Figure 3 Pathways involved in platelet aggregation and possible mechanisms involved in response to clopidogrel. Modified from Gurbel et al. Thromb Res 2007 [15] and Angiolillo et al. Eur Heart J 2008 [89]. AC, adenylate cyclase; ADP, adenosine diphosphate; AMP, adenosine monophosphates; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate;

CYP, cytochrome P450; Gi, inhibitory G protein; Gq, stimulatory G protein; IP3, inositol triphosphate; PDE, phosphodiesterase; PKA, protein kinase A; VASP, vasodilator-stimulated phosphoprotein; PI3K, phosphatidyl inositol 3-kinase; PLC, phospholipase C.

result, a number of sites of interest have been identified, as summarized in Figure 3. Although there is some variation in the rate of clopidogrel absorption from the small intestine, it appears that the main causes of variation are polymorphisms in hepatic cytochrome P450 isozymes (for example, CYP3A4, CYP3A5, and CYP2C19) and in the P2Y12 receptor itself.

In terms of polymorphisms in CYP2C19, loss-of-function polymorphisms were first shown to significantly reduce the response to clopidogrel in platelets from healthy subjects, as demonstrated by increased platelet aggregation measured using light transmission aggregometry [39]. In that study, the baseline platelet activity was not influenced by the CYP2C19 genotype. In the presence of ADP, platelet aggregation decreased gradually during treatment with clopidogrel in individuals with the wild-type *1/*1 homozygote (reaching 48.9% at day 7; $P < 0.001$ vs. baseline). By contrast, platelet aggregation did not change significantly in individuals with the *1/*2 heterozygote (71.8% at day 7; $P = 0.22$ vs. baseline). Thus, the CYP2C19*2 loss-of-function allele was associated with a marked decrease in platelet responsiveness (i.e., greater platelet aggregation) to clopidogrel.

Similar findings have been reported in a number of other studies [39–43], and CYP2C19 polymorphisms have been detected in many individuals worldwide. Indeed, the prevalence of the CYP2C19*2 mutant allele has been reported to be as high as 70% in some populations [44]. Elsewhere, the prevalence of either the *2 and *3 polymorphisms seems to be <40% (Figure 4) [45].

Of note, however, the prevalence of poor metabolizers or intermediate metabolizers of clopidogrel seems to be higher in Asian (18–23%) than in Caucasian (approximately 3%) populations, and this may be related to the common gene variants of CYP2C19 in these populations [46].

What is the Clinical Impact of Clopidogrel Resistance?

Because of the widespread use of clopidogrel to protect patients undergoing critical cardiovascular interventions, resistance to this agent presents a significant clinical problem in patients with atherothrombotic diseases.

Hochholzer et al. [47] investigated the relationship between ADP-induced platelet aggregation, measured using light transmission aggregometry, and major adverse cardiac events in 802 consecutive patients within a 30-day period after elective coronary stent placement. All patients received a loading dose of clopidogrel 600 mg followed by a maintenance dose of 75 mg daily, and were classified according to the quartiles of platelet aggregation (quartile 1, <4%; quartile 2, 4–14%; quartile 3, 15–32%; quartile 4, >32%). Although the incidence of subsequent coronary events was low (<1%) in patients in the first and second quartiles of platelet aggregation, the incidence was significantly higher in patients in the third and fourth quartiles (>3%; $P = 0.034$).
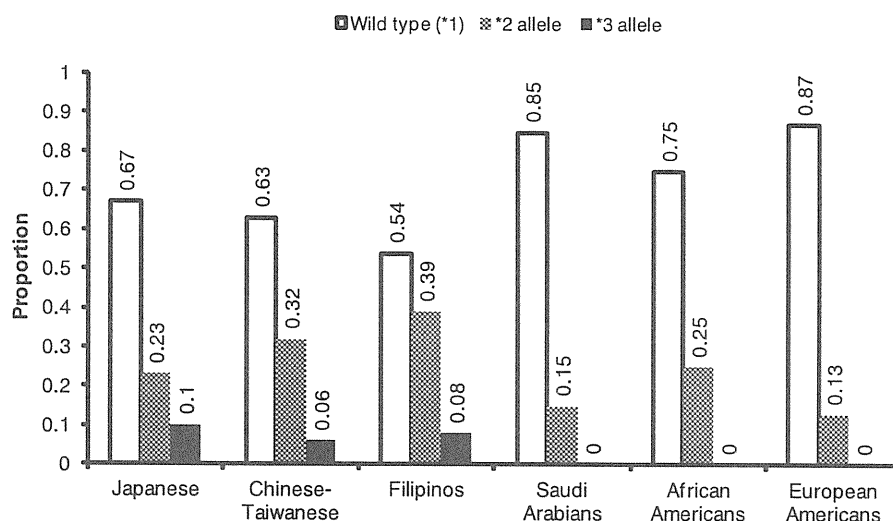
Figure 4 Estimated prevalence of polymorphisms in CYP2C19 [35].

In terms of the implications of genetic polymorphisms, the results of a number of studies have been reanalyzed to investigate whether these polymorphisms might be at least partly responsible for differences in treatment efficacy and long-term outcomes. Trenk et al. [43] reported that patients carrying at least one CYP2C19*2 allele were more prone to show impaired platelet reactivity (measured by light transmission aggregometry) upon clopidogrel treatment, which was associated with significantly worse clinical outcomes after coronary stent placement. In that study, 552 patients were homozygous for the wild-type CYP2C19 allele and 245 carried at least one *2 allele. Compared with patients homozygous for the wild-type allele, those with the *2 allele showed significantly higher levels of residual platelet aggregation when on clopidogrel (23.0% vs. 11.0%; $P < 0.0001$). In addition, patients with residual platelet aggregation >14% versus ≤14% (41.3% vs. 22.5%, respectively) showed a 3-fold greater increase (95%CI = 1.4–6.8; $P = 0.004$) in the 1-year incidence of death and MI.

In a study involving 1477 patients with acute coronary syndromes [16], the rates of the primary efficacy outcome such as death from cardiovascular causes, nonfatal MI, and nonfatal stroke were significantly higher in patients carrying loss-of-function polymorphisms of CYP2C19 (12.1% vs. 8.0%; $P = 0.01$) than in those with wild-type CYP2C19. Similar results were found for the rates of definite or probable stent thrombosis (2.6% vs. 0.8%; $P = 0.02$) over 450 days after the start of clopidogrel treatment.

In another study of 259 patients who used clopidogrel for ≥1 month after a first MI, 5-year event-free survival was significantly lower in patients with a polymorphism in CYP2C19 (HR = 3.69; 95%CI = 1.69–8.5; $P = 0.0005$) [48].

A study of 2208 consecutively enrolled patients presenting with acute MI who received clopidogrel [49] assessed the association between allelic variants of several genes related to clopidogrel (ABCB1, CYP3A5, CYP2C19, P2RY12, and ITGB3) and the risk of death from any cause, nonfatal stroke, or MI during 1 year of follow-up. A total of 225 patients died and nonfatal MI or stroke occurred in 94 patients during the follow-up period. Of note, the rate of cardiovascular events at 1 year was higher in patients carrying any two CYP2C19 loss-of-function alleles than in patients with none (21.5% vs. 13.3%; adjusted HR = 1.98; 95%CI = 1.10–3.58). Similarly, the rate of cardiovascular events was higher among patients with a variant allele of ABCB1, a gene involved in clopidogrel absorption, than in patients with the wild-type ABCB1 genotype (15.5% vs. 10.7%; adjusted HR = 1.72; 95%CI = 1.20–2.47). Interestingly, these rates were even higher among the patients who underwent PCI during hospitalization, as the rate of cardiovascular events among patients with two CYP2C19 loss-of-function alleles was 3.58 times (95%CI = 1.71–7.51) that of patients with no loss-of-function alleles. By contrast, the ABCB1 alleles had no significant, independent effect in these patients. Taken together, the findings of this study indicate that polymorphisms in a range of genes may affect the outcomes of clopidogrel therapy; however, the effect is most marked for CYP2C19. This may be attributed to differences in the effects of these polymorphisms on the function of the protein product, because polymorphisms of CYP2C19 cause loss of function, whereas those of the ABCB1 gene did not.

"Measuring" Clopidogrel Resistance

Platelet function, and hence clopidogrel activity, can be measured using a number of different approaches. The most commonly used approaches include ADP-induced platelet aggregation, VASP phosphorylation/flow cytometry, thromboelastography, the platelet drop-count method, and light transmission elastography.

It was initially considered that ADP-induced platelet aggregation could be used as a marker for clopidogrel activity. However, clopidogrel specifically inhibits the P2Y12 receptor rather than the P2Y1 receptor, which is responsible for the initial wave of ADP-induced platelet aggregation. Furthermore, because the

extent of residual P2Y1-dependent platelet aggregation varies considerably, ADP-induced aggregation may not be the most appropriate method to measure the response to clopidogrel.

Therefore, other approaches have been evaluated. In thromboelastography, a small blood sample is placed in a cuvette and aggregation is induced by gentle rotation or injection of ADP [50–52]. The platelet–fibrin clot strength is then assessed. In patients responsive to clopidogrel, the strength of the clot is weak, whereas in platelets exhibiting resistance to clopidogrel, the strength of the clots will be greater.

Another method is the platelet count drop method. Although this method is widely available in nonspecialized laboratories, it is unclear whether this approach provides meaningful clinical information, and it seems less reliable than methods based on light transmission aggregometry [53].

These three approaches measure the overall platelet aggregatory ability and can be influenced by pathways independent of the P2Y12 pathway. Thus, these methods may not fully reflect the extent of clopidogrel resistance in individuals in which the P2Y12 pathway is defective.

