0013-7227/08/$15.00/0
Printed in U.S.A.

Endocrinology 149(4):1646–1653

doi: 10.1210/en.2007-1021

Adiponectin Antagonizes Stimulatory Effect of Tumor Necrosis Factor-α on Vascular Smooth Muscle Cell Calcification: Regulation of Growth Arrest-Specific Gene 6-Mediated Survival Pathway by Adenosine 5′-Monophosphate-Activated Protein Kinase

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Adiponectin exhibits diverse protective effects against atherogenesis and antagonizes many effects of TNFα. Here, we investigated the effect of adiponectin and TNFα on vascular calcification, a critical event in the development and progression of vascular disease. In human aortic smooth muscle cells (HASMC), TNFα augmented inorganic phosphate (Pi)-induced calcification, whereas adiponectin significantly suppressed it and abolished the stimulatory effect of TNFα in a concentration-dependent manner. Similarly, adiponectin ameliorated the accelerating effect of TNFα on Pi-induced apoptosis, the essential process of HASMC calcification. Furthermore, these effects of TNFα and adiponectin were associated with AMP-activated protein kinase (AMPK)-dependent growth arrest-specific gene 6 (Gas6) expression and Akt signaling. The AMPK activator, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), induced phosphorylation of AMPK and significantly inhibited Pi-induced calcification in HASMC. Conversely, pharmacological inhibition of AMPK by compound C blocked both AMPK activation and the inhibitory effect of adiponectin on calcification, providing evidence that AMPK plays a regulatory role in vascular calcification. Reporter assay revealed that adiponectin restored Gas6 promoter activity decreased by TNFα, and the effect of adiponectin was abrogated by compound C. These results demonstrate that adiponectin antagonizes the stimulatory effect of TNFα on vascular calcification by restoration of the AMPK-dependent Gas6-mediated survival pathway. (*Endocrinology* 149: 1646–1653, 2008)

VASCULAR CALCIFICATION is often encountered in advanced atherosclerotic lesions and is a common consequence of aging (1, 2). Calcification of the coronary arteries has been shown to be positively correlated with atherosclerotic plaque burden, increased risk of myocardial infarction, and plaque instability (3–5). We recently demonstrated that apoptosis plays an important role in inorganic phosphate (Pi)-induced vascular smooth muscle cell (VSMC) calcification (6). This type of calcification is dependent on down-regulation of the growth arrest-specific gene 6 (Gas6)-mediated survival pathway.

Adiponectin is an adipocyte-derived cytokine that exhibits protective properties in the heart and blood vessels (7–10). It accumulates in injured arteries from plasma and suppresses the endothelial inflammatory response (11) and VSMC proliferation (12). Furthermore, low plasma adiponectin levels are associated with progression of coronary artery calcification in type 1 diabetic and nondiabetic subjects, independent of other cardiovascular risk factors (13). Experimental studies have shown that adiponectin reduces TNFα production in response to various stresses, whereas TNFα attenuates adiponectin production, resulting in a reduction of plasma adiponectin levels (14–16). In addition to the inverse relationship between their expression, increasing evidence supports suppressive effects on each other's function (11, 17, 18). Given the importance of the reciprocal effects of TNFα and adiponectin, it is not clear whether both play a regulatory role in VSMC calcification.

Most of the beneficial actions of adiponectin are accounted for by the activation of AMP-activated protein kinase (AMPK) (19, 20). AMPK is a serine/threonine protein kinase that plays a key role in metabolic homeostasis in all eukaryotic cell types (21). Cardioprotective effects of adiponectin, including antiapoptotic actions, are also likely to be dependent on AMPK (19, 22, 23). However, the role of AMPK in the effect of adiponectin on VSMC calcification has not been addressed.

In the present study, we investigated whether adiponectin and TNFα modulate Pi-induced VSMC calcification by regulating apoptosis. We found that TNFα had a stimulatory effect, whereas adiponectin had an inhibitory effect on Pi-induced apoptosis and calcification in human aortic smooth muscle cells (HASMC). Furthermore, these actions were mediated by regulation of Gas6 at the transcription level via AMPK activation.

First Published Online January 3, 2008

Abbreviations: AICAR, 5-Aminoimidazole-4-carboxamide ribonucleoside; AMPK, AMP-activated protein kinase; Gas6, growth arrest-specific gene 6; HASMC, human aortic smooth muscle cells; Pi, inorganic phosphate; PP2C, protein phosphatase 2C; siRNA, small interfering RNA; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling; VSMC, vascular smooth muscle cells.

Endocrinology is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.

