cabergoline increased to a similar degree during co-administration of clarithromycin. Mean plasma cabergoline concentration over 10 h post-dosing increased 2.6-fold with clarithromycin co-administration. In PD patients, plasma cabergoline concentration increased 1.7-fold during clarithromycin co-administration. Co-administration with clarithromycin may increase the blood concentration of cabergoline in healthy volunteers and in PD patients.

Keywords: cabergoline, clarithromycin, drug-drug interaction, Parkinson's disease

Introduction

Dopamine receptor agonists are widely used to treat Parkinson's disease (PD): patients who are treated early in their disease course with monotherapy show a lower incidence of motor complications and adjunctive therapy is also effective in advanced stages of the disease when combined with L-dopa (1 – 3). Structurally, dopamine receptor agonists can be divided into ergoline derivatives, non-ergoline derivatives, and apomorphines (4). Cabergoline is a synthetic ergot dopamine agonist and has the potential for the treatment of PD, acromegaly, and hyperprolactinaemia (3, 5, 6). It is metabolized mainly in the liver via hydrolysis (7, 8). Clarithromycin, a macrolide antibiotic, is a potent inhibitor of the CYP3A4 and P-glycoprotein and consequently increases the blood concentration of certain drugs. Some clinical studies suggest that fewer serious drug interactions occur with clarithromycin than with older macrolides such as erythromycin and troleandomycin (9 – 11). Clarithromycin is widely used to treat respiratory tract infections and is often used in PD patients with such infections. Pneumonia is the most frequent cause of death associated with PD. The administration of clarithromycin might potentially cause an alteration of the pharmacokinetics of cabergoline. We studied the effect of co-administration of clarithromycin on the plasma concentration of cabergoline in healthy male volunteers and PD patients to ensure the safety of cabergoline during its clinical use.

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Materials and Methods

All volunteers and patients recruited to these randomized, non-blind, crossover studies gave their written informed consent prior to participation. The protocols were reviewed and approved by the Institutional Review Board of Ehime University School of Medicine, and the studies were conducted in accordance with the Declaration of Helsinki and its subsequent amendments.

Study 1

Ten healthy male volunteers, aged 20 to 52 years, were enrolled in this study. All volunteers were Japanese and selected on the basis of normal medical history, physical examination, and clinical laboratory tests. They had not taken any medication for at least 4 weeks before starting the study. Volunteers were excluded if they had confirmed or suspected cardiovascular and/or cerebrovascular disorders and if they had any history or other disorders that might compromise their safety during the study. Volunteers were randomized to cabergoline (1 mg oral tablet) (Kissei, Nagano) once daily in the morning with or without clarithromycin (200 mg oral tablet) (Taisho Toyama, Tokyo) twice daily for 6 days and crossed over to the alternate treatment after a 40-day washout period. Domperidone (10 mg oral tablet) (Kyowa Hakko Kogyo, Tokyo) was co-administered with each dose of cabergoline for prophylaxis against vomiting. Blood samples were collected on day 6 of each cycle at 0, 1, 2, 3, 4, 6, 8, and 10 h following dosing of cabergoline with or without clarithromycin.

Study 2

Seven Japanese PD patients (4 female, 3 male), aged 30 to 80 years, were enrolled in this study. All of them had been treated with cabergoline at a stable dose for more than 3 months. They were not taking any medication or food known to reduce or increase CYP activity before starting the study. All patients had been treated with the same medications for the last 4 weeks before recruitment and these were unchanged during the study. As in Study 1, the following patients were excluded: those with confirmed or suspected cardiovascular and/or cerebrovascular disorder and if they had any history or other disorders that might compromise their safety during the study. Patients received the addition of clarithromycin (400 mg) twice daily for 6 consecutive days to their previous medications, which included stable doses of cabergoline. Their signs and symptoms of PD were evaluated using the Unified Parkinson's Disease Rating Scale (UPDRS) (12 – 14). Blood samples were taken 3 h after administration of cabergoline on day 1 (immediately before addition of clarithromycin) and on day 6 (with addition of clarithromycin). Patients were asked to return a month after the cessation of this study and another blood sample was taken 3 h after cabergoline administration. They were asked not to take any medication or food known to alter CYP activity prior to this visit and to ensure that the daily dose of cabergoline was not changed.