Another approach, but one that specifically focuses on the P2Y12 pathway, is ADP-induced inhibition of adenylate cyclase, which leads to the phosphorylation of VASP. The phosphorylation state of VASP is evaluable by flow cytometry and considered a specific intracellular marker of residual P2Y12 receptor reactivity in patients on clopidogrel. However, flow cytometers may not be readily available, particularly in a point-of-care setting. Accordingly, a number of point-of-care assays have been developed to provide rapid assessment of the potential for clopidogrel resistance in a clinical setting. Three methods based on thromboelastography, platelet count drop method and light transmission aggregometry have been established to date.

The third and most reliable method developed to date is light transmission aggregometry. In this method, blood samples that readily aggregate (for example, in the absence of a platelet inhibitor, or those containing platelets that are nonresponsive to clopidogrel) show high light transmittance because the aggregates fall out of solution, and impaired responses to platelet inhibitors are measured as a decrease in light transmission relative to platelet-poor plasma samples. In general, although this approach is considered the "gold standard" for measuring platelet reactivity, it is not specific for the P2Y12 pathway.

By contrast, the VerifyNow™ assay (Accumetrics, San Diego, CA, USA), which is based on light transmission aggregometry, is specific for the P2Y12 pathway. Indeed, the VerifyNow assay has been used in several studies to investigate the prevalence of aspirin and clopidogrel resistance [54,55]. Furthermore, it seems that this system is more reliable than the platelet drop count method or thromboelastography [56]. Compared with standard light transmission aggregometry, the VerifyNow assay measures an increase in light transmission to indicate increased aggregation, as the coated beads that bind to the aggregates fall out of solution. Of note, a version of the VerifyNow assay has been developed to specifically measure aspirin activity, independent of the P2Y12 pathway.

A recent study [57] compared the abilities of several widely used techniques for measuring on-treatment platelet reactivity (light transmission aggregometry, VerifyNow P2Y12, PlateletWorks, IMPACT-R, and the PFA-100 platelet function analysis system) to predict the clinical outcomes of 1069 patients taking clopidogrel and undergoing elective coronary stent implantation. The primary endpoint, a composite of all-cause death, nonfatal acute MI, stent thrombosis and ischemic stroke at 1 year occurred more frequently in patients with high on-treatment platelet reactivity, when assessed by light transmission aggregometry, VerifyNow and PlateletWorks, which were able to discriminate between patients with or without a primary event. It must also be acknowledged that the predictive accuracy of these tests was only modest. By contrast, the other techniques were not able to discriminate between these two groups of patients. Meanwhile, the authors reported that none of the tests provided prognostic information to identify low-risk patients at higher risk of bleeding after stent implantation.

Overcoming Clopidogrel Resistance

Based on the evidence described above for drug–drug interactions and prevalence of polymorphisms of CYP enzymes, reduced bioavailability of the active metabolite seems to be the main cause of clopidogrel resistance. Therefore, is there potential to overcome clopidogrel resistance?

Clopidogrel Dose Increase

The first option may be to increase the dose of clopidogrel. In clinical use, clopidogrel is commonly administered at a loading dose of 300 mg. Two studies have compared the clinical efficacy of a higher loading dose, namely 600 mg. In the study by Cuisset et al. [58], 292 patients undergoing stenting for non-STEMI were randomized to receive either a 300- or 600-mg loading dose $\geq$12 hours before PCI. All patients received daily clopidogrel 75 mg plus aspirin 160 mg for 1 month postintervention. ADP-induced platelet aggregation and expression of P-selectin were significantly lower in the high-loading dose group than in the low-loading dose group. Furthermore, the incidence of cardiovascular events during 1 month of follow-up was significantly lower in the high-dose group (7 events vs. 18 events; $P = 0.02$); this finding was not affected by adjustment for conventional cardiovascular risk factors ($P = 0.035$).

In a study by L'Allier et al. [59], 148 patients undergoing elective PCI were randomized to receive clopidogrel 300 mg the day before the procedure ($\geq$15 h) plus 75 mg clopidogrel in the morning of the procedure (group A), clopidogrel 600 mg in the morning of the procedure (group B), or clopidogrel 600 mg the day before ($\geq$15 h) plus 600 mg in the morning of the procedure (group C). The relative inhibition of peak and late platelet aggregation stimulated by ADP was significantly greater in group C than in groups A and B, indicating the double bolus dose of clopidogrel at 600 mg/dose achieved greater platelet inhibition than conventional single loading doses.

In a pilot study by Angiolillo et al. [60], the authors evaluated the efficacy of a daily maintenance dose of clopidogrel (150 mg) that was higher than recommended at the time (i.e., 75 mg) in

patients undergoing elective PCI. Patients in both groups continued the doses for 30 days, after which they resumed standard dosing. Of note, ADP-induced (20 μM) platelet aggregation was lower in the patients given 150 mg clopidogrel/day than in patients given 75 mg clopidogrel/day (52.1% vs. 64.0%; $P < 0.001$). Similar findings were observed in a study of 60 patients [61] given a pretreatment/loading dose of 600 mg clopidogrel within 12 h of PCI and a maintenance dose of 75 or 150 mg clopidogrel for 30 days. In that study, relative platelet aggregation in response to 5 μM ADP (45.1% vs. 65.3%; $P < 0.001$) and platelet function inhibition measured by the VerifyNow assay (60.0 vs. 117.0 P2Y12 reaction units; $P = 0.004$) were significantly better with 150 mg clopidogrel than with 75 mg clopidogrel.

Several studies have also examined the effect of high doses of clopidogrel on platelet reactivity in clopidogrel-resistance patients. For example, in the study by Bonello et al. [62], 162 patients with a VASP phosphorylation index >50% after a 600-mg loading dose were randomized to either a control group or to a VASP-guided group in which patients received additional bolus doses of clopidogrel to decrease the VASP index to below 50%. Of note, in the VASP-guided group, dose adjustment was effective in 67 of 78 patients; in these patients, the VASP index decreased from 69.3 to 37.6 ($P < 0.001$). Twenty-six patients required four doses, and these were unsuccessful in 11 patients. Of interest, the rate of major adverse cardiac events over 1 month was significantly lower in the VASP-guided group (0% vs. 10%; $P = 0.007$) and there was no difference in the rate of major or minor bleeding (total: 5% vs. 4%; $P = 1$).

In a study performed by Angiolillo et al. [63], patients with inadequate responses to 75 mg/day clopidogrel (platelet inhibition <50%) received a maintenance dose of 150 mg/day (n = 17) for 1 month. In this study, platelet inhibition measured using the VerifyNow P2Y12 assay increased significantly from 27.1% to 40.6% ($P = 0.009$ relative to a control group), but only 35% of patients reached platelet inhibition of $\geq$50%.

Similar findings were reported in a larger study [64] of 153 patients with low responsiveness to clopidogrel (platelet reactivity index $\geq$69%) who were randomized to 150 mg/day (n = 58) or 75 mg/day (n = 95) clopidogrel. After 2 weeks, 150 mg/day clopidogrel was associated with a significantly lower platelet reactivity index than 75 mg/day (43.9% vs. 58.6%; $P < 0.001$), with fewer nonresponders after treatment (8.6% vs. 44.7%; $P = 0.004$). Of note, 20 of 31 patients in the 75 mg/day group became responders (i.e., platelet reactivity <69%) after switching to 150 mg/day clopidogrel for 2 weeks.

Taken together, the findings of these studies indicate the potential for using higher loading and/or maintenance doses of clopidogrel. However, the efficacy of increasing the clopidogrel dose was of limited benefit, and many patients still had inadequate responses to clopidogrel. Indeed, in a case series reported by Pena et al. [65], of the seven patients included, four patients were resistant to 225 mg/day, two of whom were still resistant despite an increase to 300 mg/day.

Furthermore, these studies did not assess the long-term effects of administering high doses of clopidogrel, which may be necessary in clinical practice in patients exhibiting clopidogrel resistance.

Alternative Drugs

Prasugrel

Prasugrel is a novel antiplatelet agent that like clopidogrel targets the ADP receptor in platelets. Production of the deacetylated metabolite of prasugrel is mediated by esterases (Figure 5); since this hydrolysis step is very rapid in vitro and in vivo, circulating levels of prasugrel are undetectable shortly after administration [66,67]. Of note, the efficacy of prasugrel has been compared with that of clopidogrel. In the TRITON-TIMI-38 [68] study, 13,608 patients with moderate-to-high-risk ACS scheduled to undergo PCI were treated with either prasugrel (60-mg loading dose; 10-mg daily maintenance dose) or clopidogrel (300-mg loading dose; 75-mg daily maintenance dose for 6–15 months. In that study, the primary end point (death from cardiovascular causes or nonfatal MI or stroke) occurred in significantly fewer patients treated with prasugrel than patients treated with clopidogrel (9.9% vs. 12.1%, respectively; HR = 0.81; 95%CI = 0.73–0.90; $P < 0.001$). Furthermore, the rates of MI, urgent target-vessel revascularization and stent thrombosis were significantly lower with prasugrel than with clopidogrel. However, prasugrel was associated with increased risk of major bleeding and life-threatening bleeding.