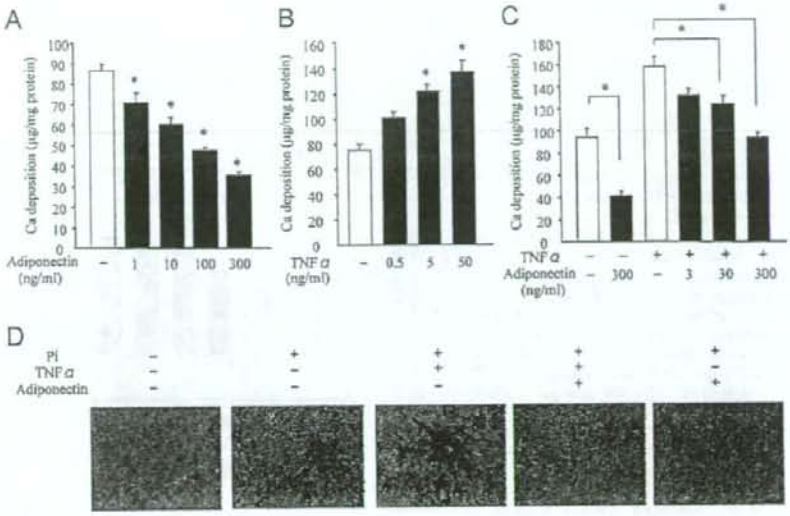

FIG. 1. Effect of adiponectin and TNFα on Pi-induced calcification. A and B, HASMC were cultured with the indicated concentrations of adiponectin (A) or TNFα (B) in calcification medium. They were added simultaneously when the medium was changed every 2 d. C, The effect of TNFα (20 ng/ml) and adiponectin with the indicated concentrations on Ca deposition was determined at 6 d. D, The effect of TNFα (20 ng/ml) and adiponectin (300 ng/ml) on Ca deposition was evaluated with von Kossa's staining at the light microscopic level. All values are presented as mean ± SE (n = 6). *, $P < 0.05$ by Bonferroni test. Each experiment was performed at least in triplicate for each condition.

Materials and Methods

Cell culture

HASMC were purchased from Clonetics Corp. (San Diego, CA). They were cultured in DMEM supplemented with 20% FBS, 100 U/ml penicillin, and 100 mg/ml streptomycin at 37 C in a humidified atmosphere with 5% CO_2. HASMC were used up to passage 8 for the experiments.

Induction and quantification of calcification

For Pi-induced calcification, Pi (a mixed solution of Na_2HPO_4 and NaH_2PO_4 whose pH was adjusted to 7.4) was added to serum-supplemented DMEM to a final concentration of 2.6 mM (calcification medium). Ca deposition was evaluated by the *o*-cresolphthalein complexone method (C-Test; WAKO, Osaka, Japan) and von Kossa's staining, as previously described (6, 24).

Determination of apoptosis

To examine the effect of TNFα (Sigma-Aldrich, St. Louis, MO) and adiponectin (R&D Systems, Minneapolis, MN) on Pi-induced apoptosis, they were added simultaneously when the medium was switched to the calcification medium. Apoptosis was detected by DNA fragmentation with a cell-death detection ELISA^{plus} kit (Roche, Manheim, Germany) and ter-

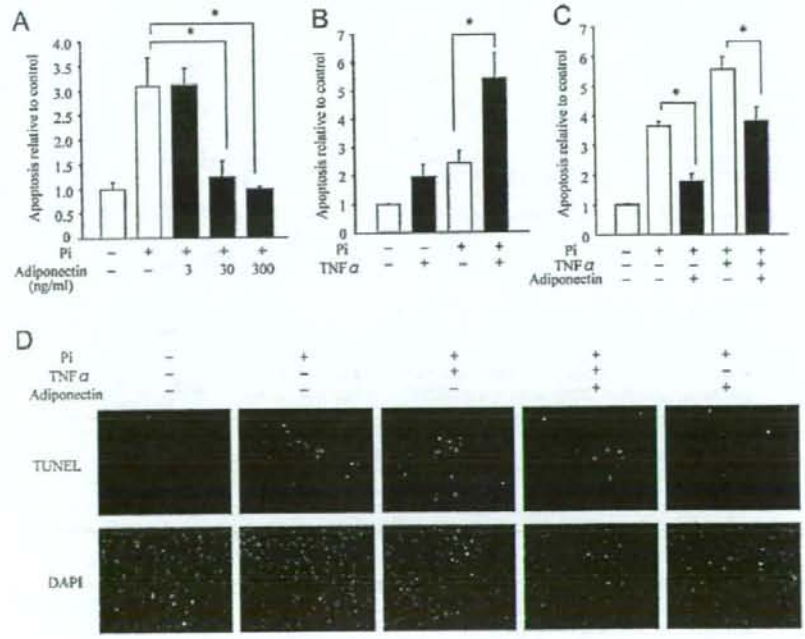

FIG. 2. Effect of adiponectin and TNFα on Pi-induced apoptosis. HASMC were cultured with the indicated concentrations of adiponectin for 6 d. Calcification medium was exchanged every 2 d. A, A quantitative index of apoptosis, determined by ELISA, is presented as the value relative to that without Pi treatment. B, HASMC were incubated with or without TNFα (20 ng/ml) in the absence or presence of 2.6 mM Pi for 6 d. C and D, On d 6, the effect of adiponectin (300 ng/ml) and TNFα (20 ng/ml) on apoptosis in calcification medium was determined by ELISA (C) and evaluated with TUNEL staining (D, *green*). Nuclei were counterstained with DAPI (*blue*). All values are presented as mean ± SE (n = 3). *, $P < 0.05$ by Bonferroni test. Each experiment was performed in triplicate for each condition.