Measurement of plasma cabergoline concentration

Blood was collected in 5-ml sodium-heparinized tubes and centrifuged at 3000 rpm for 15 min. Plasma was stored at −80°C until analysis. Cabergoline in plasma was extracted to methyl *tert*-butyl ether and was measured by liquid chromatography-tandem mass spectrometry (HPLC: HP1100 Series; Hewlett Packard, Palo Alto, CA, USA) (MS/MS: TSQ7000; Thermo-Quest, Waltham, MA, USA). Symmetry Shield RP-18 (2.1 × 150 mm, 3.5 μm; Waters, Milford, MA, USA) was applied to the column and the mobile phase consisted of acetonitrile (40%) and 20 mmol·L^{-1} ammonium formate (60%). LC/MS/MS was operated in the positive mode (15). The intra-assay coefficient of variation (CV) was 4.7 – 9.2% and the detection limit was 5 pg·ml^{-1}.

Pharmacokinetic analyses

The maximum plasma drug concentration (C_{max}) and the time to reach maximum concentration (t_{max}) were determined from actual data, mean plasma concentration over 0 – 10 h ($C_{0-10\,h}$) was calculated by averaging all readings, and area under the plasma concentration-time curve from 0 to 10 h after dosing ($AUC_{0-10\,h}$) was calculated using the linear trapezoidal rule in Study 1. In Study 2, plasma drug concentration was measured 3 h after cabergoline administration.

Statistical analyses

Results are expressed as the mean ± S.D. The plasma concentration of cabergoline was compared with and without clarithromycin co-administration by the Friedman test. C_{max} and $AUC_{0-10\,h}$ were compared using the Wilcoxon matched-pairs signed-rank test. The SPSS (version 11.5 for Windows) software was employed, with $P<0.05$ as the minimum level of significance. Sample sizes were estimated from the expectation of doubled plasma concentration for drug interaction.

Results

Study 1

All volunteers completed the schedule. Table 1 sum-

Table 1. The pharmacokinetics of cabergoline given alone and with clarithromycin

Pharmacokinetic parameter	Mean ± S.D. (95%CI)			
	Cabergoline alone		Cabergoline + clarithromycin	
C_{max} (pg·ml^{-1})	55.42 ± 16.11	(23.80 – 83.60)	152.85 ± 33.66*	(105.70 – 203.90)
$AUC_{0-10\,h}$ (pg·h·ml^{-1})	484.24 ± 144.45	(237.60 – 752.10)	1267.91 ± 331.17*	(796.45 – 1737.55)
t_{max} (h)	6.10 ± 2.42	(1.00 – 10.00)	7.20 ± 1.69	(4.00 – 10.00)
Mean $C_{0-10\,h}$ (pg·ml^{-1})	46.63 ± 15.44	(34.49 – 53.54)	121.14 ± 35.99*	(87.95 – 142.12)

$AUC_{0-10\,h}$ is the area under the plasma concentration-time curve from time 0 to 10 h; C_{max} is the maximum plasma concentration; $C_{0-10\,h}$ is the mean of plasma concentration determination over 0 – 10 h; t_{max} is the time corresponding to the maximum plasma concentration (C_{max}). * $P<0.01$ *vs* cabergoline alone (Wilcoxon matched-pairs signed-rank test).

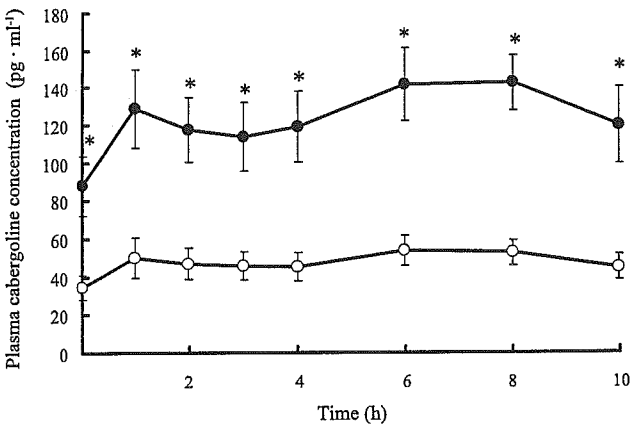

Fig. 1. Mean (±S.D.) plasma cabergoline concentration in 10 healthy volunteers after administration of cabergoline (1 mg) once daily with (closed circles) or without (open circles) clarithromycin (400 mg) twice daily for 6 days. * $P<0.01$ *vs* cabergoline alone.

