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 ≥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 (≥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 (≥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 ≥50%.

Similar findings were reported in a larger study [64] of 153 patients with low responsiveness to clopidogrel (platelet reactivity index ≥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%; $P < 0.001$).

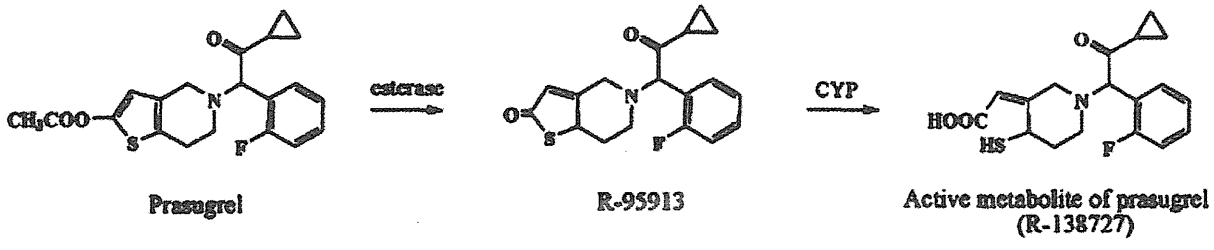

Figure 5 Mechanism of prasugrel activation.

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.

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Conflicts of Interest

The author declares no conflict of interests.

References

1. Gurbel PA, Bliden KP, Hiatt BL, et al. Clopidogrel for coronary stenting: Response variability, drug resistance, and the effect of pretreatment platelet reactivity. *Circulation* 2003;**107**:2908–2913.
2. Müller I, Besta F, Schulz C, et al. Prevalence of clopidogrel non-responders among patients with stable angina pectoris scheduled for elective coronary stent placement. *Thromb Haemost* 2003;**89**:783–787.
3. Cuisset T, Frere C, Quilici J, et al. High post-treatment platelet reactivity identified low-responders to dual antiplatelet therapy at increased risk of recurrent cardiovascular events after stenting for acute coronary syndrome. *J Thromb Haemost* 2006;**4**:542–549.
4. Gurbel PA, Bliden KP, Samara W, et al. Clopidogrel effect on platelet reactivity in patients with stent thrombosis: Results of the CREST Study. *J Am Coll Cardiol* 2005;**46**:1827–1832.
5. Price MJ, Endemann S, Gollapudi RR, et al. Prognostic significance of post-clopidogrel platelet reactivity assessed by a point-of-care assay on thrombotic events after drug-eluting stent implantation. *Eur Heart J* 2008;**29**:992–1000.
6. Patti G, Nusca A, Mangiacapra F, et al. Point-of-care measurement of clopidogrel responsiveness predicts clinical outcome in patients undergoing percutaneous coronary intervention results of the ARMYDA-PRO (Antiplatelet therapy for Reduction of MYocardial Damage during Angioplasty-Platelet Reactivity Predicts Outcome) study. *J Am Coll Cardiol* 2008;**52**:1128–1133.
7. Marcucci R, Gori AM, Paniccia R, et al. Cardiovascular death and nonfatal myocardial infarction in acute coronary syndrome patients receiving coronary stenting are predicted by residual platelet reactivity to ADP detected by a point-of-care assay: A 12-month follow-up. *Circulation* 2009;**119**:237–242.
8. Migliorini A, Valenti R, Marcucci R, et al. High residual platelet reactivity after clopidogrel loading and long-term clinical outcome after drug-eluting stenting for unprotected left main coronary disease. *Circulation* 2009;**120**:2214–2221.
9. Geisler T, Zürn C, Simonenko R, et al. Early but not late stent thrombosis is influenced by residual platelet aggregation in patients undergoing coronary interventions. *Eur Heart J* 2010;**31**:59–66.
10. Matetzky S, Shenkman B, Guetta V, et al. Clopidogrel resistance is associated with increased risk of recurrent atherothrombotic events in patients with acute myocardial infarction. *Circulation* 2004;**109**:3171–3175.
11. CAPRIE Study Investigators. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. *Lancet* 1996;**348**:1329–1339.
12. Mehta SR, Yusuf S; Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) Study Investigators. The Clopidogrel in Unstable angina to prevent Recurrent Events (CURE) trial programme; rationale, design and baseline characteristics including a meta-analysis of the effects of thienopyridines in vascular disease. *Eur Heart J* 2000;**21**:2033–2041.
13. Steinhubl SR, Berger PB, Mann JT III, et al. Clopidogrel for the Reduction of Events During Observation. Early and sustained dual oral antiplatelet therapy following percutaneous coronary intervention: A randomized controlled trial. *JAMA* 2002;**288**:2411–2420.
14. Serebruany VL, Steinhubl SR, Berger PB, et al. Variability in platelet responsiveness to clopidogrel among 544 individuals. *J Am Coll Cardiol* 2005;**45**:246–251.
15. Gurbel PA, Tantry US. Clopidogrel resistance? *Thromb Res* 2007;**120**:311–321.
16. Mega JL, Close SL, Wiviott SD, et al. Cytochrome P-450 polymorphisms and response to clopidogrel. *N Engl J Med* 2009;**360**:354–362.
17. Lau WC, Waskell LA, Watkins PB, et al. Atorvastatin reduces the ability of clopidogrel to inhibit platelet aggregation: A new drug-drug interaction. *Circulation* 2003;**107**:32–37.
18. Lennon MJ, Gibbs NM, Weightman WM, et al. A comparison of Plateletworks and platelet aggregometry for the assessment of aspirin-related platelet dysfunction in cardiac surgical patients. *J Cardiothorac Vasc Anesth* 2004;**18**:136–140.
19. van Werkum JW, Kleibeuker M, Postma S, et al. A comparison between the Plateletworks-assay and light transmittance aggregometry for monitoring the inhibitory effects of clopidogrel. *Int J Cardiol* 2010;**140**:123–126.
20. Mach F, Senouf D, Fontana P, et al. Not all statins interfere with clopidogrel during antiplatelet therapy. *Eur J Clin Invest* 2005;**35**:476–481.
21. Wenaweser P, Windecker S, Billinger M, et al. Effect of atorvastatin and pravastatin on platelet inhibition by aspirin and clopidogrel treatment in patients with coronary stent thrombosis. *Am J Cardiol* 2007;**99**:353–356.
22. Mitsios JV, Papathanasiou AI, Rodis FI, et al. Atorvastatin does not affect the antiplatelet potency of clopidogrel when it is administered concomitantly for 5 weeks in patients with acute coronary syndromes. *Circulation* 2004;**109**:1335–1338.
23. Farid NA, Small DS, Payne CD, et al. Effect of atorvastatin on the pharmacokinetics and pharmacodynamics of prasugrel and clopidogrel in healthy subjects. *Pharmacotherapy* 2008;**28**:1483–1494.
24. Ayalasomayajula SP, Vaidyanathan S, Kemp C, et al. Effect of clopidogrel on the steady-state pharmacokinetics of fluvastatin. *J Clin Pharmacol* 2007;**47**:613–619.
25. Malmström RE, Ostergren J, Jørgensen L, et al. Influence of statin treatment on platelet inhibition by clopidogrel—A randomized comparison of rosuvastatin, atorvastatin and simvastatin co-treatment. *J Intern Med* 2009;**266**:457–466.
26. Trenk D, Hochholzer W, Frundi D, et al. Impact of cytochrome P450 3A4-metabolized statins on the antiplatelet effect of a 600-mg loading dose clopidogrel and on clinical outcome in patients undergoing elective coronary stent placement. *Thromb Haemost* 2008;**99**:174–181.
27. Tan VP, Yan BP, Kiernan TJ, et al. Risk and management of upper gastrointestinal bleeding associated with prolonged dual-antiplatelet therapy after percutaneous coronary intervention. *Cardiovasc Revasc Med* 2009;**10**:36–44.
28. Ng FH, Chan P, Kwanching CP, et al. Management and outcome of peptic ulcers or erosions in patients receiving a combination of aspirin plus clopidogrel. *J Gastroenterol* 2008;**43**:679–686.
29. Ng FH, Lam KF, Wong SY, et al. Upper gastrointestinal bleeding in patients with aspirin and clopidogrel co-therapy. *Digestion* 2008;**77**:173–177.
30. Gilard M, Arnaud B, Cornily JC, et al. Influence of omeprazole on the antiplatelet action of clopidogrel associated with aspirin: The randomized, double-blind OCLA (Omeprazole CLopidogrel Aspirin) study. *J Am Coll Cardiol* 2008;**51**:256–260.
31. Cuisset T, Frere C, Quilici J, et al. Comparison of omeprazole and pantoprazole influence on a high 150-mg clopidogrel maintenance dose the PACA (Proton Pump Inhibitors And Clopidogrel Association) prospective randomized study. *J Am Coll Cardiol* 2009;**54**:1149–1153.
32. O'Donoghue ML, Braunwald E, Antman EM, et al. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: An analysis of two randomised trials. *Lancet* 2009;**374**:989–997.
33. Juurlink DN, Gomes T, Ko DT, et al. A population-based study of the drug interaction between proton pump inhibitors and clopidogrel. *CMAJ* 2009;**180**:713–718.
34. Siller-Matula JM, Spiel AO, Lang IM, et al. Effects of pantoprazole and esomeprazole on platelet inhibition by clopidogrel. *Am Heart J* 2009;**157**:148.e1–5.
35. Ho PM, Maddox TM, Wang L, et al. Risk of adverse outcomes associated with concomitant use of clopidogrel and proton pump inhibitors following acute coronary syndrome. *JAMA* 2009;**301**:937–944.
36. O'Donoghue ML, Braunwald E, Antman EM, et al. Pharmacodynamic effect and clinical efficacy of clopidogrel and prasugrel with or without a proton-pump inhibitor: An analysis of two randomised trials. *Lancet* 2009;**374**:989–997.