Meanwhile, in the PRINCIPLE-TIMI 44 study [69], 201 subjects were treated with either prasugrel (60-mg loading dose) or clopidogrel (600-mg loading dose) for inhibition of platelet aggregation and aggregation-thrombolysis in MI. The primary end point in this study was the inhibition of platelet aggregation (in response to 20 μmol/L ADP) at the end of the loading-dose phase, and this was significantly higher with prasugrel than with clopidogrel (61.3% vs. 46.1%; $P < 0.001$).
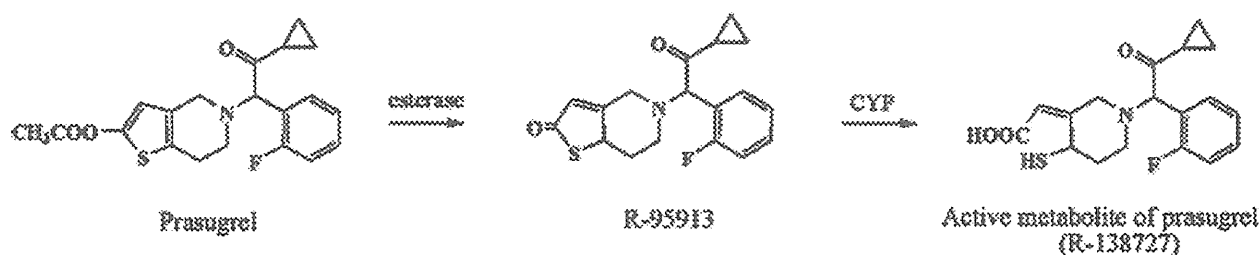

Figure 5 Mechanism of prasugrel activation.

Although there is limited evidence for whether the use of prasugrel avoids a potential interaction with PPIs via the cytochrome P450 system, a retrospective analysis of the TRITON-TIMI 38 and PRINCIPLE-TIMI 44 studies indicated that co-administration of PPIs does not affect the efficacy of prasugrel [32]. Thus, prasugrel may be more appropriate for patients using PPIs.

Interestingly, in the study reported by Pena et al. [65], as described above, four of the seven patients were switched to prasugrel, which markedly improved platelet aggregation in these patients. The authors ascribed these findings to the presence of heterozygous or homozygous *2 polymorphisms in these patients. Clearly, further studies are needed to investigate the benefits of treatment adjustments based on results of polymorphism testing performed prospectively, or following the emergence of clopidogrel resistance.

Cilostazol

Cilostazol inhibits platelet aggregation by antagonizing the activity of phosphodiesterase 3 and thereby suppresses cAMP degradation in platelets (Figure 3). It has been hypothesized that cilostazol could offer an alternative approach for patients with impaired responses to clopidogrel. However, in many countries, cilostazol is currently only approved for intermittent claudication; in Japan and other Asian countries, cilostazol is also approved for the prophylaxis of recurrent cerebral infarction.

Nevertheless, clinical studies have revealed significant advantages of using cilostazol as an adjunct to clopidogrel and aspirin in reducing restenosis in patients undergoing contemporary stent-based percutaneous interventions [70], and in reducing cardiac and cerebral adverse events [71]. Thus cilostazol may be more effective than high maintenance doses of clopidogrel [72]. However, in these studies, cilostazol was associated with more frequent discontinuation due to adverse events than clopidogrel or aspirin.

Ticagrelor

Ticagrelor is a novel platelet aggregation inhibitor for which Phase III studies have recently been completed, with promising data. Like clopidogrel, ticagrelor binds to P2Y12 to antagonize the ADP receptor on platelets, and thus inhibit platelet aggregation. However, unlike clopidogrel, ticagrelor binds reversibly to P2Y12 and does not displace ADP from the receptor, targeting 2-MeS-ADP-induced signaling [73]. In addition, ticagrelor does not require hepatic enzymatic activation, suggesting that ticagrelor may provide more consistent platelet inhibition with reduced risk for drug interactions and is unlikely to be affected by polymorphisms in the CYP system [74]. In the PLATelet inhibition and patient Outcomes (PLATO) study [75], 18,624 patients admitted to hospital with an acute coronary syndrome were randomized to receive either ticagrelor (180-mg loading dose plus 90 mg twice daily thereafter) or clopidogrel (300–600-mg loading dose plus 75 mg daily thereafter). At 12 months, the primary endpoint (a composite of death from vascular causes, MI, or stroke) had occurred in 9.8% of patients treated with ticagrelor versus 11.8% of those treated with clopidogrel (HR = 0.84; 95%CI = 0.77–0.92; $P < 0.001$). However, in that study, ticagrelor was associated with increased rates of ventricular pauses and dyspnea, although the underlying cause has not been established.

Can we Target Three Independent Pathways Rather Than Two?

Because the addition of clopidogrel to aspirin therapy was found to be clinically beneficial in terms of reducing cardiovascular adverse events, particularly in patients with inadequate response to aspirin alone, adding a third agent targeting a third pathway may provide further advantages, providing an opportunity for treatment escalation. Accordingly, the efficacy and safety of cilostazol in combination with clopidogrel and aspirin versus clopidogrel plus aspirin has been investigated in several studies. Collectively, these studies reveal significant advantages for cilostazol combination therapy in terms of cardiac death, death from any cause, or major adverse cardiac events [71,76–78] in addition to in-stent and in-segment late loss, in-segment restenosis, and target lesion revascularization [78,79]. Of particular interest is that this regimen was reported to be efficacious even in patients with resistance to clopidogrel [72,80], with improved inhibition of platelet aggregation in individuals allocated to cilostazol combination therapy compared with those on clopidogrel plus aspirin. However, these studies only included small numbers of patients and were of short durations, precluding detailed analyses of endpoints such death or incidence of major cardiovascular events. Thus, further studies are needed to confirm these findings and to determine whether other agents targeting phosphodiesterase 3 or another pathway are also effective.

Conclusions

Clopidogrel resistance is a common clinical entity that has potentially serious outcomes. It increases the risk of mortality or other adverse outcomes after cardiovascular interventions because of poor inhibition of platelet aggregation in these patients. Patients using drugs that are metabolized via the cytochrome P450 system, such as statins and PPIs, and patients with polymorphisms in these isozymes, particularly CYP2C19, are likely to show poor responses to clopidogrel. These patients may benefit from higher loading doses of clopidogrel, triple therapy with cilostazol in combination with clopidogrel plus aspirin, or switching to alternative drugs such as prasugrel, ticagrelor, or cilostazol. When genetic screening is not available, using flow cytometry or point-of-care devices to prospectively identify patients who are likely to exhibit clopidogrel resistance may guide the prescription of clopidogrel or an alternative treatment. However, further studies are needed to confirm the clinical utility of this approach. Finally, while most studies investigating clopidogrel resistance have focused on patients with acute MI, clopidogrel is also widely used in patients with other disorders such as cerebrovascular disease and PAD. Therefore, studies investigating the clinical impact of clopidogrel resistance in patients with cerebrovascular disease or PAD are needed to understand the potential risk of severe adverse events in these patients.

Acknowledgment

Conflicts of Interest

The author declares no conflict of interests.

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Relationship Between 3-O-methyldopa and the Clinical Effects of Entacapone in Advanced Parkinson's Disease

Aiko ISHIHARA[1], Takafumi MIYACHI[1], Takeshi NAKAMURA[1], Toshiho OHTSUKI[1],
Yasuhiro KIMURA[2], Kenji KIHIRA[2], Takemori YAMAWAKI[1] and Masayasu MATSUMOTO[1]

1) Department of Clinical Neuroscience and Therapeutics, Graduate School of Biomedical Sciences,
Hiroshima University, Hiroshima, Japan*
2) Department of Pharmaceutical Services, Hiroshima University Hospital, Hiroshima, Japan

ABSTRACT

The aim of this study is to clarify the relationship between serum 3-O-methyldopa (3-OMD) and the clinical effects of entacapone. The 3-OMD and maximum serum concentration (Cmax) of levodopa were measured in 21 Parkinson's Disease patients who took 100 mg levodopa / dopa decarboxylase inhibitor. After the administration of entacapone, the 3-OMD concentration and percentage of "on" time during waking hours (% of "on" time) were studied for 8 weeks. The 3-OMD concentration was reduced by 34%, and the increase in % of "on" time was 28% at the 8th week compared with baseline. We defined the COMT-index as [baseline 3-OMD concentration] / [levodopa Cmax when 100 mg levodopa was administered alone]. The COMT-index was significantly correlated with the increase in % of "on" time at the 8th week. In conclusion, the measurement of baseline 3-OMD and levodopa pharmacokinetics is useful for predicting the clinical effects of entacapone.

Key words: Parkinson's disease, 3-OMD, Entacapone, Levodopa AUC

Parkinson's disease (PD) is caused by the degeneration of nigrostriatal neurons, resulting in a deficiency of dopamine in the central nervous system (CNS). As dopamine does not readily cross the blood-brain barrier, the dopamine prodrug levodopa and dopamine agonists are mainly used to treat PD. Despite the development of dopamine agonists, levodopa is still the most effective treatment for PD[15]. However, long-term levodopa treatment causes motor complications such as wearing-off and dyskinesia[4]. These motor complications occur with an annual incidence of about 10% among PD patients[1]. Furthermore, the wearing-off phenomenon impairs the quality of life of Parkinson's disease patients[6]. Therefore, it is important that it is managed.

Levodopa is metabolized to dopamine by dopa decarboxylase and to 3-O-methyldopa (3-OMD) by catechol-O-methyltransferase (COMT) in the periphery. When levodopa is administered together with dopa decarboxylase inhibitor (DCI), levodopa availability in the CNS is increased[3], and the COMT pathway of levodopa metabolism becomes predominant in the periphery[4]. While the half-life of levodopa is approximately 1 hr, the half-life of 3-OMD is about 15 hr, which leads to the accumulation of 3-OMD in the plasma and brain under chronic levodopa treatment[10]. Like levodopa, 3-OMD is transported across the blood-brain barrier by the large neutral amino acid (LNAA) transporter and consequently competes with levodopa for uptake into the brain[14]. Moreover, a recent study indicated that 3-OMD damages neuronal cells[11]. Thus, it is assumed that the serum concentration of 3-OMD plays an important role in levodopa-treated PD patients. Entacapone, a peripheral COMT inhibitor, prolongs the retention time of levodopa in the plasma[14] and is used in PD patients with the wearing-off phenomenon. However, the determinants of serum 3-OMD concentration and the relationship between motor symptoms and serum 3-OMD concentration are not fully understood. Since entacapone was approved for use in Japan in April 2007, there is little data about its clinical effects and the relationship between its clinical effects and serum 3-OMD concentration in Japanese patients. Furthermore, there is no study about predictive factors for its clinical effect. The aims of this study are: first, to clarify the factors that determine the serum 3-OMD concentration; second, to investigate the change in serum 3-OMD concentration that occurs in Japanese PD patients administered

*Place where the work was conducted:Department of Clinical Neuroscience and Therapeutics, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan
Tel: +81-82-257-5201, Fax: +81-82-505-0490

entacapone; and third, to clarify the relationship between serum 3-OMD concentration and the clinical effects of entacapone.