marizes the mean (±S.D.) pharmacokinetic parameters for each treatment. Mean $C_{0-10\,h}$ for cabergoline was 46.6 pg·ml^{-1} without the co-administration of clarithromycin and increased significantly ($P<0.01$) to 121.1 pg·ml^{-1} with clarithromycin. At all time points, co-administration of clarithromycin increased the plasma concentration of cabergoline (Fig. 1). Individual values for C_{max} and $AUC_{0-10\,h}$ are shown in Fig. 2: mean values were significantly ($P<0.01$) increased 2.8 and 2.6 times, respectively, by clarithromycin co-administration.

Adverse effects were experienced in similar numbers of volunteers during the administration of cabergoline alone or in combination with clarithromycin (Table 2). None of these symptoms were serious and did not need any medical treatment. There was no difference in blood pressure before and after the administration of cabergoline. All of the volunteers completed the

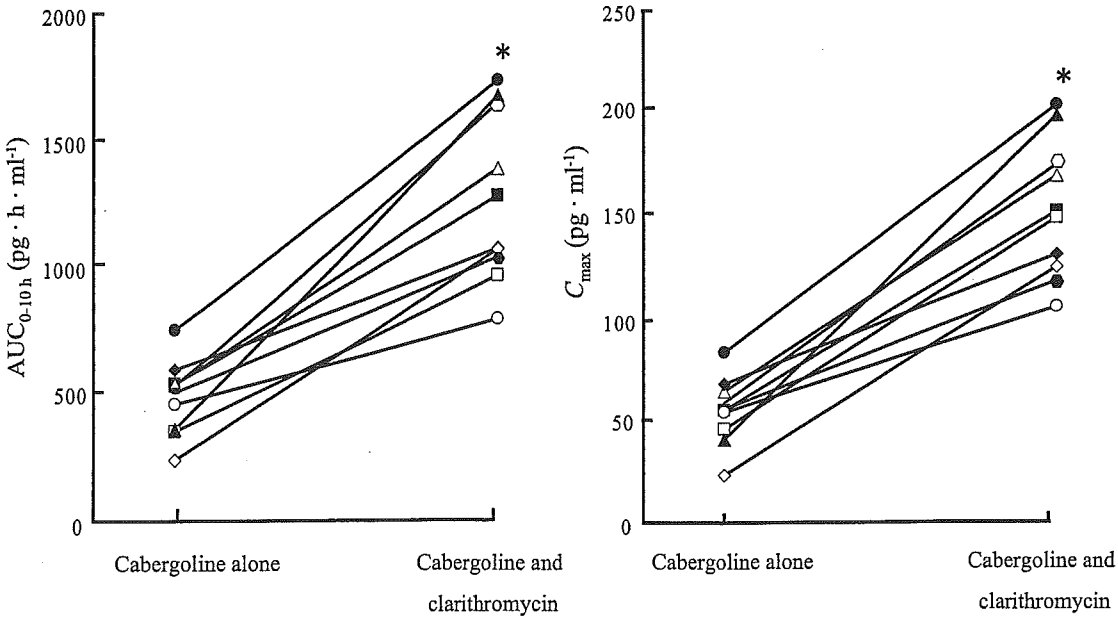

Fig. 2. Individual values for $AUC_{0-10\,h}$ and C_{max} for 10 healthy volunteers after administration of cabergoline (1 mg) once daily with or without clarithromycin (400 mg) twice daily for 6 days. * $P<0.01$ *vs* cabergoline alone.

Table 2. Symptoms and frequency of adverse events occurring in healthy volunteers

Symptoms	Cabergoline alone (n = 10)	Cabergoline and clarithromycin (n = 10)
Sleepiness	4	4
Constipation	3	4
Nausea	2	3
Dizziness	3	2
Heartburn	2	1
Diarrhea	0	1

scheduled plan.