37. Evanchan J, Donnally MR, Binkley P, Mazzaferri E. Recurrence of acute myocardial infarction in patients discharged on clopidogrel and a proton pump inhibitor after stent placement for acute myocardial infarction. *Clin Cardiol* 2010;**33**:168–171.
38. Bhatt DL, Cryer B, Contant CF, et al.; on behalf of the COGENT Investigators. The COGENT Trial. In *Proceedings of the Transcatheter Cardiovascular Therapeutics Annual Congress*, San Francisco, USA, 2009.
39. Hulot JS, Bura A, Villard E, et al. Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects. *Blood* 2006;**108**:2244–2247.
40. Fontana P, Hulot JS, De Moerloose P, et al. Influence of CYP2C19 and CYP3A4 gene polymorphisms on clopidogrel responsiveness in healthy subjects. *J Thromb Haemost* 2007;**5**:2153–2155.
41. Frere C, Cuisset T, Morange PE, et al. Effect of cytochrome P450 polymorphisms on platelet reactivity after treatment with clopidogrel in acute coronary syndrome. *Am J Cardiol* 2008;**101**:1088–1093.
42. Giusti B, Gori AM, Marcucci R, et al. Cytochrome P450 2C19 loss-of-function polymorphism, but not CYP3A4 IVS10 + 12G/A and P2Y12 T744C polymorphisms, is associated with response variability to dual antiplatelet treatment in high-risk vascular patients. *Pharmacogenet Genomics* 2007;**17**:1057–1064.
43. Trenk D, Hochholzer W, Fromm MF, et al. Cytochrome P450 2C19 681G>A polymorphism and high on-clopidogrel platelet reactivity associated with adverse 1-year clinical outcome of elective percutaneous coronary intervention with drug-eluting or bare-metal stents. *J Am Coll Cardiol* 2008;**51**:1925–1934.
44. Takahiro K, Kan C, Tatsuji I. Frequency distribution of CYP2C19, CYP2D6, and CYP2C9 mutant-alleles in several different populations. *Xenobiot Metab Dispos* 2001;**16**:69–74.
45. Ozawa S, Soyama A, Saeki M, et al. Ethnic differences in genetic polymorphisms of CYP2D6, CYP2C19, CYP3As and MDR1/ABCB1. *Drug Metab Pharmacokinet* 2004;**19**:83–95.
46. Umemura K, Furuta T, Kondo K. The common gene variants of CYP2C19 affect pharmacokinetics and pharmacodynamics in an active metabolite of clopidogrel in healthy subjects. *J Thromb Haemost* 2008;**6**:1439–1441.
47. Hochholzer W, Trenk D, Bestehorn HP, et al. Impact of the degree of peri-interventional platelet inhibition after loading with clopidogrel on early clinical outcome of elective coronary stent placement. *J Am Coll Cardiol* 2006;**48**:1742–1750.
48. Collet JP, Hulot JS, Pena A, et al. Cytochrome P450 2C19 polymorphism in young patients treated with clopidogrel after myocardial infarction: A cohort study. *Lancet* 2009;**373**:309–317.
49. Simon T, Verstuyft C, Mary-Krause M, et al. Genetic determinants of response to clopidogrel and cardiovascular events. *N Engl J Med* 2009;**360**:363–375.
50. Ganter MT, Hofer CK. Coagulation monitoring: Current techniques and clinical use of viscoelastic point-of-care coagulation devices. *Anesth Analg* 2008;**106**:1366–1375.
51. Reinhöfer M, Brauer M, Franke U, et al. The value of rotation thromboelastometry to monitor disturbed perioperative haemostasis and bleeding risk in patients with cardiopulmonary bypass. *Blood Coagul Fibrinolysis* 2008;**19**:212–219.
52. Frelinger AL 3rd, Jakubowski JA, Li Y, et al. The active metabolite of prasugrel inhibits adenosine diphosphate- and collagen-stimulated platelet procoagulant activities. *J Thromb Haemost* 2008;**6**:359–365.
53. Lordkipanidzé M, Pharand C, Schampaert E, et al. Evaluation of the platelet count drop method for assessment of platelet function in comparison with "gold standard" light transmission aggregometry. *Thromb Res* 2009;**124**:418–422.
54. Pinto Slottow TL, Bonello L, Gavini R, et al. Prevalence of aspirin and clopidogrel resistance among patients with and without drug-eluting stent thrombosis. *Am J Cardiol* 2009;**104**:525–530.
55. Kim H, Lee HK, Han K, et al. Prevalence and risk factors for aspirin and clopidogrel resistance in patients with coronary artery disease or ischemic cerebrovascular disease. *Ann Clin Lab Sci* 2009;**39**:289–294.
56. Blais N, Pharand C, Lordkipanidzé M, et al. Response to aspirin in healthy individuals. Cross-comparison of light transmission aggregometry, VerifyNow system, platelet count drop, thromboelastography (TEG) and urinary 11-dehydrothromboxane B(2). *Thromb Haemost* 2009;**102**:404–411.
57. Breet NJ, van Werkum JW, Bouman HJ, et al. Comparison of platelet function tests in predicting clinical outcome in patients undergoing coronary stent implantation. *JAMA* 2010;**303**:754–762.
58. Cuisset T, Frere C, Quilici J, et al. Benefit of a 600-mg loading dose of clopidogrel on platelet reactivity and clinical outcomes in patients with non-ST-segment elevation acute coronary syndrome undergoing coronary stenting. *J Am Coll Cardiol* 2006;**48**:1339–1345.
59. L'Allier PL, Ducrocq G, Pranno N, et al. Clopidogrel 600-mg double loading dose achieves stronger platelet inhibition than conventional regimens: Results from the PREPAIR randomized study. *J Am Coll Cardiol* 2008;**51**:1066–1072.
60. Angiolillo DJ, Bernardo E, Palazuelos J, et al. Functional impact of high clopidogrel maintenance dosing in patients undergoing elective percutaneous coronary interventions. Results of a randomized study. *Thromb Haemost* 2008;**99**:161–168.
61. von Beckerath N, Kastrati A, Wieczorek A, et al. A double-blind, randomized study on platelet aggregation in patients treated with a daily dose of 150 or 75 mg of clopidogrel for 30 days. *Eur Heart J* 2007;**28**:1814–1819.
62. Bonello L, Camoin-Jau L, Arques S, et al. Adjusted clopidogrel loading doses according to vasodilator-stimulated phosphoprotein phosphorylation index decrease rate of major adverse cardiovascular events in patients with clopidogrel resistance: A multicenter randomized prospective study. *J Am Coll Cardiol* 2008;**51**:1404–1411.
63. Angiolillo DJ, Costa MA, Shoemaker SB, et al. Functional effects of high clopidogrel maintenance dosing in patients with inadequate platelet inhibition on standard dose treatment. *Am J Cardiol* 2008;**101**:440–445.
64. Aleil B, Jacquemin L, De Poli F, et al. Clopidogrel 150 mg/day to overcome low responsiveness in patients undergoing elective percutaneous coronary intervention: Results from the VASP-02 (Vasodilator-Stimulated Phosphoprotein-02) randomized study. *JACC Cardiovasc Interv* 2008;**1**:631–638.
65. Pena A, Collet JP, Hulot JS, et al. Can we override clopidogrel resistance? *Circulation* 2009;**119**:2854–2857.
66. Williams ET, Jones KO, Ponsler GD, et al. The biotransformation of prasugrel, a new thienopyri-dine prodrug, by the human carboxylesterases 1 and 2. *Drug Metab Dispos* 2008;**36**:1227–1232.
67. Farid NA, Smith RL, Gillespie TA, et al. The disposition of prasugrel, a novel thienopyridine, in humans. *Drug Metab Dispos* 2007;**35**:1096–1104.
68. Wiviott SD, Braunwald E, McCabe CH, et al. Prasugrel versus clopidogrel in patients with acute coronary syndromes. *N Engl J Med* 2007;**357**:2001–2015.
69. Wiviott SD, Trenk D, Frelinger AL, et al. Prasugrel compared with high loading- and maintenance-dose clopidogrel in patients with planned percutaneous coronary intervention: The Prasugrel in Comparison to Clopidogrel for Inhibition of Platelet Activation and Aggregation-Thrombolysis in Myocardial Infarction 44 trial. *Circulation* 2007;**116**:2923–2932.
70. Tamhane U, Meier P, Chetcuti S, et al. Efficacy of Cilostazol in reducing restenosis in patients undergoing contemporary stent based PCI: A meta-analysis of randomised controlled trials. *EuroIntervention* 2009;**5**:384–393.
71. Han Y, Li Y, Wang S, et al. Cilostazol in addition to aspirin and clopidogrel improves long-term outcomes after percutaneous coronary intervention in patients with acute coronary syndromes: A randomized, controlled study. *Am Heart J* 2009;**157**:733–739.
72. Jeong YH, Lee SW, Choi BR, et al. Randomized comparison of adjunctive cilostazol versus high maintenance dose clopidogrel in patients with high post-treatment platelet reactivity: Results of the ACCEL-RESISTANCE (Adjunctive Cilostazol Versus High Maintenance Dose Clopidogrel in Patients With Clopidogrel Resistance) randomized study. *J Am Coll Cardiol* 2009;**53**:1101–1109.
73. Van Giezen JJ, Nilsson L, Berntsson P, et al. Ticagrelor binds to human P2Y(12) independently from ADP but antagonizes ADP-induced receptor signaling and platelet aggregation. *J Thromb Haemost* 2009;**7**:1556–1565.
74. Storey RF, Melissa Thornton S, Lawrance R, et al. Ticagrelor yields consistent dose-dependent inhibition of ADP-induced platelet aggregation in patients with atherosclerotic disease regardless of genotypic variations in P2RY12, P2RY1, and ITGB3. *Platelets* 2009;**20**:341–348.