METHODS

Patients

The study subjects were PD patients who visited our hospital from August 2007 to September 2008. The study was conducted according to the Declaration of Helsinki. The patients were given oral and written information about the study and gave their written consent.

All patients fulfilled the clinical diagnostic criteria of the UK Brain Bank for PD[8] and exhibited signs of wearing-off motor fluctuations. All patients were taking levodopa and had shown a positive response to levodopa treatment. Patients who had previously received entacapone or were suffering from dementia were excluded.

The clinical data collected included age, sex, disease duration, duration of therapy, duration of wearing-off, antiparkinsonian drug doses, and levodopa equivalent daily dose (LEDD). LEDD was calculated according to a previous report[9].

Study Design

1. First study (pharmacokinetics of the first administration of entacapone)

In order to assess the pharmacokinetics of levodopa, the patients took a tablet orally containing 100 mg levodopa and DCI (10 mg carbidopa or 25 mg benserazide) in the morning following an overnight fast. Blood specimens were then collected through an intravenous catheter at 0, 15, 30, 45, 60, 90, 120, and 180 min after the levodopa/DCI administration. The serum levodopa and 3-OMD concentrations were measured. The area under the concentration-time curve (AUC) of levodopa was calculated by the trapezoid method up to 180 min, and the maximum serum concentration (Cmax) of levodopa was calculated using the one compartment model.

The pharmacokinetic evaluation was performed the next day using the same method, but 100 mg of entacapone was now added to the regimen.

2. Second study (clinical effects and pharmacokinetics during long term entacapone therapy)

After the first study, the same patients were administered one 100 mg entacapone tablet orally with each dose of their levodopa/DCI preparation for 8 weeks. The patients completed an "on/off" self-rating diary on a daily basis at the baseline and during the 2nd, 4th, 6th, and 8th weeks. For each 30-min period between 05:00 and 24:00, the patients rated their motor physical condition by choosing: "on" (good mobility), "off" (worse to bad mobility), or asleep. The mean "on" time duration of at least 3 days was calculated from the self-rating diary. When the "on" time duration was not

prolonged by more than 30 min at the 4th week, 200 mg entacapone was administered with each dose of their levodopa/DCI preparation. The doses of other antiparkinsonian drugs were not changed throughout the study. The levodopa dose was reduced when a patient's symptoms necessitated it. Unified Parkinson's Disease Rating Scale (UPDRS) motor scores were assessed, and serum 3-OMD concentrations were measured at the baseline and in the 2nd, 4th, 6th, and 8th weeks.

Serum analysis

The serum levodopa and 3-OMD concentrations were determined by the high-performance liquid chromatography-electrochemical detection method[2] with minor modifications. In brief, 100 μl aliquot serum samples were processed by adding 100 μl of 1 M perchloric acid and 20 μl of 50 μM dihydroxybenzylamine hydrobromide as an internal standard. Then, the precipitated proteins were removed by centrifugation at 13,000 rpm for 5 min. The resultant supernatants were applied to the chromatographic system.

The chromatographic system consisted of a LC-10AT pump, a CTO-10A column oven, and a DGU-14A degasser (Shimadzu, Kyoto, Japan). The system was connected to an ECD-100 electrochemical detector (Eicom, Kyoto, Japan). The voltage was set at 750 mV vs Ag/AgCl, and chromatographic separation was performed using an EICOMPAK SC-5ODS column (Eicom, Kyoto, Japan) with a PC-03 guard column (Eicom, Kyoto, Japan) in a column oven. The mobile phase (per liter) contained 150 ml of methanol, 850 ml of citrate-acetate buffer, 400 mg of sodium octane sulfate, and 20 mg of EDTA-2Na, pumped at a fixed flow rate of 0.5 ml/min. The citrate-acetate buffer consisted of 0.017 M of sodium acetate and 0.083 M of citric acid monohydrate, and the pH of the buffer was adjusted to 2.8 with perchloric acid. Levodopa, 3-OMD, and dihydroxybenzylamine were eluted at 5.2, 11.8, and 7.2 min, respectively.

Statistical Methods

Statistical analysis was performed using a nonparametric method (Wilcoxon signed-rank test, Spearman's rank correlation and Friedman test), and statistical significance was set at $p < 0.05$. Calculations were performed using the JMP 5.0.1J software (SAS Institute Inc., Cary, N.C., USA) and SPSS 16.0J for Windows (SPSS Inc, Chicago).

RESULTS

Patients

Twenty-one advanced PD patients, comprising 9 men and 12 women, were recruited. The characteristics of the patients are shown in Table. At the baseline, 16 patients took levodopa/carbidopa and

5 patients took levodopa/benserazide preparation. The concomitant antiparkinsonian medications included anticholinergics (n = 6), selegiline (n = 13), dopamine agonists (n = 19), amantadine (n = 6), and droxidopa (n = 3). The median "on" time duration of these patients was 7.7 hr and the median percentage of "on" time during waking hours (% of "on" time) was 48%.

Table : Patient baseline characteristics (n=21)

Age (yrs)	68	(52 - 80)
Sex (n)		
Men	9	
Women	12	
Duration of PD (yrs)	12	(5 - 19)
Duration of wearing-off (yrs)	2	(1 - 10)
Duration of antiparkinsonian medication (yrs)	11	(3 - 18)
"on" time duration (hrs)	7.7	(2.2 - 10.3)
% of "on" time during waking hours (%)	48	(12 - 61)
Hoehn and Yahr stage at "on" phase	3	(1 - 5)
UPDRS motor score at "on" phase	24.5	(9 - 42)
Daily dose of levodopa/DCI (mg)	400	(250 - 625)
Dosing frequency of levodopa/DCI	4	(3 - 8)
Total LEDD (mg)	840	(467.5 - 1346)

All data are shown as median (minimum - maximum).
UPDRS : unified Parkinson's disease rating scale
DCI : dopa decarboxylase inhibitor
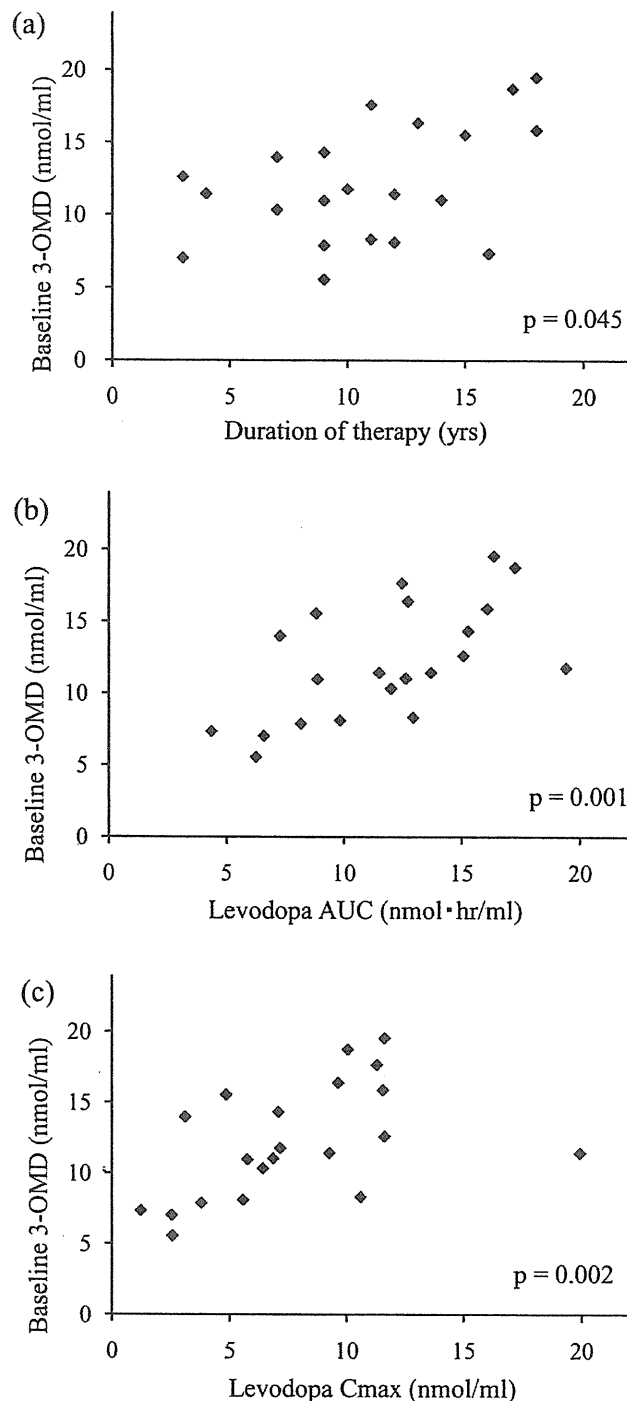LEDD : levodopa equivalent daily dosage

First study

The baseline serum 3-OMD concentration was positively correlated with duration of therapy (p = 0.045), levodopa AUC (p = 0.001) and levodopa Cmax (p = 0.002) in the absence of entacapone (Fig. 1). However, the baseline serum 3-OMD concentration was not correlated with age, sex, duration of wearing-off, the daily levodopa dosage, LEDD, Hoehn & Yahr stage, UPDRS motor score, the "on" time duration, nor % of "on" time at the baseline.