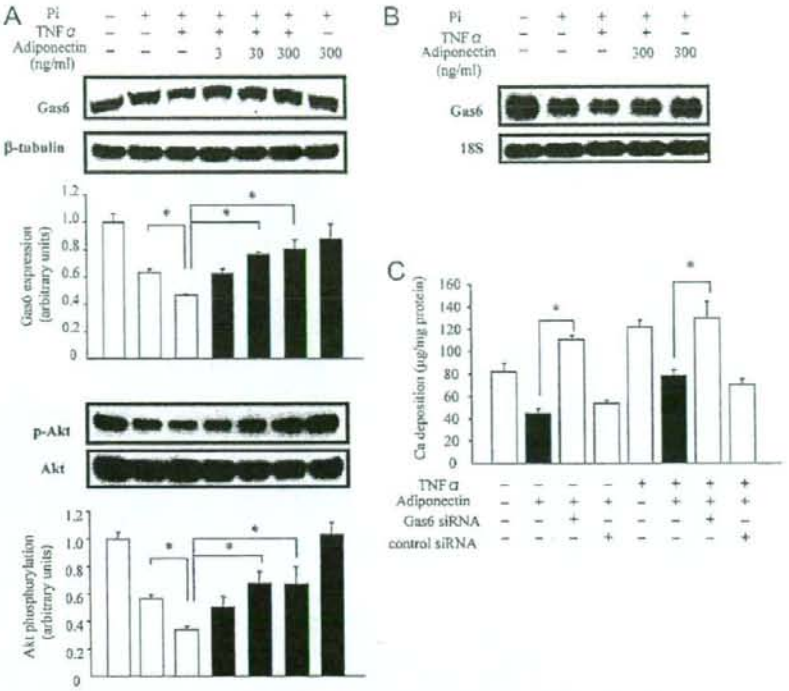

FIG. 3. Gas6 is the target of the effect of adiponectin and TNFα on Pi-induced calcification. HASMC were cultured with the indicated concentrations of adiponectin and TNFα (20 ng/ml). On d 6, cell lysates were collected and immunoblotted with antibodies that recognize Gas6, phospho-Akt (p-Akt), Akt, or β-tubulin. A, The untreated condition is the serum-supplemented status without Pi. B, Total RNA (5 μg) was harvested for Northern blot analysis after HASMC were incubated with adiponectin (300 ng/ml) and TNFα (20 ng/ml) for 6 d. When HASMC had reached 80–90% confluence, siRNA (100 nM) was transfected and then was transfected every 2 d with adiponectin (300 ng/ml) and TNFα (20 ng/ml) up to 6 d. C, Ca deposition was measured and normalized by cell protein content. All values are presented as mean ± SE (n = 3). *, $P < 0.05$ by Bonferroni test. Each experiment was performed in triplicate for each condition.

minal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) assay with ApopTag Plus obtained from Chemicon International, Ltd. (Hampshire, UK), according to the manufacturer's instructions.

Generation of promoter reporter construct and luciferase activity assay

The 1925-bp Gas6 promoter (−1827/+99) corresponding to the Gas6 promoter sequences was generated by PCR from human genomic DNA with the appropriate sets of primers (6). These inserts were cloned into a pGL3 basic vector (Promega, Charbonnières, France) by standard molecular biological techniques. The construct was verified by sequencing. HASMC were transiently transfected in 12-well plates with 0.8 μg plasmid DNA and lipofectamine 2000 (Invitrogen Corp., Paisley, UK) according to the procedure recommended by the manufacturer. Cells were treated with TNFα, adiponectin, and compound C at 24 h after transfection, followed by incubation for an additional 44 h. Firefly luciferase activity was determined using a luciferase assay system (Promega) and normalized by total cell protein.

Preparation of small interfering RNA (siRNA) targeting Gas6 and transfection

To evaluate the role of Gas6 in the inhibitory effect of adiponectin on calcification, we knocked down Gas6 using siRNA. Two kinds of siRNA were designed to target human Gas6 and nonspecific control siRNA was synthesized using standard templates (6). siRNA (100 nM) was transfected using transfection reagent (Upstate, Charlottesville, VA) when HASMC had reached 80–90% confluence and then was transfected every 2 d with TNFα and adiponectin up to 6 d. The efficiency of Gas6 siRNA was confirmed with immunoblotting (6).

RNA extraction and Northern blot analysis

Total RNA was extracted from HASMC using an RNeasy minikit (QIAGEN, Courtaboeuf, France). For Northern blot analysis, harvested RNA (5 μg) was fractionated on 1.4% formaldehyde-agarose gel and transferred to a nylon filter. The filter was hybridized at 68 C for 2 h with ^{32}P-labeled Gas6 cDNA (6) and an 18S probe in QuickHyb solution (Stratagene, La Jolla, CA) and autoradiographed.

Immunoblotting

The effect of TNFα and adiponectin on the expression of Gas6, phospho-Akt, and Akt was examined, as described previously (24). Analysis of AMPK activation was performed using an antibody specific for the phosphorylated Thr172 of AMPK (Cell Signaling Technology Inc., Beverly, MA).

Statistical analysis

All results are presented as mean ± SE. Statistical comparisons were made by ANOVA, followed by Bonferroni test. A value of $P < 0.05$ was considered statistically significant.

Results

Adiponectin and TNFα regulate Pi-induced calcification in HASMC

To investigate the effect of adiponectin and TNFα on Pi-induced calcification, HASMC were incubated with adiponectin and TNFα in the presence of 2.6 mM Pi. On d 6, Ca deposition was suppressed by adiponectin in a concentration-dependent manner (40 ± 2% of control at 300 ng/ml, Fig. 1A), whereas TNFα significantly augmented Ca deposition (182 ± 13% of control at 50 ng/ml; Fig. 1B). Furthermore, adiponectin clearly inhibited Ca deposition stimulated by TNFα in a concentration-dependent manner (Fig. 1C). This was also found by von Kossa's staining (Fig. 1D). These results suggest that adiponectin has an inhibitory effect on both Pi-induced and TNFα-stimulated calcification in HASMC.