75. Cannon CP, Harrington RA, James S, et al. Comparison of ticagrelor with clopidogrel in patients with a planned invasive strategy for acute coronary syndromes (PLATO): A randomised double-blind study. *Lancet* 2010;**375**:283–293.
76. Chen KY, Rha SW, Li YJ, et al. Triple versus dual antiplatelet therapy in patients with acute ST-segment elevation myocardial infarction undergoing primary percutaneous coronary intervention. *Circulation* 2009;**119**:3207–3214.
77. Lee SW, Park SW, Hong MK, et al. Triple versus dual antiplatelet therapy after coronary stenting: Impact on stent thrombosis. *J Am Coll Cardiol* 2005;**46**:1833–1837.
78. Lee SW, Park SW, Kim YH, et al. Comparison of triple versus dual antiplatelet therapy after drug-eluting stent implantation (from the DECLARE-Long trial). *Am J Cardiol* 2007;**100**:1103–1108.
79. Lee SW, Park SW, Kim YH, et al. Drug-eluting stenting followed by cilostazol treatment reduces late restenosis in patients with diabetes mellitus the DECLARE-DIABETES Trial (A Randomized Comparison of Triple Antiplatelet Therapy with Dual Antiplatelet Therapy After Drug-Eluting Stent Implantation in Diabetic Patients). *J Am Coll Cardiol* 2008;**51**:1181–1187.
80. Shim CY, Yoon SJ, Park S, et al. The clopidogrel resistance can be attenuated with triple antiplatelet therapy in patients undergoing drug-eluting stents implantation. *Int J Cardiol* 2009;**134**:351–355.
81. Jaremo P, Lindahl TL, Fransson SG, et al. Individual variations of platelet inhibition after loading doses of clopidogrel. *J Intern Med* 2002;**252**:233–238.
82. Mobley JE, Bresee SJ, Wortham DC, et al. Frequency of nonresponse antiplatelet activity of clopidogrel during pretreatment for cardiac catheterization. *Am J Cardiol* 2004;**93**:456–458.
83. Lepantalo A, Virtanen KS, Heikkila J, et al. Limited early antiplatelet effect of 300 mg clopidogrel in patients with aspirin therapy undergoing percutaneous coronary interventions. *Eur Heart J* 2004;**25**:476–483.
84. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Identification of low responders to a 300-mg clopidogrel loading dose in patients undergoing coronary stenting. *Thromb Res* 2005;**115**:101–108.
85. Dziewierz A, Dudek D, Heba G, et al. Inter-individual variability in response to clopidogrel in patients with coronary artery disease. *Kardiol Pol* 2005;**62**:108–117.
86. Lev EI, Patel RT, Maresh KJ, et al. Aspirin and clopidogrel drug response in patients undergoing percutaneous coronary intervention: The role of dual drug resistance. *J Am Coll Cardiol* 2006;**47**:27–33.
87. Angiolillo DJ, Fernandez-Ortiz A, Bernardo E, et al. Platelet function profiles in patients with type 2 diabetes and coronary artery disease on combined aspirin and clopidogrel treatment. *Diabetes* 2005;**54**:2430–2435.
88. Gurbel PA, Bliden KP, Hayes KM, et al. The relation of dosing to clopidogrel responsiveness and the incidence of high post-treatment platelet aggregation in patients undergoing coronary stenting. *J Am Coll Cardiol* 2005;**45**:1392–1396.
89. Angiolillo DJ, Capranzano P, Goto S, et al. A randomized study assessing the impact of cilostazol on platelet function profiles in patients with diabetes mellitus and coronary artery disease on dual antiplatelet therapy: Results of the OPTIMUS-2 study. *Eur Heart J* 2008;**29**:2202–2211.

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

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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

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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.

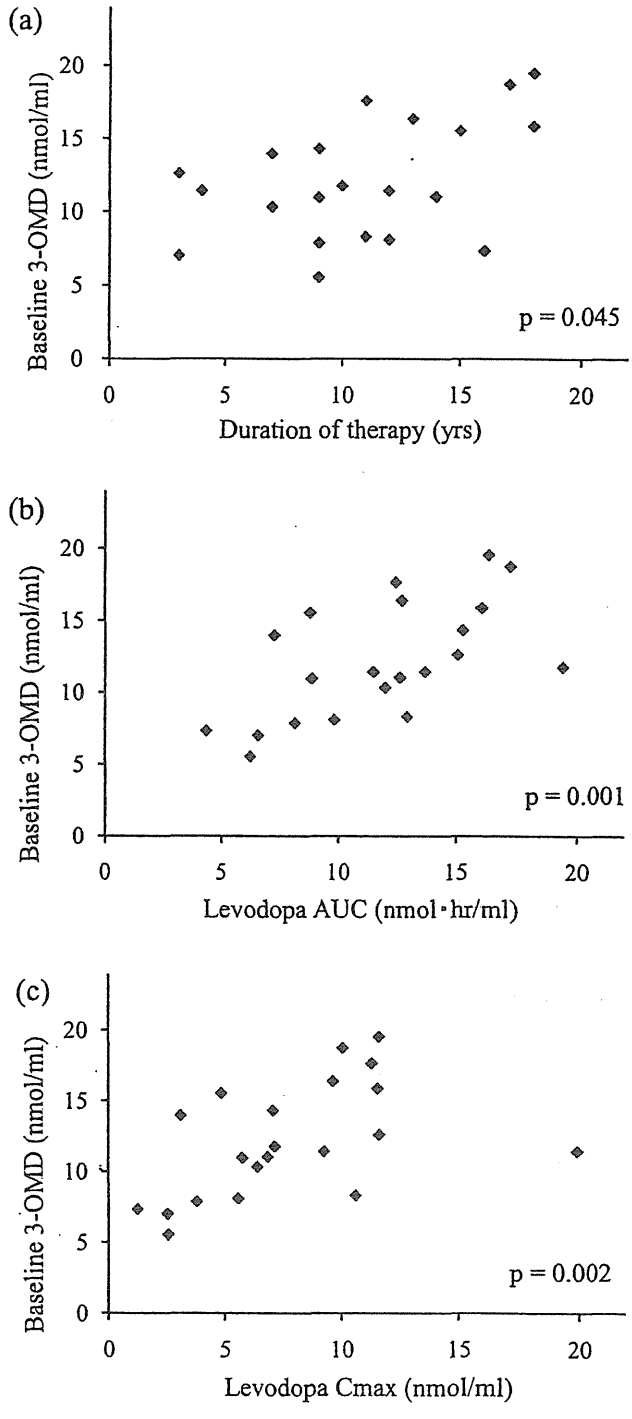

Fig. 1. The baseline serum 3-OMD concentration was positively correlated with (a) the duration of therapy (p = 0.045), (b) levodopa AUC (p = 0.001), and (c) levodopa Cmax (p = 0.002). (Spearman's rank correlation)

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.

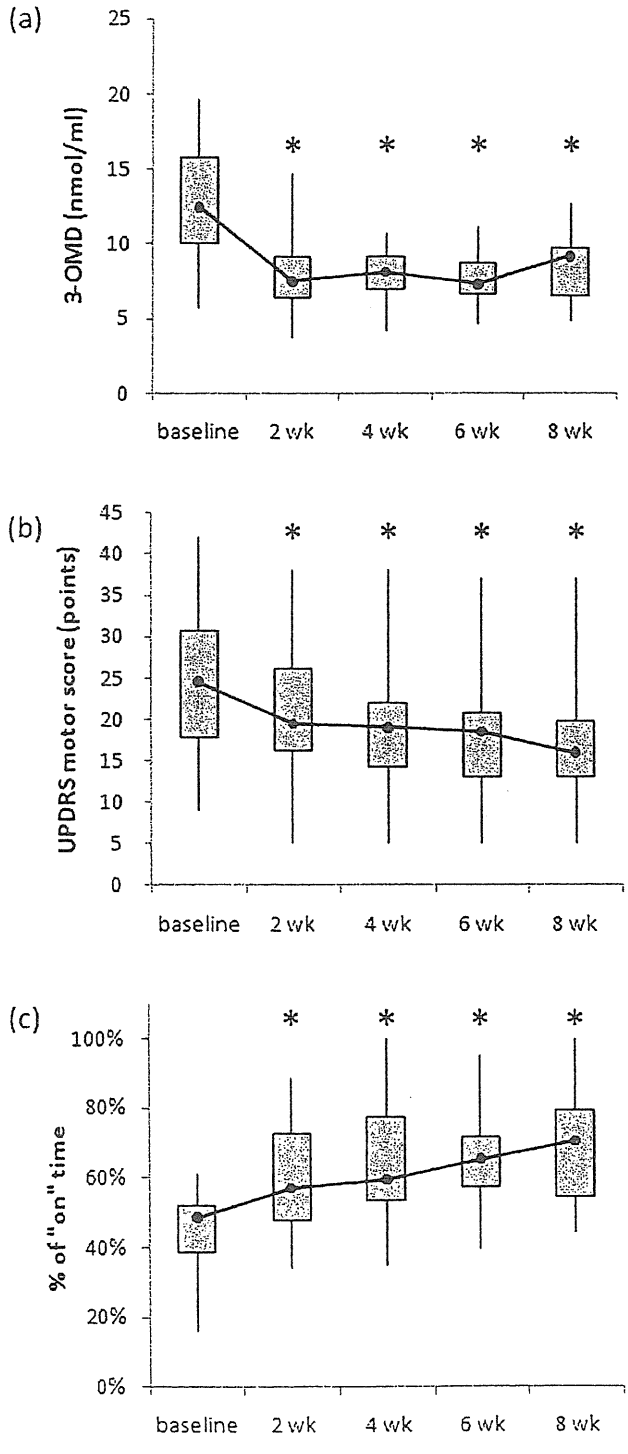

Fig. 2. Changes in (a) serum 3-OMD, (b) UPDRS motor score, and (c) % of "on" time from the baseline to the 8th week after entacapone therapy (n=18).
* Wilcoxon's signed rank test, $p < 0.001$, compared with the baseline.