The AUC of levodopa was significantly increased by 22% (median) after the first entacapone administration compared with the control value for levodopa/DCI alone (p < 0.001).

Second study

After the initiation of entacapone administration, 3 patients withdrew from the study due to study protocol deviation (2 patients did not complete the "on/off" self-rating diary, and another patient stopped taking entacapone of her own volition because of dyskinesia). The remaining 18 patients completed the study. The daily levodopa dose was not changed at any point during the study period. In 1 patient (5.6%), the dose of entacapone was increased to 200 mg because the "on" time duration was not prolonged by more than 0.5 hr at the 4th week.


Fig. 1. The baseline serum 3-OMD concentration was positively correlated with (a) the duration of therapy (p = 0.045), (b) levodopa AUC (p = 0.001), and (c) levodopa Cmax (p = 0.002). (Spearman's rank correlation)

The median serum 3-OMD concentration, the UPDRS motor score and median % of "on" time was significantly decreased with entacapone (Friedman test, p <0.001, respectively). The median serum 3-OMD concentration was significantly decreased from the 2nd week to the 8th week and was decreased by 3.5 nmol/ml (34%) at the 8th week (Wilcoxon's signed rank test, p < 0.001) (Fig. 2a). The UPDRS motor score was also

significantly decreased by 7 points (median) at the 8th week (Wilcoxon's signed rank test, $p < 0.001$) (Fig. 2b) compared with the baseline. The median "on" time duration was significantly improved by 4.4 hr at the 8th week (Wilcoxon's signed rank test, $p < 0.001$) and the median % of "on" time was

also significantly improved by 28% at the 8th week (Wilcoxon's signed rank test, $p < 0.001$) (Fig. 2c) compared with the baseline.
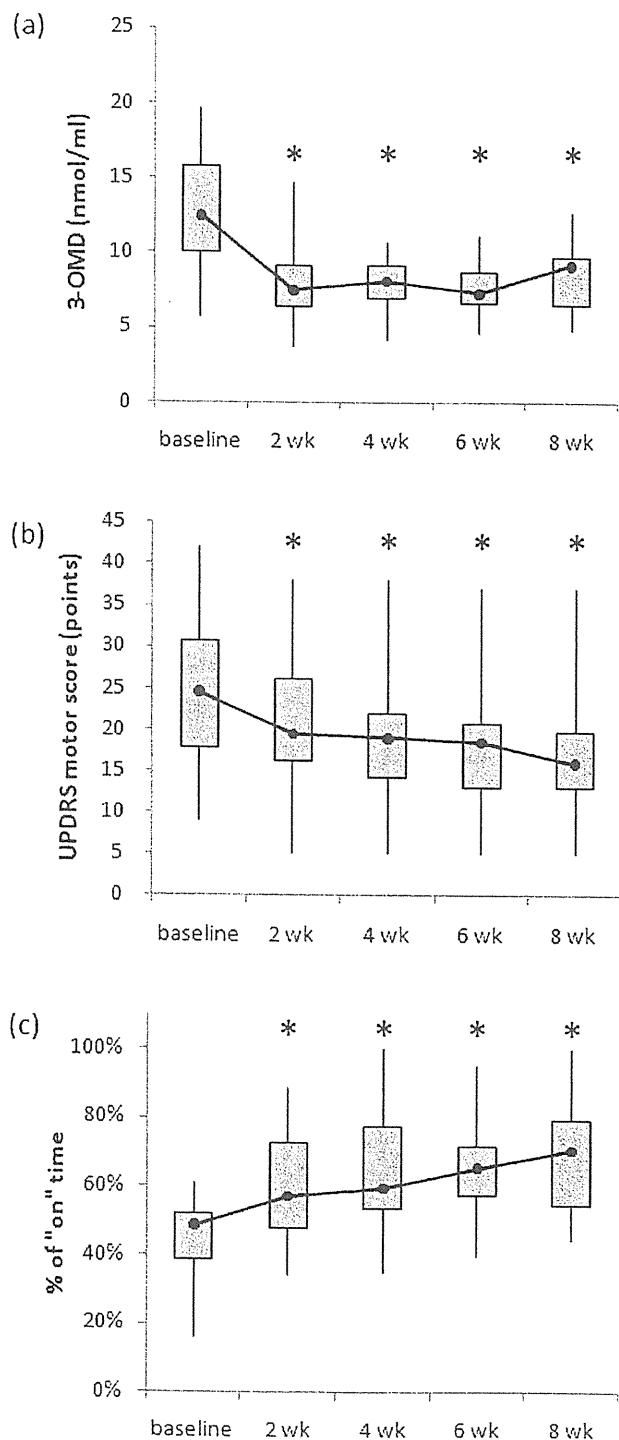
Fig. 2. Changes in (a) serum 3-OMD, (b) UPDRS motor score, and (c) % of "on" time from the baseline to the 8th week after entacapone therapy (n=18).
* Wilcoxon's signed rank test, $p < 0.001$, compared with the baseline.
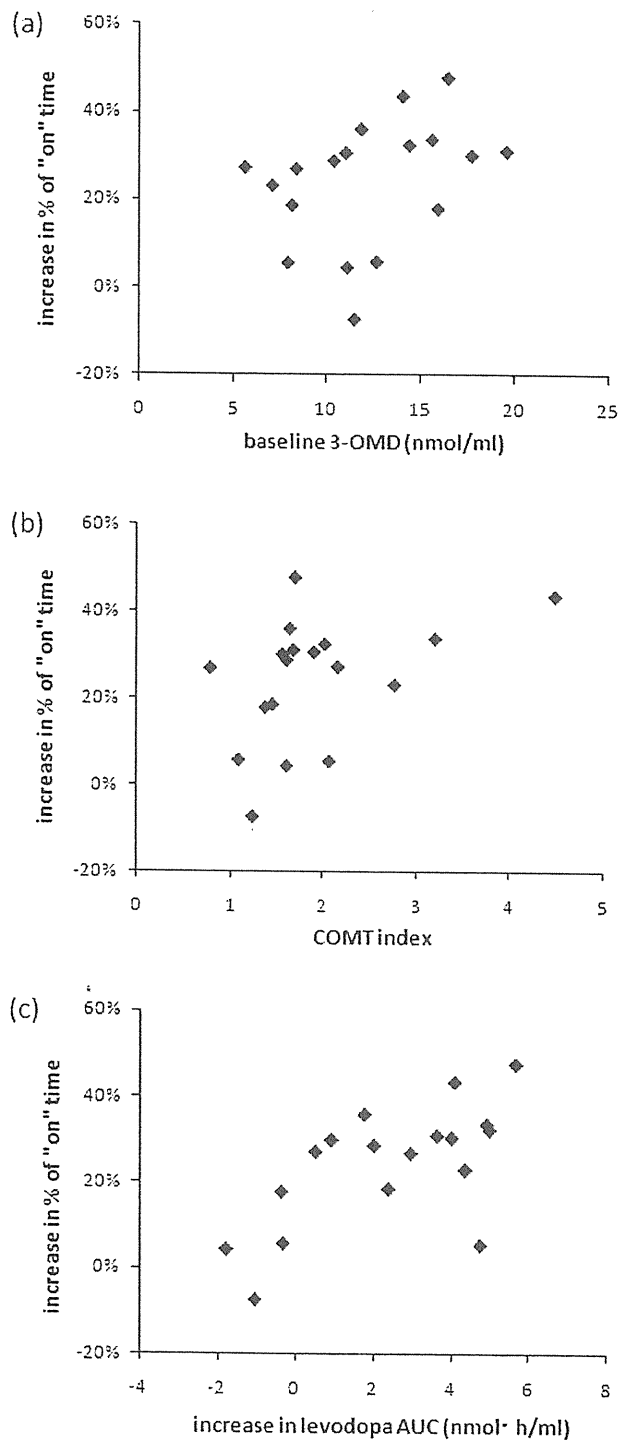
Fig. 3. (a) Association between the increase in % of "on" time and serum 3-OMD concentration. (p=0.053)
(b) Association between the increase in % of "on" time and COMT index. (p=0.027)
(c) Association between the increase in % of "on" time and the change in levodopa AUC. (p=0.006)
Evaluation at the 8th week (n=18).
(Spearman's rank correlation)

The baseline serum 3-OMD concentration tended to be correlated with the increase in % of "on" time at the 8th week (p = 0.053) (Fig. 3a). Neither the rate of decline in the serum 3-OMD concentration nor the reduction in the serum 3-OMD concentration was correlated with the increase in % of "on" time. None of age, sex, disease duration, duration of therapy, duration of wearing-off, LEDD, Hoehn & Yahr stage, or UPDRS motor score was correlated with the increase in % of "on" time.

Since levodopa is metabolized to 3-OMD by COMT, the concentration of 3-OMD is determined by COMT activity and the amount of levodopa absorbed. The ratio of the serum 3-OMD concentration to the levodopa concentration might reflect COMT activity more correctly than serum 3-OMD concentration. Thus, we defined the COMT-index as [baseline 3-OMD concentration] / [levodopa Cmax when 100 mg levodopa was administered alone]. The COMT-index was significantly correlated with the increase in % of "on" time at the 8th week (p = 0.027) (Fig. 3b).

The increase in levodopa AUC after the first entacapone administration was positively correlated with the increase in % of "on" time at the 8th week (p = 0.006) (Fig. 3c).

DISCUSSION

We prospectively studied the association between the 3-OMD, a levodopa metabolite, concentration and the effects of entacapone in Japanese advanced PD patients.