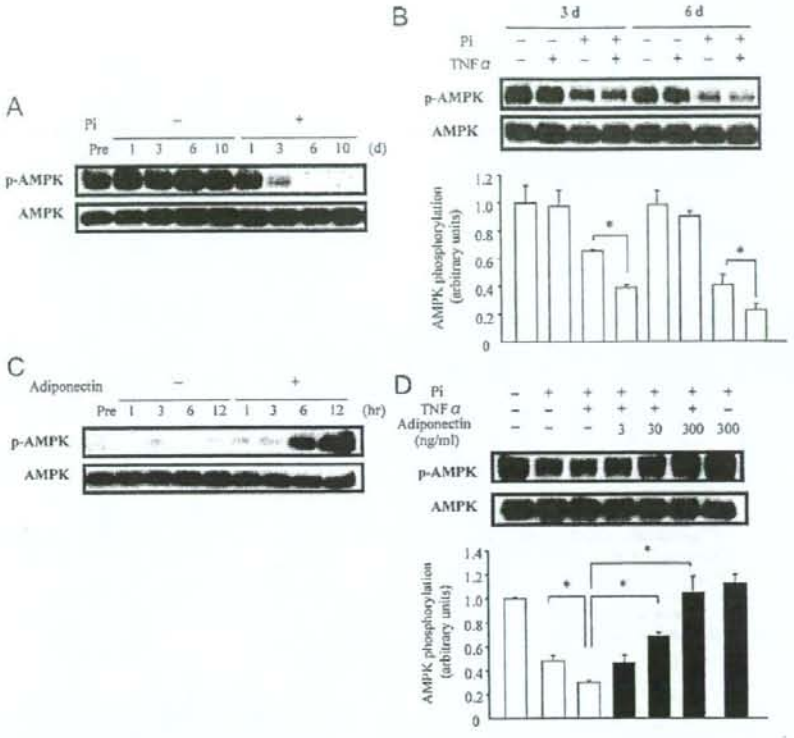

FIG. 4. Effect of adiponectin and TNFα on AMPK activity during Pi-induced calcification. HASMC were cultured in the absence or presence of Pi (2.6 mM) for up to 10 d. After the indicated incubation period, cell lysates were harvested and immunoblotted with antibodies to phospho-AMPK (p-AMPK) and AMPK. A, The untreated condition is the serum-supplemented status without Pi. B, Immunoblotting analysis showing the effect of TNFα (20 ng/ml) on p-AMPK and AMPK expression in the absence or presence of serum containing Pi (2.6 mM). C, Serum-starved HASMC were incubated with or without adiponectin (300 ng/ml) for 12 h. HASMC were cultured with the indicated concentrations of adiponectin and TNFα (20 ng/ml). D, On d 6, cell lysates were harvested and immunoblotted with antibodies to p-AMPK and AMPK. All values are presented as mean ± SE (n = 3). *, $P < 0.05$ by Bonferroni test. Each experiment was performed in triplicate for each condition.

Adiponectin antagonizes stimulatory effect of TNFα on Pi-induced apoptosis by restoration of Gas6-mediated survival pathway

Because apoptosis has been shown to be an important pathway regulating Pi-induced calcification (6, 24), we examined the effect of adiponectin and TNFα on apoptosis in HASMC. Adiponectin, at concentrations exerting inhibitory effects on calcification, significantly reduced apoptosis, as quantified by cytoplasmic histone-associated DNA fragments (Fig. 2A). On the other hand, apoptosis was enhanced by TNFα in the presence of Pi (Fig. 2B). As shown in Ca deposition, adiponectin antagonized the stimulatory effect of TNFα on apoptosis. This inhibition was also observed by TUNEL assay (Fig. 2, C and D).

We previously demonstrated that Pi-induced apoptosis was mediated by down-regulation of the Gas6-mediated survival pathway (6, 24). Therefore, we examined the effects of adiponectin and TNFα on this pathway. Both Gas6 mRNA and protein expression were down-regulated by TNFα in the presence of Pi, whereas adiponectin clearly restored their expression (Fig. 3, A and B). Next, because the Gas6-mediated survival pathway is Akt-dependent, the effect of adiponectin and TNFα on Akt phosphorylation was examined. As shown in the Gas6 expression, the similar effect of adiponectin and TNFα was observed in Akt phosphorylation that is high at basal level in the untreated condition containing serum (Fig. 3A). We confirmed that total Akt was not changed by adiponectin and TNFα treatment (Fig. 3A). On the other hand, adiponectin and TNFα did not affect Gas6 expression and Akt phosphorylation in the condition without Pi treatment (data not shown).

Furthermore, to evaluate the role of Gas6 in the inhibitory effect of adiponectin on calcification, we examined whether the knockdown of Gas6 abrogated the effects of adiponectin using siRNA. On d 6, transfection of Gas6 siRNA markedly decreased its expression (data not shown), as reported previously (6). The inhibitory effect of adiponectin on Pi- and TNFα-induced calcification was reversed by Gas6 siRNA, supporting the critical role of Gas6 in the effect of adiponectin on calcification (Fig. 3C).

AMPK plays a critical role in VSMC calcification and is regulated by adiponectin and TNFα

It has been reported that AMPK is a central signaling molecule in adiponectin's action (19, 20). We investigated whether AMPK is involved in the effect of adiponectin on Pi-induced calcification. First, we examined the activity of AMPK during calcification. Immunoblot analysis showed that phosphorylated AMPK was markedly down-regulated in the presence of Pi for 10 d, whereas the expression of total AMPK was not changed (Fig. 4A). TNFα further inhibited its phosphorylation in the presence of Pi, without changing total AMPK (Fig. 4B). In the case of adiponectin, AMPK phosphorylation was remarkably stimulated in a time-dependent manner (Fig. 4C). As shown in Fig. 4D, adiponectin further restored AMPK phos-