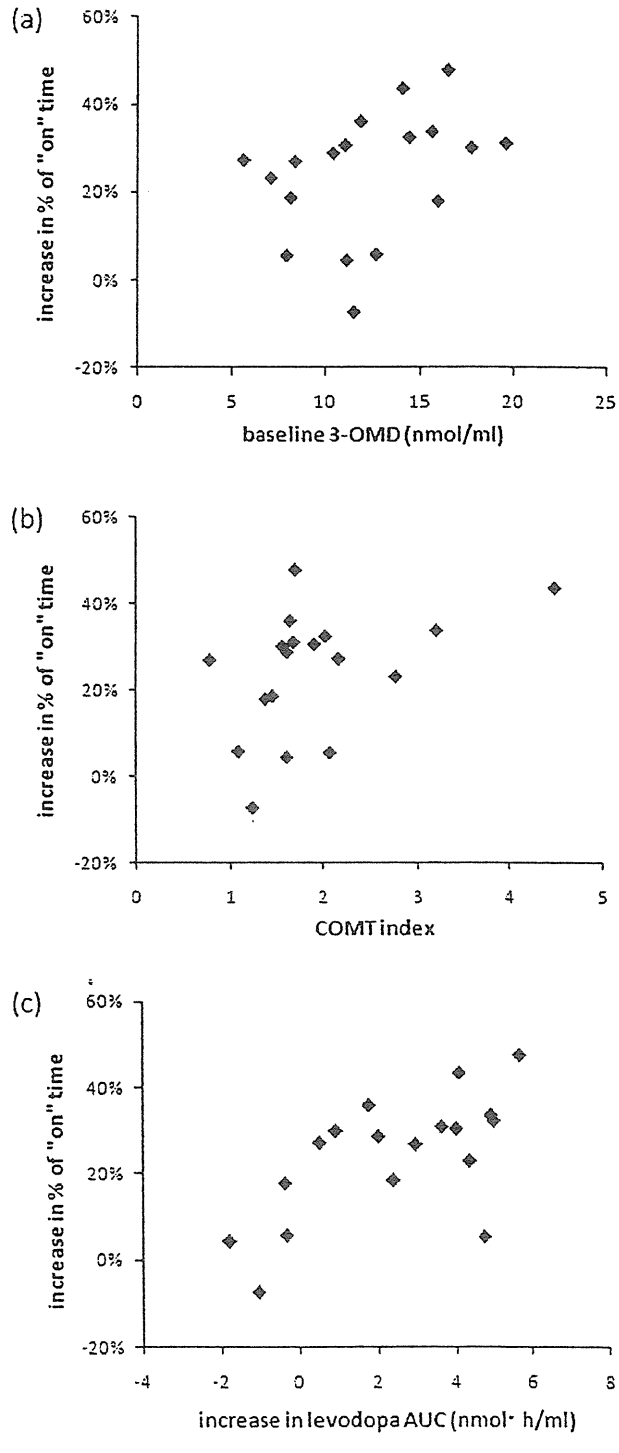

Fig. 3. (a) Association between the increase in % of "on" time and serum 3-OMD concentration. (p=0.053)
(b) Association between the increase in % of "on" time and COMT index. (p=0.027)
(c) Association between the increase in % of "on" time and the change in levodopa AUC. (p=0.006)
Evaluation at the 8th 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.

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REFERENCES

1. **Ahlskog, J.E. and Muenter, M.D.** 2001. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. Mov. Disord. **16**:448-458.
2. **Baruzzi, A., Contine, M., Albani, F. and Riva, R.** 1986. Simple and rapid micromethod for the determination of levodopa and 3-O-methyldopa in human plasma by high-performance liquid chromatography with coulometric detection. Journal of Chromatography **375**:165-169.
3. **Boomsma, F., Meerwaldt, J.D., Man in't Veld, A.J., Hovestadt, A. and Schalekamp, M.A.** 1989. Treatment of idiopathic parkinsonism with L-dopa in the absence and presence of decarboxylase inhibitors: effects on plasma levels of L-dopa, dopa decarboxylase, catecholamines and 3-O-methyl-dopa. J. Neurol. **236**:223-230.
4. **Caraceni, T., Scigliano, G. and Musicco, M.** 1991. The occurrence of motor fluctuations in parkinsonian patients treated long term with levodopa: role of early treatment and disease progression. Neurology **41**:380-384.
5. **Cedarbaum, J.M.** 1987. Clinical pharmacokinetics of anti-parkinsonian drugs. Clin. Pharmacokinet. **13**:141-178.
6. **Chapuis, S., Ouchchane, L., Metz, O., Gerbaud, L. and Durif, F.** 2005. Impact of the motor complications of Parkinson's disease on the quality of life. Mov. Disord. **20**:224-230.
7. **Defer, G.L., Widner, H., Marie, R.M., Remy, P. and Levivier, M.** 1999. Core assessment program for surgical interventional therapies in Parkinson's disease (CAPSIT-PD). Mov. Disord. **14**:572-584.
8. **Hughes, A.J., Daniel, S.E., Kilford, L. and Lees, A.J.** 1992. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. J. Neurol. Neurosurg. Psychiatry **55**:181-184.
9. **Katzenschlager, R., Head, J., Schrag, A., Ben-Shlomo, Y., Evans, A. and Lees, A.J.** 2008. Fourteen-year final report of the randomized PDRG-UK trial comparing three initial treatments in PD. Neurology **71**:474-480.
10. **Kuruma, I., Bartholini, G., Tissot, R. and Pletscher, A.** 1971. The metabolism of L-3-O-methyldopa, a precursor of dopa in man. Clin. Pharmacol. Ther. **12**:678-682.
11. **Lee, E.S., Chen, H., King, J. and Charlton, C.** 2008. The role of 3-O-methyldopa in the side effects of L-dopa. Neurochem. Res. **33**:401-411.
12. **LeWitt, P.A., Jennings, D., Lyons, K.E., Pahwa, R., Rabinowicz, A.L., Wang, J., Guarnieri, M., Hubble, J.P. and Murck, H.** 2009. Pharmacokinetic-pharmacodynamic crossover comparison of two levodopa extension strategies. Mov. Disord. **24**:1319-1324.
13. **Mizuno, Y., Kanazawa, I., Kuno, S., Yanagisawa, N., Yamamoto, M. and Kondo, T.** 2007. Placebo-controlled, double-blind dose-finding study of entacapone in fluctuating parkinsonian patients. Mov. Disord. **22**:75-80.
14. **Nutt, J.G.** 2000. Effect of COMT inhibition on the pharmacokinetics and pharmacodynamics of levodopa in parkinsonian patients. Neurology **55(11 Suppl 4)**:S33-37; discussion S38-41.
15. **Olanow, C.W., Agid Y., Mizuno, Y., Albanese, A., Bonuccelli, U., Damier, P., De Yebenes, J., Gershanik, O., Guttman, M., Grandas, F., Hallett, M., Hornykiewicz, O., Jenner, P., Katzenschlager, R., Langston, W. J., LeWitt, P., Melamed, E., Mena, M.A., Michel, P.P., Mytilineou, C., Obeso, J.A., Poewe, W., Quinn, N., Raisman-Vozari, R., Rajput, A.H., Rascol, O., Sampaio, C. and Stocchi, F.** 2004. Levodopa in the treatment of Parkinson's disease: current controversies. Mov. Disord. **19**:997-1005.
16. **Ruottinen, H.M. and Rinne, U.K.** 1996. A double-blind pharmacokinetic and clinical dose-response study of entacapone as an adjuvant to levodopa therapy in advanced Parkinson's disease. Clin. Neuropharmacol. **19**:283-296.

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ORIGINAL ARTICLE

Blood pressure variability and prognosis in acute ischemic stroke with vascular compression on the rostral ventrolateral medulla (RVLM)

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One of the known causes of hypertension is vascular compression on the rostral ventrolateral medulla (RVLM). However, it remains unknown whether RVLM vascular compression causes the significant variability in blood pressure observed during acute ischemic stroke. The purpose of this study was to evaluate differences in blood pressure variability and prognosis in acute ischemic stroke patients based on the presence or absence of RVLM vascular compression. We evaluated 56 patients with acute ischemic stroke. Blood pressure was measured every 6 h for 72 h after admission and evaluated with successive variation (SV). The presence of RVLM vascular compression was evaluated using time-of-flight 3D magnetic resonance imaging. Neurological severity was evaluated using the National Institutes of Health Stroke Scale (NIHSS) at admission and 14 days after admission, and clinical improvement was determined by taking the difference in the NIHSS scores between admission and at 14 days. Patient clinical outcome was evaluated with the modified Rankin scale on discharge. Vascular compression of the RVLM was identified in 15 patients (26.8%). The proportion of patients showing clinical improvement was significantly higher in the non-compression group (odds ratio, 0.21 (95% CI=0.06–0.78); P=0.01). The SV value for systolic blood pressure was significantly higher in the compression group (P<0.0001). We found that patients with RVLM vascular compression had a greater variability in blood pressure during the acute ischemic stroke phase, which may be related to poorer prognosis.
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Keywords: acute ischemic stroke; blood pressure; rostral ventrolateral medulla

INTRODUCTION

Regardless of nationality or ethnicity, hypertension is a lifestyle-related disease afflicting patients throughout the world and is a major risk factor for stroke. Hypertension is a multifactorial disease. It has been reported that a large population of patients with vascular compression on the rostral ventrolateral medulla (RVLM) have hypertension.[1–4] Surgical decompression of the RVLM reduced sympathetic nerve activity and normalized systemic blood pressure.[5,6] Therefore, it is suggested that vascular compression on the RVLM influences the development or maintenance of hypertension. The RVLM has been experimentally shown to be a site of cardiac and vasomotor regulation. The mechanism of increased blood pressure in patients with vascular compression on the RVLM remains to be completely elucidated. It is currently hypothesized that chronic stimulation of this region with vascular compression can cause constitutive activation of the sympathetic nervous system and the development of hypertension.[7,8]