Study 2

Table 3 shows the clinical characteristics of the PD patients. They were aged 56 to 75 (mean 67.4 ± 6.02) years. There were 4 females and 3 males, and all had a diagnosis of idiopathic PD. The mean duration of the illness was 5.93 ± 5.65 years. Administration of clarithromycin increased the plasma concentration of cabergoline in all patients (Fig. 3). The mean plasma concentration of cabergoline was 145.4 ± 52.9 pg · ml^{-1} without clarithromycin and 252.7 ± 100.6 pg · ml^{-1} with clarithromycin, therefore increasing 1.74-fold with clarithromycin co-administration (P<0.01).

No patients showed adverse effects, for example, nausea, vomiting, dizziness, hypotension, during the administration of clarithromycin.

One month after cessation of clarithromycin, blood samples were collected in 4 patients who could revisit for this study. The plasma concentration of cabergoline returned to the baseline level prior to clarithromycin administration in all 4 patients (Fig. 3).

With this increase in plasma cabergoline concentration, 3 of 7 patients showed an improvement in their PD symptoms. UPDRS improved in these 3 patients from 7 to 5, 36 to 30, and 44 to 40 in cases 2, 6, and 7, respectively. The score did not change in the other 4 patients, remaining at 45, 3, 1, and 61 in cases 1, 3, 4, and 5, respectively. When UPDRS improved, it was in part II or III for motor examination, especially tremor at rest or rigidity.

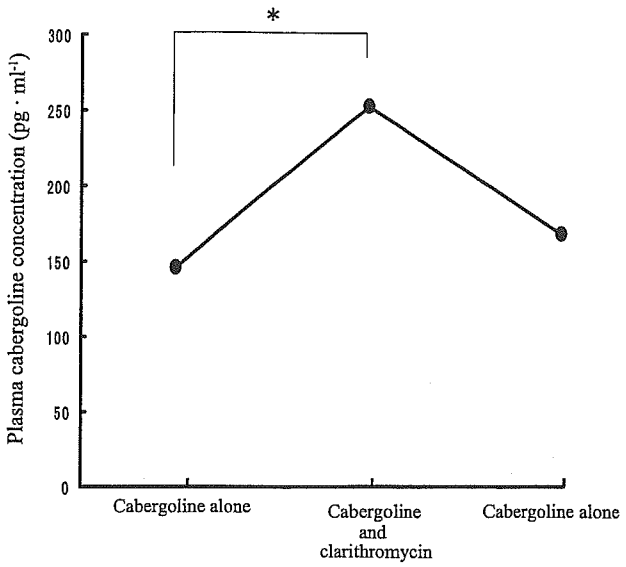

Fig. 3. Mean plasma cabergoline concentration in PD patients after 3-h therapeutic administration of stable therapeutic doses of cabergoline alone or in combination with clarithromycin (400 mg) twice daily for 6 days (n = 7) and again 1 month after discontinuationof clarithromycin (n = 4). * P<0.01.

Discussion

Cabergoline binds dopamine D_2 receptors selectively and causes functional dopaminergic activity in the brain. A distinctive characteristic of cabergoline is its long elimination half-life ($t_{1/2\beta}$) (8). In 12 healthy volunteers, mean t_{max} for cabergoline was 2.5 h and mean $t_{1/2\beta}$ was 109.7 ± 41.3 h following administration with food (16). Cabergoline has been shown to be metabolized through