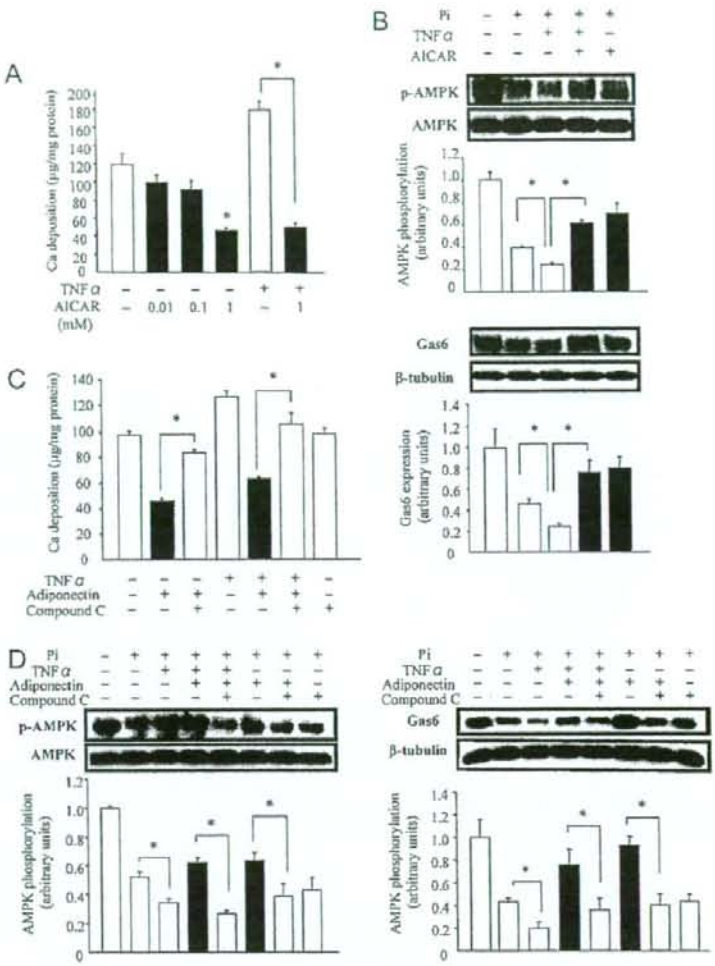

FIG. 5. AMPK plays an important role in Pi-induced calcification. HASMC were treated with or without AICAR (1 mM), a pharmacological activator of AMPK and TNFα (20 ng/ml) in calcification medium for 6 d. A and B, Ca deposition (n = 6) (A) was measured, and immunoblotting with antibodies to p-AMPK, AMPK, Gas6, and β-tubulin (B) was performed (n = 3). HASMC were cultured with or without compound C (1 μM), a chemical inhibitor of AMPK, adiponectin (300 ng/ml), and TNFα (20 ng/ml) in calcification medium for 6 d. C and D, Ca deposition (C) was evaluated (n = 6), and immunoblotting with antibodies to p-AMPK, AMPK, Gas6, and β-tubulin (D) was performed (n = 3). All values are presented as mean ± SE. *, $P < 0.05$ by Bonferroni test. Each experiment was performed in triplicate for each condition.

phorylation that was inhibited by Pi and TNFα in a calcification-promoting condition.

To clarify the causal relationship between AMPK and calcification, we tried to activate AMPK by treatment with 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR) (25). In HASMC, AICAR significantly inhibited Ca deposition in a concentration-dependent manner (Fig. 5A). In addition, TNFα-stimulated Ca deposition was also blunted by AICAR. Interestingly, AICAR restored Gas6 expression down-regulated by Pi and TNFα (Fig. 5B). Next, to investigate whether the effect of adiponectin is dependent on AMPK, we tried to block AMPK using compound C, a chemical inhibitor of AMPK. As shown in Fig. 5C, compound C clearly abrogated the inhibitory effect of adiponectin both on Pi- and TNFα-induced calcification. The increase in Gas6 expression as well as AMPK phosphorylation by adiponectin was also abolished by compound C (Fig. 5D). These results suggest that AMPK regulates Gas6 expression, followed by regulation of Ca deposition in HASMC.

Transcription activity of Gas6 is regulated by adiponectin and TNFα via AMPK

To investigate whether Gas6 expression is transcriptionally regulated by adiponectin, TNFα, and AMPK, a promoter study was undertaken. Reporter assay using the −1.9-kb Gas6-luciferase DNA construct revealed that adiponectin completely reversed the down-regulation of Gas6 transcription activity by TNFα. Furthermore, compound C abrogated the effect of adiponectin on Gas6 transcription activity, indicating that adiponectin and TNFα regulate Gas6 expression at the transcription level via AMPK activity (Fig. 6).

Discussion

The present study showed that adiponectin has a protective effect against Pi-induced calcification and, furthermore,

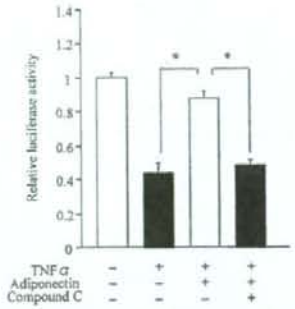

FIG. 6. Effect of adiponectin and TNFα on Gas6 promoter activity. HASMC were transfected with the Gas6 promoter-luciferase construct using lipofectamine 2000. Twenty-four hours after transfection, adiponectin (300 ng/ml), compound C (1 μM), and TNFα (20 ng/ml) were added. Cells were incubated for an additional 44 h. Luciferase activity was normalized to that of vehicle-treated cells. All values are presented as mean ± SE (n = 4). *, $P < 0.05$ by Bonferroni test. Each experiment was performed in triplicate for each condition.