We clarified that the baseline serum 3-OMD concentration was associated with the duration of therapy. Our study suggested that long-term levodopa therapy increases COMT activity. Serum 3-OMD concentration was associated with the AUC and Cmax of levodopa, but not with the daily levodopa dosage. The rationale for this was that most patients (15 of 21 patients) took a daily dosage of 300 - 400 mg levodopa, but each of them would have had a different levodopa absorption rate. Because the AUC and Cmax of levodopa reflect the amount of levodopa absorption, serum 3-OMD, a levodopa metabolite, concentration was associated with the AUC and Cmax of levodopa.

Our study showed that the 3-OMD concentration was decreased by about 30% and the UPDRS motor score at the "on" phase was also decreased by 7 points by treatment with entacapone in Japanese patients. As entacapone generally does not increase the Cmax of levodopa after a single coadministration of levodopa[16], motor function is not improved by a single administration of entacapone. However, repeated administration of entacapone may increase the overall levodopa concentration and improve the motor function of patients. Moreover, a recent

study[11] showed that 3-OMD impaired the locomotor activity of rats and that 3-OMD can damage neuronal cells. In another study[12], when treatment with controlled-release carbidopa and levodopa was compared with treatment with a combination of carbidopa, levodopa and entacapone, the mean pharmacokinetic values of levodopa were similar, but the "off" time duration was shorter for the entacapone group than the controlled-release levodopa group. As it is assumed that the 3-OMD concentration was decreased in the entacapone group, the difference between the clinical effects observed in the 2 groups might have been due to differences in the 3-OMD concentration.

Therefore, we assumed that PD patients benefit from entacapone for the following reasons: first, entacapone increases the overall serum levodopa concentration; second, the amount of levodopa crossing the blood-brain barrier might be increased due to reduced competition with 3-OMD for the LNAA transporter; and finally, neuronal cell damage caused by 3-OMD also might be reduced.

In a previous study of Japanese Parkinson's disease patients with wearing-off motor fluctuations, the mean "on" time duration improvement was 1.4 hr for the entacapone group[13]. The reason why patients obtained a longer "on" time duration in our study may be attributable to differences in the categories in the self-rating diary. In a previous study[13], "on" was defined as good to excellent mobility and "off" as bad mobility. On the other hand, our diary defined "on" as good mobility and "off" as worse to bad mobility (partial to complete "off"). Partial "off" is a condition in between "off" and "on" [7]; thus, partial "off" might be easily converted to the "on" state by entacapone. In addition, open-label study also contributed to the longer "on" time. We defined the COMT-index as an index of COMT activity. The COMT-index was significantly correlated with the increase in the % of "on" time, in other words, patients with high COMT activity would show increased clinical effects of entacapone. We considered that patients with low COMT activity would show poor clinical effects of entacapone because there might be other factors for the wearing-off phenomenon in those patients. Therefore, the COMT-index could predict the clinical effect of entacapone before the administration of entacapone.

The increase in levodopa AUC after the first entacapone administration was also correlated with the increase in % of "on" time at the 8th week. We considered that patients with a good pharmacological response on first entacapone administration would have a good clinical response and that the increase in levodopa AUC could also predict the clinical effect of entacapone.

However, a two-day blood study is necessary to calculate the increase in levodopa AUC after the first entacapone administration. On the other hand, the COMT-index can be calculated with only a one-day blood study and might be more clinically useful than the increase in levodopa AUC.

The limitations of this study were as follows. The sample size was small, and we conducted an open-label study. We excluded patients with dementia because the "on" time parameter was designed so that it could be recorded by the patients themselves. Therefore, selection bias could not be avoided and might have interfered with the collection of accurate information.

In conclusion, our study showed that the measurement of serum 3-OMD concentration and levodopa pharmacokinetics and the calculation of COMT index are useful for predicting the clinical effects of entacapone. A further large study will be needed to confirm our conclusion.

ACKNOWLEDGEMENTS

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ORIGINAL ARTICLE

Cancer-associated ischemic stroke is associated with elevated D-dimer and fibrin degradation product levels in acute ischemic stroke with advanced cancer

Tomoyuki Kono, Toshiho Ohtsuki, Naohisa Hosomi, Ikuko Takeda, Shiro Aoki, Yoshimasa Sueda, Kayoko Ishihara, Takeshi Nakamura, Takemori Yamawaki and Masayasu Matsumoto

Department of Clinical Neuroscience and Therapeutics, Hiroshima University Graduate School of Biomedical Sciences, Hiroshima, Japan

Aim: Although several studies have reported various causes of ischemic stroke in patients with cancer, only a few have evaluated the clinical relevance of ischemic stroke pathogenesis to cancer. The aim of the present study was to elucidate the clinical characteristics of cancer-associated ischemic stroke.

Methods: We evaluated 154 ischemic stroke patients without cancer and 57 ischemic stroke patients with cancer who had either received continuous treatment for cancer within 5 years before to the onset of ischemic stroke, or who had been diagnosed with cancer within 1 year after the onset of ischemic stroke. Cancer patients were grouped into "cancer-associated ischemic stroke," the "conventional ischemic stroke," or "other."

Results: A total of 15 patients (26%) were classified into the cancer-associated ischemic stroke in cancer patients. In univariate analysis of the cancer-associated ischemic stroke and the others, there were significant differences in the prevalence of hypertension, hyperlipidemia and advanced cancer (clinical stage IV), and the levels of D-dimer, fibrin degradation product and hemoglobin. With multivariate regression analysis of those factors, the prevalence of hypertension, hyperlipidemia and advanced cancer (clinical stage IV), and the levels of D-dimer and fibrin degradation product remained as statistically independent factors, which were associated with cancer-associated ischemic stroke ($n = 111$, $\chi^2 = 67.21$, $P < 0.0001$).

Conclusion: In acute ischemic stroke, the cancer-associated ischemic stroke is associated with elevated D-dimer and fibrin degradation products, even after controlling hypertension, hyperlipidemia and advanced cancer (clinical stage IV). **Geriatr Gerontol Int 2012; ••: ••–••.**

Introduction

Systemic thromboembolism associated with cancer was first described by Armand Trousseau and colleagues in 1865. In a more recent autopsy study of 3426 cancer cases, excluding primary brain tumors, 15% of the cases examined had experienced cerebrovascular events.[1] The

causes of ischemic stroke in patients with cancer might differ from those in patients without cancer. Cancer can predispose patients to hypercoagulable states and might cause the development of deep vein thrombosis (DVT) and non-bacterial thrombotic endocarditis (NBTE).[2] NBTE is one of the most common causes of ischemic stroke in cancer patients,[3] and it has been found in 27% of cancer patients with ischemic stroke on post-mortem analysis.[1]

A number of previous studies have evaluated the characteristics of ischemic stroke patients with cancer. Cestari *et al.* classified the subtypes of ischemic stroke with cancer and found conventional ischemic stroke in just 35 of 96 patients (36%).[4] In addition, there were 36 of 52 patients (69%) with NBTE or embolism of an undetermined source in the subtype of embolic stroke.[4] In another study, embolic signals were detected with transcranial Doppler (TCD) in 45.9% of ischemic stroke patients with cancer.[5] In embolic stroke patients with cancer, a hypercoagulable state attributable to the cancer might have been one of the major causes of ischemic stroke.

However, these studies included a large number of elderly cancer patients, and it is possible that there was a certain proportion of these elderly patients in whom conventional ischemic stroke might have been attributable to atrial fibrillation (Af), atherosclerotic disease or other causes. This highlights the possibility that the causes of ischemic stroke in cancer patients are more complex than previously anticipated. To effectively detect and prevent ischemic stroke in cancer patients, it is necessary to define the causal relationship between ischemic stroke and cancer. However, the characteristics of ischemic stroke attributable to cancer that might allow its discrimination from conventional ischemic stroke remain undetermined.

The aim of the present study was to elucidate the clinical characteristics of cancer-associated ischemic stroke to differentiate it from the other type of ischemic stroke.

Methods

Design

This was a retrospective study of acute ischemic stroke patients admitted to Hiroshima University Hospital, Hiroshima, Japan, between January 2006 and August 2010. Ischemic stroke was confirmed by computed tomography (CT) and/or magnetic resonance imaging (MRI). We classified the patients as with cancer if they had active cancer or had been diagnosed with any cancer within 1 year after the onset of ischemic stroke, excluding primary intracranial tumors. Active cancer was defined by the presence of any continuous treatment for cancer within 5 years before the onset of

ischemic stroke. The patients were classified as non-cancer patients if they had never been diagnosed with cancer, had undergone surgical removal of cancer more than 5 years before stroke onset and had shown no recurrence of cancer until their stroke onset, or had no clinical information regarding their cancer.

Data collection

Patients were classified as hypertensive when they had been diagnosed with hypertension before stroke onset, and/or were taking antihypertensive medication. Patients were classified as having diabetes mellitus (DM) if they had glycated hemoglobin (HbA_{1c}) $\geq 6.5\%$ and fasting blood glucose ≥ 126 mg/dL, and/or were taking oral hypoglycemic agents or insulin. Patients were classified as hyperlipidemic if they had total cholesterol ≥ 220 mg/dL, low-density lipoprotein cholesterol ≥ 140 mg/dL and triglyceride level ≥ 150 mg/dL, and/or were taking antihyperlipidemic medication. Af was diagnosed with a standard electrocardiogram (ECG), 24-h ECG recording or 14-day ambulatory ECG monitoring.[6] The clinical stage of cancer was evaluated at the onset of ischemic stroke based on the tumor–node–metastasis (TNM) classification (for solid cancer) or the modified Ann Arbor (Cotswold's) staging (for malignant lymphoma).[7] In cases with an onset of ischemic stroke before cancer diagnosis, the clinical stage of cancer was evaluated at the time of cancer diagnosis. When a patient had multiple primary cancers, we evaluated the most advanced cancer.