Table 3. Patients clinical and demographic characteristics

Case	Gender	Age (year)	Cabergoline dose (mg/day)	L-dopa dose (mg/day)	Hoehn-Yahr stage		Disease duration (year)
					ON	OFF	
1	Male	71	3	400 (levodopa/carbidopa)	2.5	3	10.0
2	Female	68	2	200 (levodopa/benserazide)	1	1	2.7
3	Female	56	3	250 (levodopa/carbidopa)	0	2	17.0
4	Female	69	2	0	1	1	1.6
5	Male	69	1	300 (levodopa/benserazide)	3	3	2.6
6	Male	64	1	300 (levodopa/carbidopa)	2.5	2.5	5.0
7	Female	75	2	250 (levodopa/carbidopa)	3	3.5	2.6

hydrolysis and P450-mediated metabolism appears to be minimal (7, 8). In our study, the plasma concentration of cabergoline (C_{max}, $C_{0-10\,h}$ and $AUC_{0-10\,h}$) increased about 2.7 times with clarithromycin co-administration. Clarithromycin has been shown to increase the plasma concentration of certain drugs via the inhibition of P-glycoprotein (17). This suggests that the concentration of cabergoline might be increased by clarithromycin mainly through the inhibition of P-glycoprotein-mediated excretion.

Cabergoline is used in the treatment of PD in combination with other antiparkinsonian drugs. Pharmacokinetic interaction between cabergoline and levodopa has been investigated in two studies in PD patients. Plasma cabergoline concentrations were assayed over a 24-h period 3 weeks after a stable dose of cabergoline (2 mg daily). Pharmacokinetic parameters of cabergoline were unmodified by the addition of levodopa (levodopa/carbidopa, 250/25 mg daily) (18). The pharmacokinetics of levodopa before and after the concomitant administration of cabergoline has been studied in 12 PD patients (19). Plasma levodopa concentrations were measured over an 8-h period before cabergoline was added and 8 weeks after starting cabergoline. No modification of levodopa pharmacokinetics (absorption, bioavailability, $t_{1/2\beta}$) was observed when levodopa and cabergoline were co-administered. The pharmacokinetics of cabergoline and selegiline have been determined in 6 PD patients not treated with levodopa when the two drugs were given alone or in combination (20). No pharmacokinetic drug-drug interaction was found between cabergoline and selegiline, since all measured pharmacokinetic parameters of both drugs remained unchanged comparing monotherapy and combination therapy. The effect of food on the pharmacokinetics of cabergoline has also been investigated. Under both fasting and fed conditions C_{max}, AUC, $t_{1/2\beta}$, and t_{max} for cabergoline remained the same (16).

Cabergoline is an ergot alkaloid and clarithromycin is known to interfere with ergotamine metabolism. Ergot alkaloids are commonly used for migraine and have vasoactive properties. There has been a case report of clarithromycin-associated ischemia in the treatment of migraine with an ergot alkaloid (21). Itraconazole is a triazole used as local and systemic antifungal agent and is a potent inhibitor of CYP3A4 and P-glycoprotein. Christensen and colleagues (22) reported a case of PD showing increased plasma cabergoline concentration during concomitant treatment with itraconazole. Plasma cabergoline concentration was increased approximately 3-fold during treatment with itraconazole for 1 week. In our study, the concentration of cabergoline increased 2.7-fold in PD patients during the treatment of clarithromycin for 6 days. Our results were compatible with the previous study of itraconazole. Clarithromycin and itraconazole are both inhibitors of CYP3A4 and P-glycoprotein, although itraconazole is a more potent inhibitor (23, 24). Clarithromycin might elevate the concentration of cabergoline by the inhibition of both CYP3A4 and P-glycoprotein.

Drug-drug interactions between levodopa and DOPA decarboxylase inhibitors (carbidopa, benserazide), levodopa and COMT inhibitors, or levodopa and selegiline are applied practically in the treatment of PD. Co-administration of carbidopa increased the plasma concentration of levodopa approximately 400% (25) and entacapone 140% (26) in patients with PD. When adjunctive selegiline is used with levodopa, the extracellular concentration of dopamine increased several fold in the brain of monkeys (27). In the treatment of PD, drug-drug interaction is used for more efficient drug therapy. In our study, no patients or healthy volunteers showed dose-dependent serious adverse effects. Nonetheless, physicians should be aware of the potential for interaction between clarithromycin and cabergoline: in particular, their co-administration should be avoided in PD patients who show marked dyskinesia or levodopa-induced psychosis.

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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.