has an antagonistic effect on TNFα-augmented calcification. Based on our previous finding that Pi-induced calcification is dependent on apoptotic cell death in HASMC, we examined the role of adiponectin and TNFα in Pi-induced apoptosis. As expected, we found that adiponectin had an inhibitory effect and TNFα had a stimulatory effect on Pi-induced apoptosis. This study also demonstrated the

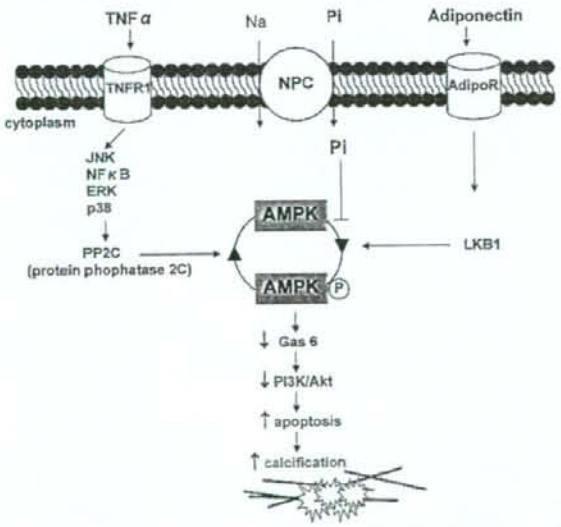

FIG. 7. Scheme of the effect of TNFα and adiponectin on Pi-induced calcification. In HASMC, exogenous Pi is internalized by sodium-dependent phosphate cotransporter (NPC, such as Pit-1) and inhibits AMPK phosphorylation, followed by down-regulation of the Gas6-mediated survival pathway. This pathway stimulates apoptosis, leading to subsequent development of calcification. TNFα directly suppresses AMPK activation by promoting PP2C activation via TNF receptor-1 (TNFR1). On the other hand, adiponectin activates LKB1-AMPK pathway via adiponectin receptors (AdipoR). AMPK activation modulated by TNFα and adiponectin contributes to the regulation of Pi-induced calcification.

regulation of Gas6 expression by TNFα and adiponectin, a suppressive effect and a promoting effect, respectively, at the transcriptional level. Akt, a critical downstream effecter of Gas6, was activated by adiponectin, whereas TNFα had an opposite action on its phosphorylation. Given that adiponectin and TNFα did not affect Gas6 expression and Akt phosphorylation in the absence of Pi (data not shown), the effects of adiponectin and TNFα on these molecules may dependent on Pi-induced responses. These results suggest that Gas6 is the target of adiponectin and TNFα in regulating Pi-induced apoptosis, accompanied by modulation of the Akt-dependent survival pathway.

As reported previously (6), Pi-induced VSMC calcification is associated with both phenotypic transition to osteoblastic cells via sodium-dependent phosphate cotransporter and apoptotic cell death. In our preliminary experiments, the expression of osteopontin, an osteoblastic marker, was not affected by TNFα and adiponectin (data not shown). Although this result suggests little influence of TNFα and adiponectin on osteoblastic differentiation of VSMC, extensive and systematic investigation including other markers of osteoblastic differentiation is needed to conclude this issue.

Multiple lines of clinical evidence show that adiponectin has protective actions on the cardiovascular system (26, 27). Circulating levels of adiponectin in humans are as high as 500–30,000 μg/ml (28). Therefore, the concentration of adiponectin (300 ng/ml) used in this study are within physiological levels. Especially, consistent with our findings, adiponectin has been implicated in apoptosis of cardiovascular cells (19, 23, 29). Adiponectin inhibits apoptosis in cardiac myocytes and fibroblasts that are exposed to hypoxia-reoxygenation stress (19). In endothelial cells, adiponectin has been reported to inhibit serum starvation-induced apoptosis (23). *In vivo* experiments have also shown that adiponectin-deficient mice develop larger myocardial infarcts due to increased myocardial cell apoptosis and TNFα expression (17). Taking these observations together with our results, the antiapoptotic actions of adiponectin contribute to the inhibition of VSMC calcification.

Most effects of adiponectin have been attributed to the activation of AMPK, which affects many aspects of cellular metabolism including glucose uptake (30, 31), glucose utilization (32), and fatty acid oxidation (33, 34). Recently, AMPK activation in VSMC has been suggested as a target to prevent or treat vascular disease (35, 36). AICAR-induced AMPK activation inhibited angiotensin II-stimulated VSMC proliferation, and administration of AICAR prevented neointimal formation in a rat balloon injury model (35). AMPK activation in VSMC elicited cell cycle arrest at the G1 phase and inhibited cell proliferation via p53 up-regulation (36). Furthermore, in the heart, the inhibitory effects of adiponectin on ischemic injury-induced apoptosis have been shown to be dependent on AMPK activation (19). The results of *in vitro* studies also revealed that AMPK signaling is essential for the antiapoptotic activities of adiponectin on endothelial cells (23). These observations are consistent with the finding of the present study that AMPK activated by adiponectin stimulated Gas6 expression to restore the survival pathway, leading to the suppression of calcification.