Blood cell counts and blood coagulation factors (e.g. prothrombin time [PT; s], PT international normalized ratio [PT–INR], activated partial thromboplastin time [APTT; s], fibrinogen [mg/dL], D-dimer [µg/mL], fibrin degradation product [FDP; µg/mL], and antithrombin 3 [AT3; %]) were evaluated within 24 h of admission. D-dimer levels were measured by the latex agglutination method using a Sysmex XE7000 and the RIAS AUTO D-dimer NEO reagent (Kobe, Japan).

Ischemic stroke subtypes were classified by two stroke neurologists using the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria and imaging examinations (e.g. brain MRI, carotid ultrasonography, transthoracic echocardiography [TTE]), which were carried out within 1 week after hospitalization.[8] In addition, patients with the cancer-associated ischemic stroke were further categorized in accordance with the patients for whom no causes of stroke other than cancer could be identified in the subtype of undetermined etiology, despite extensive evaluations including brain MRI, carotid ultrasonography and TTE. Patients were classified as conventional ischemic stroke if their ischemic stroke was a lacunar infarction or if they had Af, myocardial infarction, rheumatic valvular disease, valvular replacement, cardiomyopathy, severe stenosis

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(>50%) of the infarct-related artery, arterial dissection or aortitis.

Statistical analysis

Medians (minimum to maximum) were used to describe continuous data, and frequency and percentage were used for categorical data. Univariate analyses were carried out to evaluate differences among the groups with regards to baseline characteristics, risk factors and laboratory data. Statistical analysis was carried out using JMP software version 9.0 for Windows (SAS Institute, Cary, NC, USA). Values were compared between patient groups using Fisher's exact test for categorical variables. Differences in continuous variables among the groups were examined using the Kruskal–Wallis test. When there was a statistically significant difference, the Wilcoxson signed rank test was applied to examine the difference between each group. Multivariate logistic regression was utilized to assess the relative importance of variables found to be related to the cancer-associated ischemic stroke, in initial univariate analyses. All analyses were two-tailed, and a value of $P < 0.05$ was considered statistically significant.

Results

A total of 211 consecutive acute ischemic stroke patients (79 women and 132 men) were identified between January 2006 and October 2010. The median age was 73 years (range 25–92). The patients' baseline characteristics are listed in Table 1. With our classifications, 15 patients (26%) were in the cancer-associated ischemic stroke group, 39 (68%) were in the conventional ischemic stroke group and three (6%) were not assigned to either classification among the cancer patients. No patient in the present study was assigned to both classifications. Of the three patients not assigned to either classification, one was classified as having a stroke of an undetermined etiology, because the general condition of the patient deteriorated before further evaluation. The two other patients in this group showed disseminated intravascular coagulation (DIC). There were no significant differences in prevalence of vascular risk factors in the cancer patients compared with that in the non-cancer patients. The most common vascular risk factor in the cancer patients was hypertension. It was similar to non-cancer patients. Ischemic stroke onset preceded cancer diagnosis in eight patients. The median duration from cancer diagnosis to stroke onset was 8 months (range −11 to 120) in the cancer patients. The patients in the cancer-associated ischemic stroke group had a lower rate of hypertension and hyperlipidemia than those in the conventional ischemic stroke group ($P < 0.05$).

Table 1 Baseline characteristics

	Non-cancer (n = 154)	Cancer (n = 57)	CAIS (n = 15)	CIS (n = 39)	Other (n = 3)
Age, years median (range)	73 (25, 92)	75 (50, 91)	74 (50, 87)	76 (56, 91)	67 (56, 86)
Sex					
Female, n (%)	65 (42)	14 (25)	6 (40)	7 (20)	1 (33)
Risk factors					
Hypertension, n (%)	100 (65)	33 (58)	5 (33)	26 (67)	2 (67)
DM, n (%)	54 (35)	19 (33)	3 (20)	16 (41)	0 (0)
Hyperlipidemia, n (%)	74 (48)	17 (30)	1 (7)	16 (41)	0 (0)
Af, n (%)	55 (36)	19 (33)	0 (0)	19 (49)	0 (0)
Ischemic stroke subtypes					
Small-vessel disease, n (%)	22 (14)	5 (9)	0 (0)	5 (13)	0 (0)
Large artery atherosclerosis, n (%)	30 (20)	12 (21)	0 (0)	12 (31)	0 (0)
Cardioembolism, n (%)	62 (40)	18 (32)	0 (0)	18 (46)	0 (0)
Other determined etiology, n (%)	16 (10)	6 (10)	0 (0)	4 (10)	2 (67)
Undetermined etiology, n (%)	24 (16)	16 (23)	15 (100)	0 (0)	1 (33)
Duration from cancer diagnosis to ischemic stroke onset, month median (range).	NA	8 (−11, 120)	13 (0, 69)	7 (−11, 120)	7 (0, 11)

Af, atrial fibrillation; CAIS, cancer-associated ischemic stroke; CIS, conventional ischemic stroke; DM, diabetes mellitus; NA, not assessed.

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Table 2 Distributions of primary cancers

	Cancer (n = 57)	CAIS (n = 15)	CIS (n = 39)	Other (n = 3)
Lung	8	2	6	0
Stomach	7	1	6	0
Liver	6	2	4	0
Colon	5	0	5	0
Prostate	4	0	4	0
Malignant lymphoma	4	1	3	0
Pancreas	3	2	0	1
Gall bladder	3	2	1	0
Kidney	3	2	1	0
Esophagus	3	0	2	1
Pharynx	2	0	1	1
Breast	3	1	2	0
Others	6	2[†]	4[‡]	0

[†]These two patients had uterus cancer and malignant melanoma. [‡]These four patients had thyroid cancer, gastrointestinal stromal tumor, bladder cancer or soft tissue tumor of the elbow. CAIS, cancer-associated ischemic stroke; CIS, conventional ischemic stroke.

The distributions of the primary cancers are shown in Table 2. Primary cancers were located in the lung in eight patients (14%) and in the stomach in seven patients (12.2%). Through histological classification, it was determined that 39 patients (68%) had adenocarcinoma. There was no significant difference in the distribution of adenocarcinoma between the cancer-associated ischemic stroke and the conventional ischemic stroke groups (Fig. 1). There was a higher prevalence of advanced cancer (clinical stage IV) in the cancer-associated ischemic stroke group than in the conventional ischemic stroke group ($P < 0.0001$; Fig. 2).

Blood cell counts, including hemoglobin and platelet counts, were evaluated in all patients. D-dimer and FDP levels were evaluated in 177 (84%) and 118 patients (56%), respectively. There was no significant difference in platelet counts among the groups. Hemoglobin was low in both cancer groups compared with that of the non-cancer group. D-dimer and FDP levels were significantly higher in the cancer-associated ischemic stroke group than in the non-cancer group and conventional ischemic stroke group ($P < 0.05$; Fig. 3). To assess the association between blood coagulation and clinical stage of primary cancer, differences between D-dimer and FDP levels were evaluated in the different clinical stages. Levels of D-dimer and FDP in the patients with cancer of clinical stage IV were significantly higher than those in patients of clinical stages I–III (D-dimer: 1.3 μg/mL [0.1–29.0] in stages I–III and 8.3 μg/mL [0.4–81.5] in stage IV and FDP: 3.1 μg/mL [0.8–50.5] in
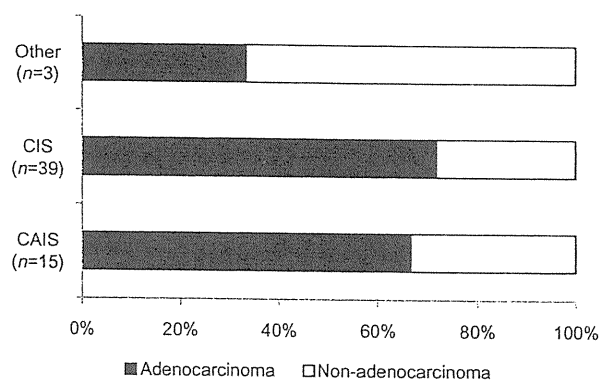

Figure 1 Histological types of primary cancer in the ischemic stroke classification in association with cancer. The difference in the distribution of adenocarcinoma between the cancer-associated ischemic stroke (CAIS) group and the conventional ischemic stroke (CIS) group was not significant.
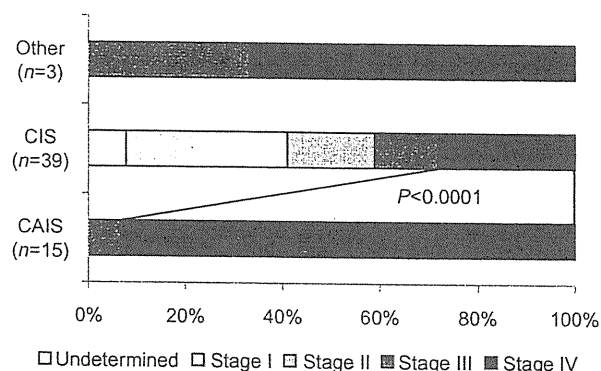

Figure 2 Clinical stage of primary cancer in the cancer-associated ischemic stroke group. There was a higher prevalence of advanced cancer (clinical stage IV) in patients with cancer-associated ischemic stroke (CAIS) compared with patients with conventional ischemic stroke (CIS).

stages I–III and 15.1 μg/mL [1.2–100.2] in stage IV, $P < 0.05$, respectively).