More than 80% of acute stroke patients have elevated blood pressure. Several days following the incidence of stroke, however, the blood pressure in these patients returns to baseline levels.[9] This blood pressure elevation varies depending on the subtype of ischemic stroke and on the patient's medical history.[10] In general, the cause of elevated blood pressure during the acute phase of ischemic stroke is presumed to be an increase in sympathetic nerve activity and stress from the ischemic insult that disrupts intracerebral autoregulation to maintain cerebral blood flow.[11] During acute ischemic stroke, the rapid decrease in blood pressure reduces cerebral blood flow in parallel to a decrease in the perfusion pressure that is sufficiently large to expand infarct volumes and worsen neurologic symptoms. Therefore, such an excessive lowering of blood pressure during acute ischemic stroke is not desirable. Even in studies examining the correlation between blood pressure and prognosis in the acute ischemic stroke phase, the variability in blood pressure has been shown to be an independent prognostic factor for a poor outcome.[12,13]

RVLM vascular compression may cause significant variability in blood pressure during acute ischemic stroke by sympathetic nerve activation.[14] However, to our knowledge, it remains unclear whether RVLM vascular compression influences blood pressure during acute ischemic stroke. Therefore, the purpose of this study was to evaluate differences in blood pressure variability and prognosis during acute ischemic stroke in the presence or absence of RVLM vascular

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compression and to examine the influence of chronic sympathetic nerve activation on acute ischemic stroke.

METHODS

Subjects and study design

This study was conducted in consecutive patients with acute ischemic stroke admitted between April 2008 and March 2010 to Hiroshima University Hospital within 24 h of stroke onset. Exclusion criteria included the inability to undergo magnetic resonance imaging (MRI) examination, the administration of thrombolysis, an National Institutes of Health Stroke Scale (NIHSS) score $\geqslant 23$ on admission, the use of anti-hypertensive or vasopressor medicines within 72 h after admission, and surgery within 72 h after admission. In addition, we excluded patients with medullary infarctions, which might have effects on RVLM. The stroke subtype was determined based on MRI findings, electrocardiography, and carotid artery and cardiac ultrasound findings by at least two stroke specialists according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) classification.[15] The acute treatment was determined based on the stroke subtype in accordance with the established guidelines. Neurological severity was evaluated using the NIHSS upon admission and 14 days after admission.[16] Their clinical outcome was evaluated with the modified Rankin scale at the time of discharge.[17] The study was approved by the institutional review board of Hiroshima University Hospital.

Hypertension was defined as the use of any anti-hypertensive medicines before admission or a confirmed blood pressure of $\geqslant 140/90$ mm Hg at rest after 2 weeks from stroke onset. Diabetes mellitus was defined as HbA1c $\geqslant 6.5\%$, fasting blood sugar $\geqslant 126\,mg\,dl^{-1}$, or the use of any anti-diabetic medicines. Hyperlipidemia was defined as total cholesterol $\geqslant 220\,mg\,dl^{-1}$, low-density lipoprotein cholesterol $\geqslant 140\,mg\,dl^{-1}$ on admission, or the use of any anti-hyperlipidemic medications. For 72 h after admission, blood pressure and pulse rate were measured every 6 h on the unaffected arm in a resting supine position using a standard automated sphygmomanometer without any anti-hypertensive medicines. Blood pressure and pulse rate variability was evaluated with successive variation (SV), defined as the square root of the averaged squared differences between two successive measurements.[18]

The presence or absence of RVLM vascular compression was evaluated on a 3T MRI unit (GE, Fairfield, CT, USA) using 3D time-of-flight (slice thickness of 1.0 mm, TR/TE/flip angle, 23/3.4/18°). The RVLM was located at the root-entry zone of cranial nerves IX and X (Figure 1). The location was defined as follows: upper and lower borders of the root-entry zone were determined by the uppermost and lowest fibers of the IX/X nerve bundle entering the medulla; the anterior border of the root-entry zone was defined as the transition of the olivary convexity to the concavity of the retro-olivary sulcus; and the posterolateral border was located at the junction of parenchymal brain tissue and individual nerve fibers.[2] Arterial compression (vertebral artery or posterior-inferior cerebellar artery) within this defined area was considered 'positive' for RVLM, and patients with no arterial compression were considered 'negative.' We defined arterial compression as deformation of the medulla surface. Therefore, instances in which the artery just contacted the surface of the medulla were excluded from the criteria for RVLM vascular compression. The presence of RVLM vascular compression was evaluated by two neurologists who were unaware of each patient's medical history.

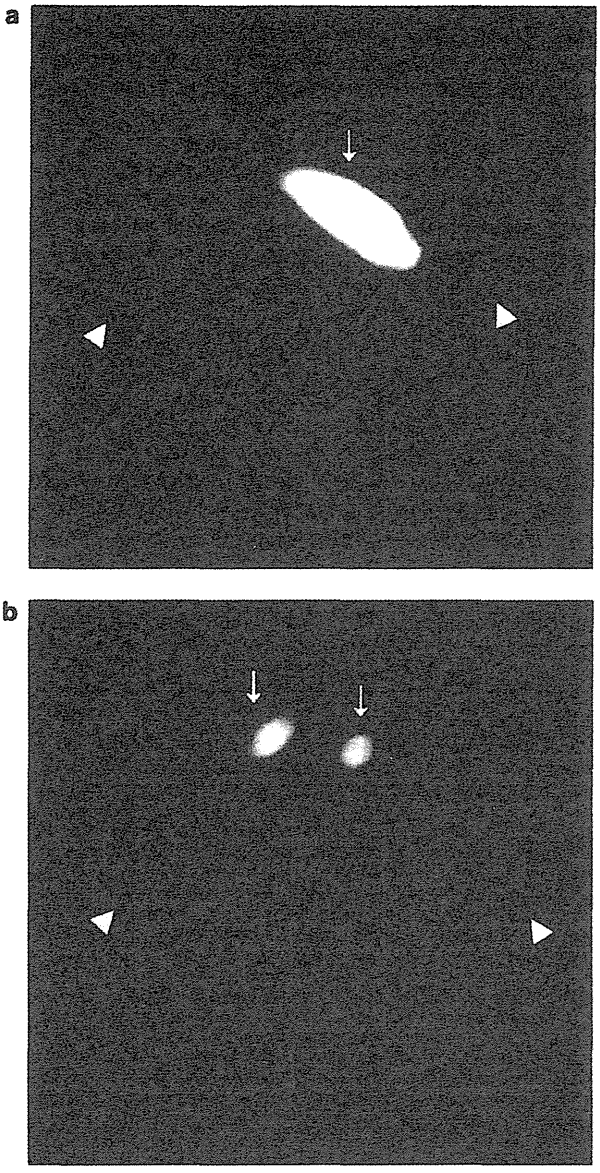

Figure 1 Axial 3D time-of-flight (TOF) images are shown (**a**) compression case, (**b**) non-compression case. The RVLM extends in a craniocaudal direction from the root entry zone of cranial nerve IX down to the upper part of cranial nerve XI. The RVLM is dorsolateral to the olive and medioventral to the root entry zone of cranial nerves IX and X (arrowhead). The compression of vertebral artery (arrow) or posterior-inferior cerebellar artery within this defined area was considered 'positive' for RVLM.

Statistical analysis

Data are shown as the mean ± s.d. or median (minimum-maximum) for continuous variables. Statistical analysis for comparison of the two groups was performed using Student's *t*-test or the Mann–Whitney *U*-test for continuous variables and the χ^2 test or Fisher's exact test for categorical variables. *P*-values <0.05 were considered statistically significant. Receiver operating characteristic curves were configured to establish cut-off points for SV that optimally predicted RVLM compression.

RESULTS

Of the 86 enrolled patients, 11 did not undergo MRI, 4 had missing data, 3 received thrombolysis, 4 had an NIHSS score $\geqslant 23$ on admission, 5 received anti-hypertensive or vasopressor medicines, and 3 had surgery within 48 h of hospital admission. Thus, all analyses were performed on the remaining 56 patients. MRI was performed at a mean time of 32.3 h after symptom onset (range, 4–50 h). Table 1 shows the baseline characteristics of all patients. Vascular compression on the RVLM was identified in 15 (26.8%) patients (hereafter, referred to as the compression group). Age was not significantly different between the patients with and without RVLM vascular compression. In the compression group, the proportion of males was significantly higher (93.3 *vs.* 63.4%, $P=0.03$). The prevalence of hypertension was significantly higher in the compression group (93.3 *vs.* 61.0%, $P=0.02$). At the time of admission, there was no significant difference

Table 1 Baseline characteristics, blood pressure and NIHSS on admission, and ischemic stroke subtypes

Variables	*Compression (n=15)*	*Non-compression (n=41)*	P-*value*
Age, mean years ± s.d.	71.6 ± 9.37	73.2 ± 9.5	0.59
Male, n (%)	14 (93.3%)	26 (63.4%)	0.03
Risk factors			
Hypertension, n (%)	14 (93.3%)	25 (61.0%)	0.02
Diabetes mellitus, n (%)	9 (60.0%)	17 (41.5%)	0.22
Hyperlipidemia, n (%)	7 (46.7%)	27 (65.9%)	0.19
Baseline NIHSS	4 (0–8)	4 (0–20)	0.29
Baseline SBP, mean mm Hg ± s.d.	159.1 ± 8.9	149.5 ± 26.1	0.17
Baseline DBP, mean mm Hg ± s.d.	81.1 ± 14.7	75.4 ± 15.2	0.88
Ischemic stroke subtype			
Atherothrombotic, n (%)	5 (33.3%)	13 (31.7%)	
Cardioembolic, n (%)	2 (13.3%)	17 (41.5%)	
Small vessel, n (%)	3 (20.0%)	5 (12.2%)	0.17
Other etiology, n (%)	5 (33.3%)	6 (14.6%)	

Abbreviations: DBP, diastolic blood pressure; NIHSS, National Institutes of Health Stroke Scale; SBP, systolic blood pressure.