In the present study, we further demonstrated that adiponectin significantly augmented the transcriptional activity of Gas6

that was decreased by TNFα. Indeed, suppression of AMPK by compound C clearly abrogated this beneficial effect of adiponectin. This result suggests that AMPK participates in the transcriptional regulation of Gas6 by adiponectin and TNFα. Several studies support that AMPK regulates the expression of particular genes at the transcriptional level (37–39). For example, AMPK activation by AICAR enhanced activator protein 1-mediated proopiomelanocortin promoter activities, which were completely abolished by compound C (37). AMPK has been shown to mediate the transcription signal that leads to the repression of phosphoenolpyruvate carboxykinase expression, a key enzyme of gluconeogenesis, through phosphorylation of a transcription factor, AICAR-responsive element binding protein (38). It has also been observed that AICAR treatment is able to reduce nuclear factor-κB-regulated transcription, which is activated by TNFα (39).

Consistent with our findings, it has been recently reported that TNFα directly suppresses AMPK activation by promoting protein phosphatase 2C (PP2C) activity via TNF receptor-1 (40). PP2C has been proposed as one of modulators of the covalent regulation of AMPK (41). Increased PP2C levels account for the reduced AMPK activity and phosphorylation after TNFα treatment (40). On the other hand, LKB1 [also known as serine/threonine kinase II (STK II)] is the well-known, principal upstream kinase of AMPK (42, 43) that is regulated by adiponectin (44). AMPK activation by adiponectin is considered to be mediated by the cell surface receptors adiponectin receptors 1 and 2 (45). Another adiponectin receptor, T-cadherin, has recently been identified (46). In preliminary experiments, we found that all of the three adiponectin receptors were endogenously expressed in HASMC, and Pi did not affect their expression (data not shown). Taking these observations together, we hypothesized the mechanism of regulation by adiponectin and TNFα on Pi-induced vascular calcification (Fig. 7). However, further intensive investigations are required to elucidate the role of each player in VSMC calcification.

In summary, adiponectin inhibited VSMC calcification and antagonized the stimulatory effect of TNFα. This action was caused by preventing apoptosis via AMPK activation, followed by restoration of the Gas6-mediated survival pathway. AMPK regulated Gas6 expression at the transcriptional level. AMPK activation regulated by adiponectin and TNFα in vascular calcification might be a key to the management of cardiovascular disease.

Acknowledgments

We thank Yuki Ito for technical assistance.

Received July 25, 2007. Accepted December 26, 2007.

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This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (No. 18590801 and No. 19590854), Novartis Foundation for Gerontological Research, Kanzawa Medical Research Foundation, Ono Medical Research Foundation, and Takeda Research Foundation.

Disclosure Statement: The authors have nothing to disclose.

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Endocrinology **is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Geriatr Gerontol Int 2008; 8: 93–100

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

White matter lesions as a feature of cognitive impairment, low vitality and other symptoms of geriatric syndrome in the elderly

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Aim: White matter lesions (WML) are common findings on magnetic resonance imaging (MRI) in elderly persons. In this study, we analyzed the relation of WML with global cognitive function, depression, vitality/volition, and 19 symptoms of geriatric syndrome in Japanese elderly patients who attended three university geriatric outpatient clinics.

Methods: Two hundred and eighty-six subjects (103 men and 183 women; mean ± standard deviation age, 74.5 ± 7.8 years) were included in this study. MRI scans were performed for the diagnosis of WML, and the severity of periventricular and deep white matter hyperintensities (PVH and DWMH) was rated semiquantitatively. Concurrently, all subjects underwent tests of cognitive function, depressive state and vitality, and were examined for 19 symptoms of geriatric syndrome.

Results: The study subjects showed cognitive decline, depression and low vitality, all to a mild extent. Univariate linear regression analysis showed a negative correlation between the severity of WML and cognitive function or vitality. Multiple logistic analysis revealed that the severity of WML was a significant determinant of cognitive impairment and low vitality, after adjustment for confounding factors such as age, sex and concomitant diseases. PVH and/or DWMH score was significantly greater in subjects who exhibited 13 out of 19 symptoms of geriatric syndrome. Logistic regression analysis indicated that WML were associated with psychological disorders, gait disturbance, urinary problems and parkinsonism.

Conclusion: WML were associated with various symptoms of functional decline in older persons. Evaluating WML in relation to functional decline would be important for preventing disability in elderly people.

Keywords: deep white matter hyperintensity, geriatric syndrome, periventricular hyperintensity, white matter lesion.

Accepted for publication 10 December 2007.

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doi: 10.1111/j.1447-0594.2008.00454.x

Introduction

Brain magnetic resonance imaging (MRI) has markedly enhanced the chance of detecting characteristic hyperintense signals in the periventricular and subcortical areas on T2-weighted images, even in asymptomatic older persons.[1] These lesions are known as white matter lesions (WML), leukoaraiosis or white matter (periventricular and subcortical) hyperintensities.[2-4] WML, which accompany symptoms of gait abnormalities,[5-7] urinary symptoms[5,8,9] and cognitive impairment,[4,10,11] are reported to be associated with aging,[12-14] hypertension,[14] diabetes[15] and atherosclerosis.[5] There is poor understanding of the pathogenesis of the lesions, and it remains unknown whether WML are mere innocuous radiological changes that appear as a result of the aging process,[2,3,10] or whether they are one of the causal factors of the functional decline in elderly people.

Geriatric syndrome is a group of symptoms that are related to daily life, and the comorbidity triggers the loss of independence of elderly persons. Hence, evaluation of geriatric syndrome is important for the physical and mental care of the elderly. To address the pathological significance of WML in the global cognitive and psychological functions, and in geriatric syndrome in representative Japanese elderly subjects, we organized a group of geriatric outpatient clinics, and investigated the clinical manifestations of WML in those patients. Especially, we analyzed the relation of WML with global cognitive function, depressive state, vitality/volition and 19 symptoms of geriatric syndrome.