In univariate analysis on the cancer-associated ischemic stroke and the other groups, there were significant differences in the prevalence of hypertension, hyperlipidemia, and advanced cancer (clinical stage IV) and the levels of D-dimer, FDP and hemoglobin. Then, we carried out multivariate logistic regression analysis on the cancer-associated ischemic stroke and the others with those factors. As a result, the prevalence of hypertension, hyperlipidemia, and advanced cancer (clinical stage IV), and the levels of D-dimer and fibrin degradation product remained as statistically independent factors, which associated with cancer-associated ischemic stroke ($n = 111$, $\chi^2 = 67.21$, $P < 0.0001$; Table 3).

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Figure 3 Blood cell counts and coagulation factors in the cancer-associated ischemic stroke group. There was no significant difference in platelet counts between the cancer-associated ischemic stroke (CAIS) and the conventional ischemic stroke (CIS) groups. Although hemoglobin level was low in both cancer patient groups compared with that of the non-cancer patients, there was no significant difference between the patients with CAIS and those with CIS. D-dimer and fibrin degradation product (FDP) levels were significantly higher in the CAIS group than the other groups. Boxplot graph was made with median, 10th, 25th, 75th and 90th values. Significant differences among groups: $*P < 0.05$, $**P < 0.0001$.
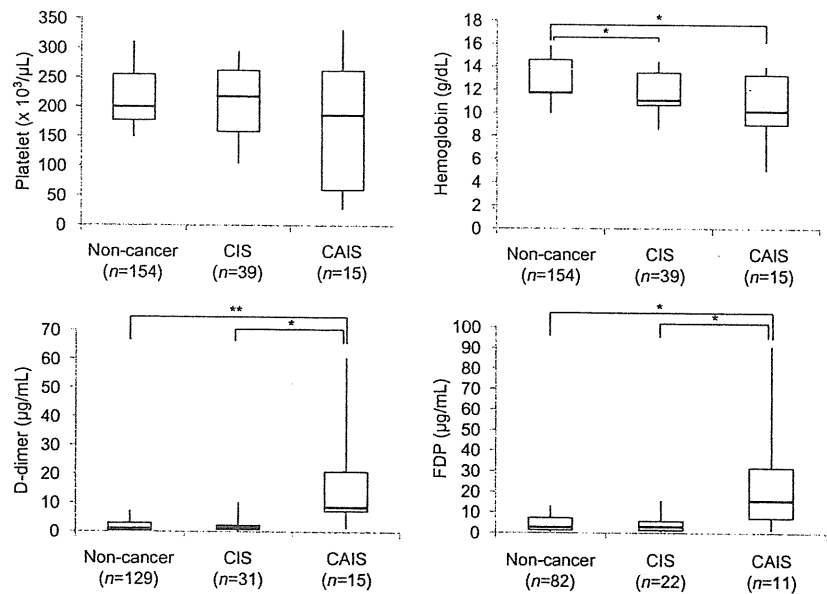
Table 3 Multivariate logistic regression analysis on the cancer-associated ischemic stroke and the others

Factors	χ^2	P-value
Hypertension	11.13	0.0008
Hyperlipidemia	4.16	0.0414
Advanced cancer	48.47	<0.0001
D-dimer	12.60	0.0004
FDP	9.24	0.0024
Hemoglobin	2.15	0.9988

$n = 111$, $\chi^2 = 67.21$, $P < 0.0001$. FDP, fibrin degradation product.

Discussion

In the present study, we evaluated the clinical characteristics of ischemic stroke patients with cancer. Of the 58 patients examined, 16 (28%) were classified as having cancer-associated ischemic stroke. The characteristics of cancer-associated ischemic stroke were evaluated by comparison with the non-cancer group and conventional ischemic stroke group. There was no significant difference in the prevalence of adenocarcinoma between the cancer-associated ischemic stroke and the conventional ischemic stroke groups. However, there was a higher prevalence of advanced cancer (clinical stage IV) in patients with cancer-associated ischemic stroke than in patients with conventional ischemic stroke. There was a lower prevalence of hypertension and hyperlipidemia in patients with the cancer-associated ischemic stroke than in patients with the conventional ischemic stroke. There were no significant differences between the two groups in hemoglobin and platelet counts. FDP

and D-dimer levels were significantly higher in patients with the cancer-associated ischemic stroke than in the non-cancer group and the conventional ischemic stroke group. In acute ischemic stroke, cancer-associated ischemic stroke was associated with elevated D-dimer and fibrin degradation products, even after controlling hypertension, hyperlipidemia and advanced cancer (clinical stage IV).

In the present study, we attempted to select the patients with cancer-associated ischemic stroke using the TOAST criteria. Kim *et al.* have also tried to classify ischemic stroke patients with cancer using conventional and cryptogenic stroke mechanisms.[9] In their classification, ischemic stroke patients with undetermined etiologies according to the TOAST criteria were categorized solely with reference to cryptogenic stroke mechanisms. Although their classification method is almost equal to our method, they classified five NBTE patients as a conventional ischemic stroke mechanism. NBTE is widely recognized as a unique cause of cardioembolism frequently observed in cancer patients, as has been reported in a post-mortem series.[1] Although transesophageal echocardiography (TEE) is the most appropriate method to diagnose cardiac sources of embolism, such as NBTE,[10] it is difficult to carry out TEE in all cancer patients, as TEE is an invasive examination. In the present study, we carried out TEE in just 37% of the patients who were suspected to have had a cardioembolic stroke from the results of imaging analysis and who could tolerate esophageal intubation. Although our execution rate of TEE was higher than that in previous reports,[4,11] it is quite likely that there is a selection bias. Therefore, we tried to classify cancer-associated ischemic stroke without using the finding of TEE. As a

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result, three patients with NBTE in the cancer-associated ischemic stroke group were included, because there were not embolic sources, despite extensive examination without TEE. From this result, this classification method could be adequate in a clinical site.

D-dimer is a plasmin-derived degradation product of cross-linked fibrin that is elevated in patients with hypercoagulability. D-dimer levels were elevated in a wide variety of conditions with intravascular clotting, including ischemic stroke itself. Previous studies have reported that D-dimer is higher in stroke patients with cancer than in stroke patients without cancer.[4] In the present study, we have shown that, among ischemic stroke patients, D-dimer levels were more elevated in patients with cancer-associated ischemic stroke than non-cancer and conventional ischemic stroke. The patients with NBTE showed high D-dimer levels. There were three patients with NBTE found with TEE in the cancer-associated ischemic stroke group. Their D-dimer levels were 8.7 μg/mL, 23.1 μg/mL and 55.5 μg/mL; these values were markedly higher than those of the other patients in this group. It might be because NBTE can result from a hypercoagulable stage. Furthermore, we investigated the possibility that patients with advanced cancer show high levels of D-dimer. Interestingly, the levels of D-dimer were higher in the patients with stage IV cancer than in those with clinical stages I–III. From the present results, D-dimer and FDP remained as independently significant, even after controlling the prevalence of hypertension, hyperlipidemia and advanced cancer (clinical stage IV). Therefore, although the elevation of blood coagulation factors in the patients with the cancer-associated ischemic stroke might be attributable to a higher clinical stage of primary cancer, it was independently associated with the cancer-associated ischemic stroke.

Uemura *et al.* reported that hemoglobin levels were reduced in cancer patients compared with patients with no malignancy.[12] Their explanation for the reduced hemoglobin levels in cancer patients was the general condition of these patients as a result of cancer cachexia or gastrointestinal bleeding in gastrointestinal cancer patients. The present results support those of Uemura *et al.*; hemoglobin levels in the cancer groups were lower than the non-cancer group in the present study. However, there was no significant difference in hemoglobin levels between the patients with cancer-associated ischemic stroke and those with conventional ischemic stroke. The present results suggested that hemoglobin levels cannot be a marker used to distinguish between cancer-associated ischemic stroke and conventional ischemic stroke in cancer patients.

Circulating mucinous material has been reported in embolic stroke patients with mucin-producing adenocarcinomas.[13–15] Seok *et al.* reported that embolic signal was observed in patients with metastasis or adenocarcinoma.[5] Of the patients in the present study, 68% were adenocarcinoma patients. However, there was no significant difference in histological types between the cancer-associated ischemic stroke and the conventional ischemic stroke patients. These results require confirmation in a larger number of patients.

In the present results, there was a significant difference in the clinical stage of cancer, but not the histological type between the patients with cancer-associated ischemic stroke and those with conventional ischemic stroke. A few previous reports have evaluated the relationship between cancer-associated ischemic stroke and its clinical stage. Kim *et al.* showed a high prevalence of distant metastasis in cryptogenic stroke mechanisms.[9] This report supports the present results in that there was a high prevalence of advanced cancer in the patients with cancer-associated ischemic stroke.

The present study has some limitations. First, this was a single-center retrospective study. Our hospital functions as both a regional cancer center and an advanced emergency center. The proportion of ischemic stroke patients with cancer might differ between our hospital and the general population. Therefore, there might be a selection bias for ischemic stroke patients with cancer. Second, the method of classifying cancer-associated ischemic stroke is not generalized. With our classification system, all of the ischemic stroke patients with Af were classified as having cardioembolism in conventional ischemic stroke. However, there is a possibility that some of these stroke cases might have been attributable to a hypercoagulable state as a result of cancer rather than to Af. Further selection criteria are necessary to select the Af patients in whom the ischemic stroke was a result of hypercoagulability as a result of cancer. Therefore, further research investigating the association between ischemic stroke and cancer is needed. Finally, although TEE is necessary to diagnose NBTE, TEE cannot be carried out in all cases because of patients' general conditions, anatomical reasons (e.g. esophageal or gastric cancer and esophageal varix) and/or lack of patient cooperation.

In conclusion, 26% of the patients studied were classified as having cancer-associated ischemic stroke. Based on our results, elevated D-dimer and FDP levels can be associated with cancer-associated ischemic stroke, even after controlling hypertension, hyperlipidemia and advanced cancer (clinical stage IV).

Acknowledgments

Disclosure statement

None.

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