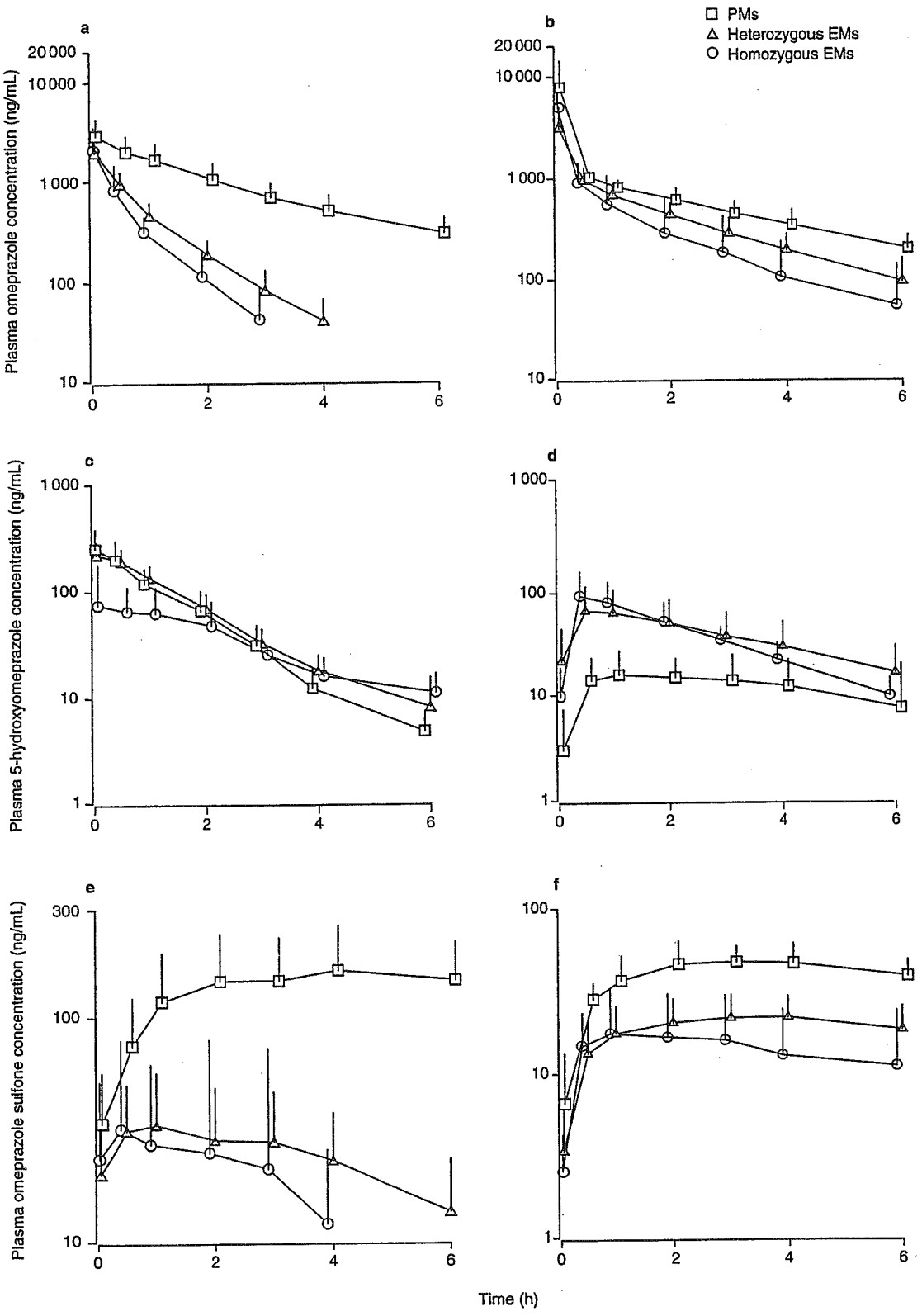

Fig. 1. The time course of plasma omeprazole concentrations (mean + SD) in the young (**a**) and the elderly (**b**), plasma 5-hydroxyomeprazole concentrations in the young (**c**) and the elderly (**d**), and plasma omeprazole sulfone concentrations in the young (**e**) and the elderly (**f**) subjects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

Table II. The area under the plasma concentration-time curve (AUC) of omeprazole, 5-hydroxyomeprazole and omeprazole sulfone, and the ratio of 5-hydroxyomeprazole AUC to omeprazole sulfone AUC[a]