Table 2 Relationship between RVLM vascular compression and clinical improvement at 14 days

	Improved (NIHSS ⩾4 improvement or NIHSS=0)	*Unimproved*	*OR (95% CI) probability*
Compression	4 (26.7%)	11 (73.3%)	0.21 (0.06–0.78)
Non-Compression	26 (63.4%)	15 (36.6%)	*P*=0.01

Abbreviations: CI, confidence interval; NIHSS, National Institutes of Health Stroke Scale; RVLM, rostral ventrolateral medulla.

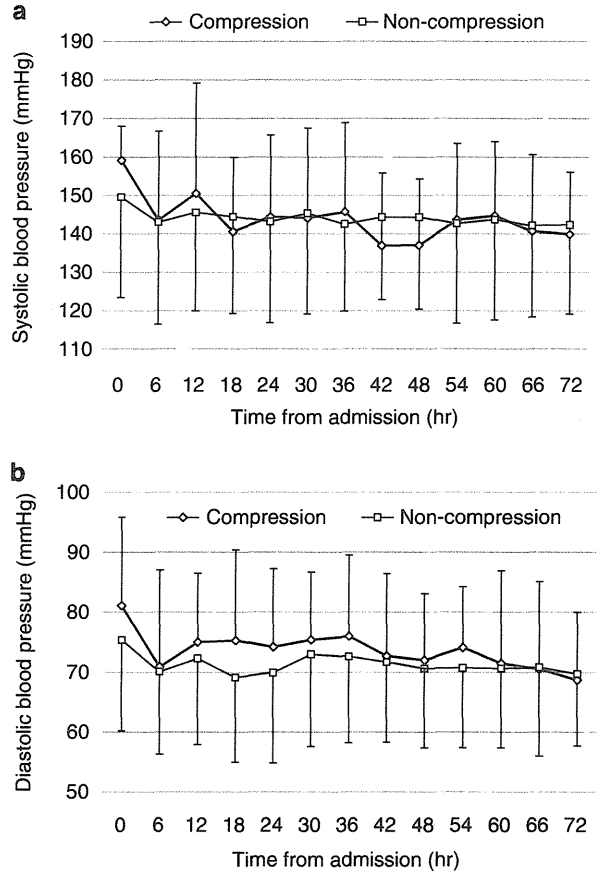

Figure 2 Average blood pressures during 72 h of hospitalization in the compression group (n=15) and the non-compression group (n=41). (**a**) Systolic blood pressure, (**b**) diastolic blood pressure.

in NIHSS scores, and no significant difference was observed in systolic or diastolic blood pressure between the groups. In addition, the subtype of ischemic stroke was not significantly different between the groups (P=0.17).

The patients were defined as clinically improved when their NIHSS score decreased more than four points or recovered to 0 on day 14 (ref. 19). The proportion of clinically improved patients was 26.7% in the compression group and 63.4% in the non-compression group (Table 2), with a significantly lower rate of improvement in the compression group (odds ratio, 0.21 (95% CI=0.06–0.78); P=0.01).

Figures 2 and 3 show the systolic and diastolic blood pressures and the SV values of the patients up to 72 h after admission. At all time points, no significant differences were observed in mean systolic or diastolic blood pressures between the two groups. However, the SV value of the systolic blood pressure was significantly higher in the compression group during the first 24 h after admission (P<0.0001) and during the 72 h period after admission (P<0.0001). Conversely, there were no significant differences in the diastolic blood pressure SV value between the groups at any time point. The pulse rate SV value was significantly higher in the compression group during the 72 h period after admission (8.7 ± 2.7 b.p.m. *vs.* 7.3 ± 2.2 b.p.m., P<0.05)

In the explanatory analysis, the best cutoff SV value of the systolic blood pressure during the 72 h period after admission obtained from the Receiver operating characteristic curve was 15.3 mm Hg, which predicted RVLM compression with a sensitivity of 67% and a specificity of 88% (area under the curve=0.783; P=0.0002). In the compression group, the patients with SV <15.3 mm Hg tended to show a more favorable outcome, defined using modified Rankin scale, than the patients with SV ⩾15.3 mm Hg (P=0.10) (Figure 4).

DISCUSSION

This study found that blood pressure variability during the acute ischemic stroke phase was significantly greater in patients with RVLM vascular compression than in those without. In addition, decreased improvement in neurologic symptoms was observed in patients with RVLM vascular compression compared with patients without RVLM vascular compression. In the compression group, patients with high SV values were more likely to have unfavorable outcomes. The association between blood pressure variability and acute ischemic stroke outcome has previously been reported.[12,13] To our knowledge, this study is the first to clearly show an association between RVLM vascular compression, blood pressure variability and functional prognosis.

It has been reported that a large proportion of patients with RVLM vascular compression have hypertension.[1–4] Mechanical stimulation of the RVLM causes glutamate release from RVLM vasomotor neurons, thereby increasing sympathetic nerve activity.[20] RVLM vascular compression was observed in 7–22.2% of healthy individuals without

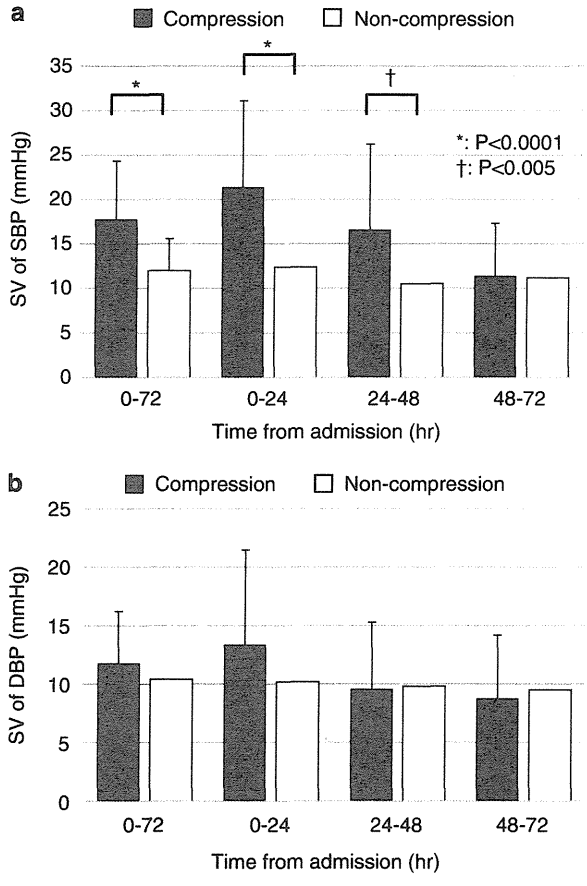

Figure 3 Successive variation (SV) of blood pressure during 72 h of hospitalization in the compression group (n=15) and the non-compression group (n=41). (**a**) Systolic blood pressure (SBP), (**b**) diastolic blood pressure (DBP).

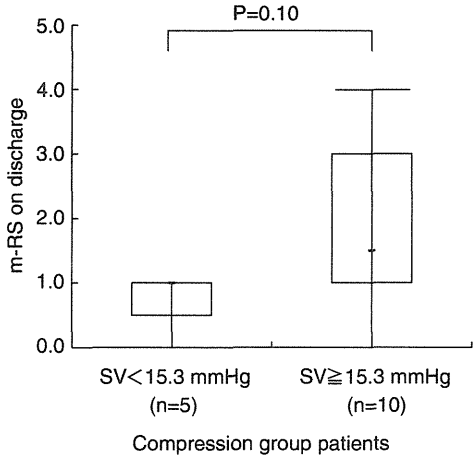

Figure 4 Box plot showing median modified Rankin scale (m-RS) on discharge (solid bar), interquartile range (bar width), and minimum/maximum values across SV < 15.3 mm Hg or ≥ 15.3 mm Hg in compression group.

hypertension and in 74–90% of patients with hypertension.[3,4,21,22] Our results demonstrated that the prevalence of hypertension was 93.3% (14/15) in patients with RVLM vascular compression, and it was higher than that in patients without RVLM vascular compression, which is consistent with previous studies. The prevalence of hypertension was 60.5% (25/41) in patients without RVLM vascular compression, which is similar to other previous population-based studies of ischemic stroke.[23,24] We propose that RVLM vascular compression is one of the important factors related to hypertension. However, it is still unknown whether RVLM vascular compression is the cause of hypertension because no study has prospectively followed young normotensive subjects with RVLM vascular compression to evaluate the incidence of hypertension. Conversely, several reports have demonstrated that surgical decompression of the RVLM reduced sympathetic nerve activity and normalized systemic blood pressure.[5,6] In addition, in our study, the patient with RVLM vascular compression who did not show hypertension (n=1) had higher blood pressure variability than patients without RVLM vascular compression and had a poor prognosis on discharge. In the other target organs (the heart), it has been reported that the regression of left ventricular hypertrophy depends on not only blood pressure levels, but also the reduction of cardiac sympathetic drive.[25] Therefore, we think that chronic sympathetic nerve activation influences target organ damage even when patients are not hypertensive.

In the present study, the compression group had a significantly higher SV value and a significantly lower proportion of clinically improved patients. In the compression group, the sympathetic nerve may be chronically activated. Hypertension is likely one of the markers of this chronic sympathetic nerve activation, which explains the significantly higher rate of hypertension in the compression group. In patients with chronic hypertension, cerebral blood flow decreases rapidly with cerebral ischemia in association with an increasing cerebral oxygen extraction fraction.[26] Therefore, blood pressure variability may change cerebral blood flow. This pathological response may deteriorate the improvement of neurological symptoms in patients with RVLM vascular compression.

Our findings demonstrated that the difference in blood pressure between the groups was not significant, although it tended to be slightly higher in the compression group. Interestingly, the variability in blood pressure was significantly greater in the compression group. The sympathetic nervous system is generally persistently activated during acute ischemic stroke, leading to increased blood pressure regardless of the presence or absence of RVLM vascular compression. Therefore, it is difficult to detect a difference in blood pressure with or without RVLM vascular compression during the acute ischemic stroke phase. By contrast, RVLM vascular compression clearly activates the sympathetic nervous system, leading to increased burst-like firing of sympathetic nerves,[27] which is thought to have a role in blood pressure variability.

It has been reported that patients with RVLM vascular compression have increased sympathetic nerve activity.[8] In addition, the increased sympathetic nerve activity may induce high blood pressure variability because it was reduced after ganglion blockade with trimethaphan.[28] In parallel to this response, when sympathetic nerve activity increases, the baroreflex counteracts this activation in normal subjects. However, it is still unknown whether the increase in sympathetic nerve activity is caused by increased sympathetic nerve traffic, increased vascular sensitivity, or impaired baroreflex buffering in patients with RVLM vascular compression. It has been reported that baroreflex buffering and baroreflex-mediated vasopressin release are severely impaired in patients with RVLM vascular compression.[7] When the impaired baroreflex is associated with RVLM vascular compression, blood pressure variability may increase with increased sympathetic nerve activity. It has been suggested that restoration of baroreflex sensitivity may prevent stroke in the animal model.[29] It is possible that the

restoration of baroreflex sensitivity reduced blood pressure variability and, therefore, stroke incidence. However, blood pressure variability was not examined in the experiment by Liu *et al.* Therefore, it is unknown whether stroke prevention with the restoration of baroreflex sensitivity was mediated by the reduction of blood pressure variability. In our study, we evaluated the association of blood pressure variability at the acute ischemic stroke phase and its prognosis for patients with or without RVLM vascular compression. However, we were unable to evaluate blood pressure variability at the pre-ischemic stroke period. Therefore, further studies are needed to clarify the association between blood pressure variability and the occurrence of stroke in patients with RVLM vascular compression.

Various measures of variability of individual blood pressure profiles have been used. The most common measures of variations are the extreme values, such as maximum, minimum, range (difference between maximum and minimum), s.d., or coefficient of variation (s.d. over mean).[30] In the present study, we selected SV as a parameter of within-patient blood pressure variability. This parameter includes the serial variation on a time sequence, whereas other measures, such as s.d. and coefficient of variation, ignore the sequential nature of such a data set. Therefore, when analyzing the sequential nature of data, as in the present study, it is better to use SV. If not, time-invariant measures, such as s.d. or coefficient of variation, can result in a misleading prediction of prognosis and is less informative for blood pressure management.

In the present study, we evaluated RVLM vascular compression with MRI images. However, it is possible that cases in which there appeared to be RVLM vascular compression had a low degree of compression that did not result in sympathetic nerve activation. Thus, we examined the association between SV and prognosis in the compression group, which was divided into two groups using the best cutoff SV value of the systolic blood pressure during the 72 h period after admission. As a result, the patients with high SV values tended to show more unfavorable prognosis than the patients with low SV values. Therefore, large-scale studies are required to confirm the influence of the SV value on the compression group prognosis.

The present study has several limitations. First, a selection bias may exist because of the small sample size at a single institution. Therefore, it will be necessary to conduct a similar multi-center study to confirm whether these results can be generalized. In addition, our results may not reflect all ischemic stroke patients because of the frequent exclusion of severe cases. It is highly possible that the severe cases have poor prognoses regardless of the presence of RVLM vascular compression or that the influence of RVLM vascular compression might be low. To study these issues, we are planning a multi-center, large-scale trial to define the association of RVLM vascular compression with the prognosis of ischemic stroke patients. Another limitation of the current study is that we did not examine the correlation between RVLM vascular compression and SV value with the use of anti-hypertensive medications before stroke onset. In particular, medications that inhibit sympathetic nerve activation may influence blood pressure and blood pressure variability. However, the half-lives of these anti-hypertensive medications are not more than 24 h, and their effects decrease with time. In our study, the SV value for blood pressure variability was calculated at 72 h after admission. Therefore, pre-medication with anti-hypertensives should have had a limited effect on the SV value. Finally, the correlation with ischemic location was not fully investigated. A previous study demonstrated that patients with infarctions involving the insular cortex tended to suffer from autonomic dysfunction,[31] which may influence blood pressure. However, in the present study, few patients had infarctions involving the insular cortex, and the proportion of patients with infarctions involving the insular cortex was similar between the compression and non-compression groups (two in the compression group (13.3%) and five in the non-compression group (12.2%)). Thus, the influence of ischemic location in the present study may also be limited.

In conclusion, this study found that patients with RVLM vascular compression had greater variability in blood pressure during the acute ischemic stroke phase, which may be a factor related to poorer prognosis. In the future, larger scale prospective studies are required to confirm the influence of RVLM vascular compression in acute ischemic stroke.

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1 Jannetta PJ, Segal R, Wolfson Jr SK. Neurogenic hypertension: etiology and surgical treatment. I. Observations in 53 patients. *Ann Surg* 1985; **201**: 391–398.
2 Naraghi R, Gaab MR, Walter GF, Kleineberg B. Arterial hypertension and neurovascular compression at the ventrolateral medulla. A comparative microanatomical and pathological study. *J Neurosurg* 1992; **77**: 103–112.
3 Naraghi R, Geiger H, Crnac J, Huk W, Fahlbusch R, Engels G, Luft FC. Posterior fossa neurovascular anomalies in essential hypertension. *Lancet* 1994; **344**: 1466–1470.
4 Morimoto S, Sasaki S, Miki S, Kawa T, Itoh H, Nakata T, Takeda K, Nakagawa M, Kizu O, Furuya S, Naruse S, Maeda T. Neurovascular compression of the rostral ventrolateral medulla related to essential hypertension. *Hypertension* 1997; **30**: 77–82.
5 Frank H, Schobel HP, Heusser K, Geiger H, Fahlbusch R, Naraghi R. Long-term results after microvascular decompression in essential hypertension. *Stroke* 2001; **32**: 2950–2955.
6 Frank H, Heusser K, Geiger H, Fahlbusch R, Naraghi R, Schobel HP. Temporary reduction of blood pressure and sympathetic nerve activity in hypertensive patients after microvascular decompression. *Stroke* 2009; **40**: 47–51.
7 Jordan J, Toka HR, Heusser K, Toka O, Shannon JR, Tank J, Diedrich A, Stabroth C, Stoffels M, Naraghi R, Oelkers W, Schuster H, Schobel HP, Haller H, Luft FC. Severely impaired baroreflex-buffering in patients with monogenic hypertension and neurovascular contact. *Circulation* 2000; **102**: 2611–2618.
8 Sendeski MM, Consolim-Colombo FM, Leite CC, Rubira MC, Lessa P, Krieger EM. Increased sympathetic nerve activity correlates with neurovascular compression at the rostral ventrolateral medulla. *Hypertension* 2006; **47**: 988–995.
9 Bath P, Chalmers J, Powers W, Beilin L, Davis S, Lenfant C, Mancia G, Neal B, Whitworth J, Zanchetti A. International society of hypertension (ISH): statement on the management of blood pressure in acute stroke. *J Hypertens* 2003; **21**: 665–672.
10 Toyoda K, Okada Y, Fujimoto S, Hagiwara N, Nakachi K, Kitazono T, Ibayashi S, Iida M. Blood pressure changes during the initial week after different subtypes of ischemic stroke. *Stroke* 2006; **37**: 2637–2639.
11 Shiokawa O, Sadoshima S, Fujii K, Yao H, Fujishima M. Impairment of cerebellar blood flow autoregulation during cerebral ischemia in spontaneously hypertensive rats. *Stroke* 1988; **19**: 615–622.
12 Stead LG, Gilmore RM, Vedula KC, Weaver AL, Decker WW, Brown Jr RD. Impact of acute blood pressure variability on ischemic stroke outcome. *Neurology* 2006; **66**: 1878–1881.
13 Yong M, Kaste M. Association of characteristics of blood pressure profiles and stroke outcomes in the ECASS-II trial. *Stroke* 2008; **39**: 366–372.
14 Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S, Piccaluga E, Turiel M, Baselli G, Cerutti S, Malliani A. Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circ Res* 1986; **59**: 178–193.
15 Adams Jr HP, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, Marsh III EE. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. *Stroke* 1993; **24**: 35–41.
16 Brott T, Adams Jr HP, Olinger CP, Marler JR, Barsan WG, Biller J, Spilker J, Holleran R, Eberle R, Hertzberg V, Rorick M, Moomaw CJ, Walker M. Measurements of acute cerebral infarction: a clinical examination scale. *Stroke* 1989; **20**: 864–870.
17 van Swieten JC, Koudstaal PJ, Visser MC, Schouten HJ, van Gijn J. Interobserver agreement for the assessment of handicap in stroke patients. *Stroke* 1988; **19**: 604–607.
18 Schachinger H, Langewitz W, Schmieder RE, Ruddel H. Comparison of parameters for assessing blood pressure and heart rate variability from non-invasive twenty-four-hour blood pressure monitoring. *J Hypertens* 1989; **7** (suppl 3): S81–S84.
19 National Institute of Neurological Disorders, Stroke rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med* 1995; **333**: 1581–1587.