Genotype	AUC (ng • h/mL)			AUC ratio
	omeprazole[b]	5-hydroxyomeprazole[c]	omeprazole sulfone[c]	5-hydroxy-omeprazole/ omeprazole sulfone
Young group				
Homozygous EMs	1441 ± 938	316 ± 124	97 ± 153	10.3 ± 12.1
Heterozygous EMs	1761 ± 474	435 ± 145	171 ± 107	3.2 ± 2.0
PMs	6892 ± 2730**	232 ± 160*	971 ± 598**	0.24 ± 0.07*
Elderly group				
Homozygous EMs	3292 ± 2376	217 ± 99	70 ± 66	5.5 ± 5.1
Heterozygous EMs	3242 ± 1156	216 ± 136	114 ± 40	2.8 ± 3.7
PMs	5650 ± 1861*	72 ± 45*	255 ± 72**	0.30 ± 0.20*

a Values are expressed as mean ± SD.

b AUC from time zero to infinity

c AUC from time zero to the last quantifiable plasma concentration.

EMs = extensive metabolisers; **PMs** = poor metabolisers; * $p < 0.05$, ** $p < 0.0001$ vs homozygous and heterozygous EMs.

For V_{ss} (mean ± SD), there was a significant difference between the young and the elderly groups (219 ± 115 and 107 ± 44.5 mL/kg, respectively; $p < 0.0001$), but no significant difference was found between the three genotypes (178 ± 142, 173 ± 79 and 110 ± 51 mL/kg for homozygous EMs, heterozygous EMs and PMs, respectively).

Discussion

We investigated the effect of aging on the relationship between the genotype and the phenotype of CYP2C19 by measuring the pharmacokinetic parameters of omeprazole after a single intravenous bolus dose. Although omeprazole is usually administered as multiple oral doses in a clinical situation, the drug was used as a probe drug to measure the effect of aging on the *in vivo* CYP2C19 activity in the present study. A number of studies showed the effect of aging on the pharmacokinetics of a drug such as phenazone (antipyrine), which undergoes hepatic metabolism.[12,13,18,19] Phenazone is frequently used as a model for the metabolising capacity of the liver and its clearance is reported to decline with age.[20,21] In phenazone metabolism, at least six CYP subfamilies are responsible for the formation of its metabolites, and combined activities of multiple CYPs are considered to contribute to phenazone clearance.[22,23] In contrast, omeprazole is metabolised to 5-hydroxyomeprazole and omeprazole sulfone by CYP2C19 and CYP3A4, respectively, and the former is mainly responsible for metabolism because many studies,[2,4-6] including the present study, have shown that omeprazole pharmacokinetics are dependent on the CYP2C19 genotype.

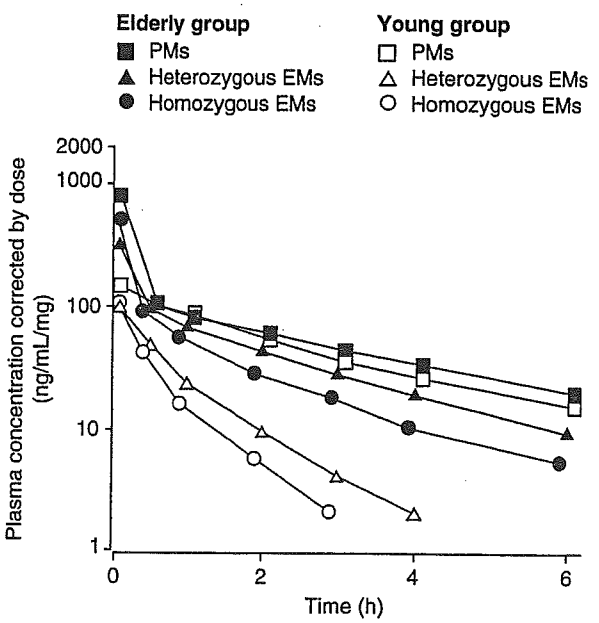

Fig. 2. The time course of dose-corrected plasma concentrations (mean) of subjects with each genotype in the young and the elderly groups. The elderly and the young subjects received a single intravenous dose of omeprazole 10mg and 20mg, respectively. **EMs** = extensive metabolisers; **PMs** = poor metabolisers.