20 Morimoto S, Sasaki S, Miki S, Kawa T, Nakamura K, Ichida T, Itoh H, Nakata T, Takeda K, Nakagawa M, Yamada H. Pressor response to compression of the ventrolateral medulla mediated by glutamate receptors. *Hypertension* 1999; **33**: 1207–1213.
21 Akimura T, Furutani Y, Jimi Y, Saito K, Kashiwagi S, Kato S, Ito H. Essential hypertension and neurovascular compression at the ventrolateral medulla oblongata: MR evaluation. *AJNR Am J Neuroradiol* 1995; **16**: 401–405.
22 Pierpaolo L, Mastronardi L, Strano S, Di Biasi C, Trasimeni G. Neurovascular conflict and essential arterial hypertension: MR evaluation. *AJNR Am J Neuroradiol* 1996; **17**: 195–196.
23 Petty GW, Brown Jr RD, Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO. Ischemic stroke subtypes: a population-based study of incidence and risk factors. *Stroke* 1999; **30**: 2513–2516.
24 Kolominsky-Rabas PL, Weber M, Gefeller O, Neundoerfer B, Heuschmann PU. Epidemiology of ischemic stroke subtypes according to TOAST criteria: incidence, recurrence, and long-term survival in ischemic stroke subtypes: a population-based study. *Stroke* 2001; **32**: 2735–2740.
25 Morgan HE, Baker KM. Cardiac hypertrophy. Mechanical, neural, and endocrine dependence. *Circulation* 1991; **83**: 13–25.
26 Temma T, Kuge Y, Sano K, Kamihashi J, Obokata N, Kawashima H, Magata Y, Saji H. PET O-15 cerebral blood flow and metabolism after acute stroke in spontaneously hypertensive rats. *Brain Res* 2008; **1212**: 18–24.
27 Schobel HP, Frank H, Naraghi R, Geiger H, Titz E, Heusser K. Hypertension in patients with neurovascular compression is associated with increased central sympathetic outflow. *J Am Soc Nephrol* 2002; **13**: 35–41.
28 Zhang R, Iwasaki K, Zuckerman JH, Behbehani K, Crandall CG, Levine BD. Mechanism of blood pressure and R-R variability: insights from ganglion blockade in humans. *J Physiol* 2002; **543**: 337–348.
29 Liu AJ, Ma XJ, Shen FM, Liu JG, Chen H, Su DF. Arterial baroreflex: a novel target for preventing stroke in rat hypertension. *Stroke* 2007; **38**: 1916–1923.
30 Rothwell PM, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, Sever PS, Poulter NR. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *Lancet* 2010; **375**: 895–905.
31 Tokgozoglu SL, Batur MK, Top uoglu MA, Saribas O, Kes S, Oto A. Effects of stroke localization on cardiac autonomic balance and sudden death. *Stroke* 1999; **30**: 1307–1311.

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Cerebral Microbleeds Predict Impending Intracranial Hemorrhage in Infective Endocarditis

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Key Words
Cerebral microbleeds · Infective endocarditis · Intracranial hemorrhage · MRI

Abstract
Background: Cerebral microbleeds (CMBs) detected by T2*-weighted MRI are a potential indicator of hypertension, microvascular disease and hemorrhagic stroke. An association between infective endocarditis (IE) and CMBs has been reported recently, but the clinical significance remains unclear. We hypothesized that CMBs in patients with IE are associated with vascular vulnerabilities such as mycotic aneurysm or pyogenic vasculitis. ***Methods:*** We retrospectively reviewed 26 consecutive patients with definite IE who underwent T2*-weighted MRI and were admitted to 2 medical centers in Osaka, Japan, between January 2006 and June 2010. We examined the incidence of symptomatic intracranial hemorrhage (ICH) occurring after initial MRI examination and investigated the association between ICH, CMBs and other clinical characteristics. ***Results:*** CMBs were identified in 14 patients (54%), and 72% of CMBs were found in the lobar region. Symptomatic ICH was observed in 8 patients (31%) during the 3-month follow-up period after initial MRI examination. In multiple logistic regression analyses, the presence of preceding ICH [odds ratio (OR) 40.0, 95% confidence interval (CI) 2.5–2,870] and the presence of CMBs (OR 34.0, 95% CI 1.3–17,300) were independent predictors of the development of ICH. Using cutoff values for CMBs of ≥2 and ≥3, the adjusted ORs for ICH increased (OR 42.1, 95% CI 1.9–24,300, and OR 70.1, 95% CI 2.5–105,000, respectively). ***Conclusions:*** In addition to prior ICH, the presence of CMBs was a strong predictor of impending ICH in patients with IE. CMBs might represent vascular vulnerability related to IE.

Introduction

Patients with infective endocarditis (IE) often experience cerebrovascular complications, and this confers a worse prognosis. Intracranial hemorrhage (ICH) occurs in approximately 2–10% of patients with IE [1–4]. In patients with IE and ICH, the prognosis is very poor [1, 3], and cardiac surgery should be delayed [5, 6].

Recent studies have reported a high frequency of asymptomatic cerebral microbleeds (CMBs) in patients with IE [2, 7, 8]. CMBs detected by T2*-weighted MRI may serve as markers of hypertensive vasculopathy, cerebral amyloid angiopathy and hemorrhagic transformation after thrombolysis [9–11], and as a predictor of multiple simultaneous ICHs [12]. However, the clinical sig-

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nificance of CMBs in patients with IE remains unclear. We hypothesized that CMBs in patients with IE are associated with vascular vulnerabilities such as mycotic aneurysm or pyogenic vasculitis. We wished to assess the risk of ICH in patients presenting with IE, and we further examined the possible association between ICH and CMBs.

Materials and Methods

Patient Selection and MRI Protocol

We conducted a retrospective, descriptive study at 2 medical centers in Osaka, Japan (Osaka University Hospital and Osaka National Hospital). Patients were classified as having IE if they met the modified Duke criteria for definite endocarditis [13]. Between January 2006 and June 2010, 44 consecutive patients were hospitalized for IE. Brain CTs were routinely performed in all patients with IE on admission. Brain MRI including gradient echo T2*-weighted sequences was also performed on admission in those patients who had neurological symptoms (n = 13) or an asymptomatic abnormal finding on initial CT (n = 3), were 60 years old or above (n = 8) or were judged to be at high risk of stroke by attending physicians (n = 2). Consequently, these 26 patients who underwent MRI on admission were included in this study. Baseline clinical information was systematically extracted from patient medical records.

The study outcome was the presence of symptomatic ICH 3 months after MRI. Patients were followed for 3 months, and subsequent brain CT or MRI was performed when the patient presented a new neurological symptom. Patients were diagnosed with ICH if the subsequent CT or MRI showed a new parenchymal hematoma or subarachnoid hemorrhage. The 18 patients without initial MRI were also followed for 3 months to investigate the incidence of symptomatic ICH.

MRI examinations were performed with a 1.5-tesla MRI system with gradient echo T2*- (repetition time/echo time 617/20 ms, flip angle 25°, matrix 256 × 256), diffusion-, T1-, T2- and T2 fluid-attenuated inversion recovery-weighted sequences using a 5-mm slice thickness and a 1-mm slice gap. MRI images were independently assessed by 2 of 3 neurologists (S.O., M.S. and B.H.) blinded to patient characteristics. CMBs were defined as homogeneous, round foci <10 mm diameter, of low signal intensity on T2*-weighted MRI. Identified CMBs were categorized by cerebral hemisphere and location (lobar, deep or infratentorial). Differences in analysis were resolved by consensus. Additionally, other abnormal MRI findings were collected, including (1) cerebral infarction, (2) parenchymal hematoma, (3) subarachnoid hemorrhage, (4) abscess and (5) aneurysm.

Statistical Analyses

Statistical analyses were performed using the software JMP 8.02 (SAS Institute Inc., Cary, N.C., USA). Interobserver agreement was evaluated using the simple κ coefficient. Fisher's exact test was used to compare qualitative variables, and the Mann-Whitney U test was applied to compare quantitative variables. Associations with $p < 0.20$ in the univariate analyses were included in multivariate logistic regression analyses. Statistical significance was defined as $p < 0.05$.

Table 1. Clinical characteristics of patients with IE with and without ICH

	ICH (+) (n = 8)		ICH (–) (n = 18)		p value
Age, years	51	(35–72)	65	(56–73)	0.182
Males	5	(63)	13	(72)	0.667
Risk factors					
Hypertension	0		5	(28)	0.281
Diabetes mellitus	0		2	(8)	1.000
Atrial fibrillation	1	(13)	6	(33)	0.375
Congenital heart diseases	2	(25)	0		0.086
Prosthetic valves	1	(13)	0		0.308
Bacteria					0.694
Staphylococcus sp.	3	(38)	5	(28)	
Streptococcus sp.	3	(38)	10	(56)	
Others	2	(25)	3	(17)	
Treatments					
Cardiac operation	5	(63)	12	(67)	1.000
Anticoagulant	1	(13)	2	(11)	1.000
Antiplatelet	1	(13)	3	(17)	1.000
β-Blocker	1	(13)	3	(17)	1.000
Statins	0		2	(11)	1.000
ACEI/ARB	2	(25)	7	(39)	0.667
Steroid/immunosuppressant	0		3	(17)	0.529
Antibiotics					
Penicillin derivative	7	(87)	14	(78)	1.000
Cephem derivative	1	(13)	4	(22)	1.000
Gentamicin	4	(50)	12	(67)	0.665
MRI findings					
Preceding cerebral infarction	5	(63)	10	(56)	1.000
Preceding ICH	5	(63)	2	(11)	0.014
Aneurysm	0		2	(11)	1.000
Microbleed	7	(88)	7	(39)	0.036
Lobar region	7	(88)	5	(28)	0.009
Deep region	4	(50)	2	(11)	0.051
Infratentorial region	6	(75)	2	(11)	0.003

Age is shown as median and interquartile range in parentheses. Other values are numbers of patients and percentages in parentheses. ACEI = Angiotensin-converting enzyme inhibitor; ARB = angiotensin II receptor blocker.

Results

Among 26 patients, interobserver agreement regarding the presence of CMBs had a κ coefficient of 0.85. Other MRI findings also demonstrated high interobserver agreement, with κ coefficients of 0.92 and 0.80 for preceding cerebral infarction and ICH, respectively. Table 1 shows patient characteristics. An initial brain MRI indicated IE-related lesions in 16 patients, namely cerebral infarction in