Table III. Results of the linear regression analysis to examine the joint effects of covariates on the pharmacokinetic parameters[a]

Parameter	Mutation allele	Age	Sex	BMI
CL (L/h)	−0.434/<0.001	−0.472/<0.001	−0.182/0.096	−0.056/0.616
HI	−0.499/<0.001	−0.462/<0.001	−0.153/0.146	−0.033/0.757
MRT (h)	0.628/<0.001	0.276/0.033	−0.142/0.223	0.030/0.803
$t_{1/2\beta}$ (h)	0.441/<0.001	0.421/0.001	0.051/0.651	0.165/0.162
V_{ss} (mL/kg)	−0.208/0.071	−0.529/<0.001	−0.131/0.278	Not examined[b]

a Values are expressed as a partial correlation coefficient/p-value.

b BMI was excluded from the variables in the analysis for V_{ss} because the parameter was corrected by bodyweight.

BMI = body mass index; **CL** = systemic clearance; **HI** = hydroxylation index; **MRT** = mean residence time; $t_{1/2\beta}$ = terminal elimination half-life; V_{ss} = volume of distribution at steady state.

In the present study, omeprazole was administered intravenously to avoid aging-related changes in drug absorption,[12,13] and the dose for the elderly group was reduced to half of that for the young group because plasma omeprazole concentration was expected to be higher in the elderly group. Although the number of sampling points was not enough to evaluate the initial phase, the plasma omeprazole concentration-time curve suggested that a one-compartment model may be suitable for the young group, but a two-compartment model may be needed for the elderly group. With more frequent sampling between the time just after injection and 30 minutes post-dose, a bioexponential decline could also be observed in young subjects. Since the concentration at time zero was estimated using the initial two points, AUC of the elderly subjects was more underestimated, and CL and V_{ss} were overestimated, than those of the young subjects. In the present study, the CL of young EMs was smaller than the reported range (24–37.2 L/h).[24] Previous studies showed that omeprazole AUC in Chinese EMs was greater than that in Caucasian EMs after a single oral dose[25] and after repeated oral dose;[26] these results suggest that Japanese EMs may also have greater AUC and smaller CL compared with Caucasian EMs.

The linear regression models were used to examine the joint effects of covariates on the pharmacokinetic parameters of omeprazole and to evaluate whether possible variables besides genotype or age may exist as important determinants of the parameters. The models showed that sex was not a significant determinant in the pharmacokinetic parameters examined in this study. In the heterozygous EMs of the young or the elderly groups consisting of both sexes, the CL (mean ± SD) were 12.3 ± 1.9/13.1 ± 5.4 L/h and 4.2 ± 1.4/3.0 ± 1.1 L/h for young and elderly males/females, respectively. The V_{ss} (mean ± SD) was 190 ± 29/265 ± 86 mL/kg and 133 ± 38/127 ± 61 mL/kg for young and elderly males/females, respectively. Both parameters showed no sex-related difference. The lack of sex-related significance was consistent with previous studies reporting no effect of sex on the activity of CYP2C19.[27,28] The number of mutation alleles and age were significant covariates for all pharmacokinetic parameters, except V_{ss}. For V_{ss}, a significant difference was noted between the young and the elderly groups; this finding corresponds with a previous study showing an age-related reduction in the apparent volume of distribution during the terminal phase of omeprazole.[14]

Although most pharmacokinetic parameters examined in this study showed significant differences between EMs and PMs in the elderly group as well as in the young group, the relationship between the CYP2C19 genotype and omeprazole pharmacokinetics in the elderly group differed considerably from that in the young group. According to the Hardy-Weinberg equation, the frequencies of two mutations can account for this difference in PMs in a Japanese population[29] and it is unlikely that some unidentified mutations affect the metabolic capacity of the elderly EMs. In fact, some of the elderly EMs showed pharmacokinetic parameters similar to the elderly PMs, resulting in larger variance in the elderly EMs. Most pharmacokinetic parameters of the