Methods

Subjects

This was a multicenter study performed at three different university geriatric outpatient clinics in Japan under the organization of a Longevity Science Research Grant from the Ministry of Health, Labor and Welfare of Japan (H15-Choju-013). Two hundred and eighty-six consecutive subjects (103 men and 183 women; mean ± standard deviation [SD] age, 74.5 ± 7.8 years) were included in this study: 187 at Kyorin University Hospital, 74 at Chiba University Hospital, and 25 at Nagoya University Hospital, from January 2004 to January 2005.

The diagnosis of dementia was made according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV). The definition of hypertension was systolic blood pressure (BP) of more than 140 mmHg or diastolic BP of more than 90 mmHg, or receiving antihypertensive drugs. The definition of diabetes was glycosylated hemoglobin A1c of more than 6.5%, or receiving antidiabetic drugs. The definition of hyperlipidemia was total cholesterol of more than 5.72 mmol/L, triglyceride of more than 1.70 mmol/L, or receiving antihyperlipidemic drugs.

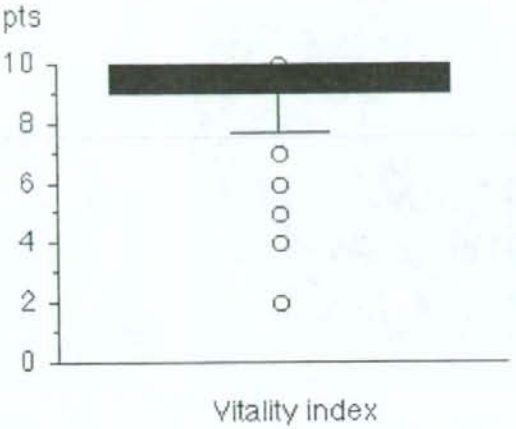

Figure 1 Distribution of vitality index. All subjects underwent assessment of vitality index as a measure of vitality related to activities of daily living (waking pattern, communication, feeding, getting on and off the toilet, rehabilitation and other activities; 2 points each; range, 0–10).

All subjects underwent the following assessment of global cognitive and psychological function. Cognitive function was evaluated by Mini-Mental State Examination (MMSE).[16] In this examination, we focused on calculation (serial subtraction of 7 from 100) to evaluate attention and working memory (part of the frontal lobe function). We also performed verbal fluency or word recollection test by asking the subjects to name as many vegetables as possible, which is also indicative of the frontal lobe function. Depression was evaluated by the 15-item Geriatric Depression Scale (GDS-15), which consists of 15 dichotomous questions for screening depressive symptoms in elderly subjects (range, 0–15).[17] Vitality index was used to measure vitality or volition in daily life (waking pattern, communication, feeding, getting on and off the toilet, rehabilitation and other activities; 2 points each; range, 0–10).[18] A full score can be maintained until one is severely disabled in cognition or function. The distribution of vitality index in the subjects of this study is shown in Figure 1.

We examined symptoms of geriatric syndrome: 19 dichotomous questions about hallucinations, delusions, insomnia, vertigo, paralysis, numbness, gait disturbance, tripping, falls, pollakiuria, urinary incontinence, constipation, decreased appetite, weight loss, apathy, speech impairment, swallowing difficulty, tremor and muscle stiffness.

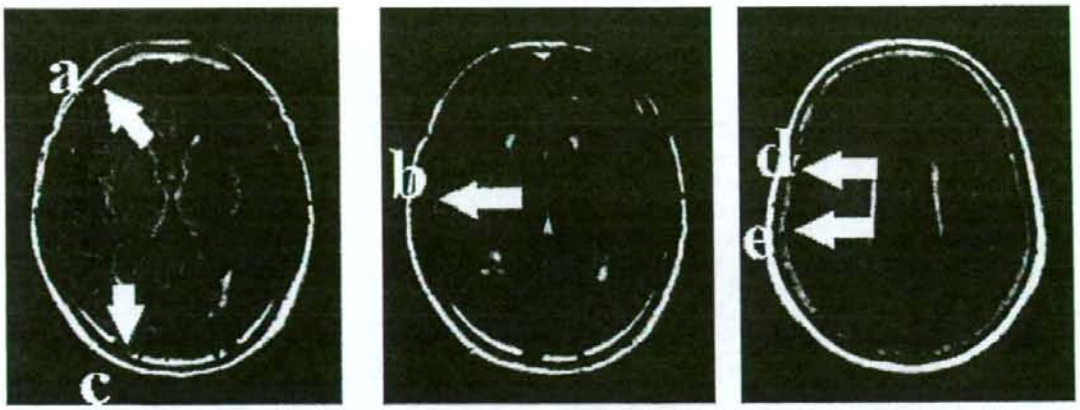

Figure 2 Evaluation of periventricular hyperintensity (PVH). PVH were evaluated in six regions in three slices: (a) adjacent to the frontal horns, (b) lateral ventricular body, (c) occipital horns, (d) frontal central semiovale in the parietal region and (e) occipital centrum semiovale in the parietal region in both hemispheres. Each area was rated as five grades according to the method of Junque *et al.*: (0) no hyperintensities; (1) <25% of the brain area; (2) 25–50%; (3) 50–75%; and (4) >75%.[11] The sum of all grades in the six regions was defined as the PVH score (range, 0–24).

Magnetic resonance imaging

Magnetic resonance imaging scans were performed for the diagnosis of WML and cerebral infarction on 1.5-T scanners (Toshiba, Nasu, Japan). T1-weighted images (repetition time [TR], 496 ms; echo time [TE], 12 ms), T2-weighted images (TR, 4280 ms; TE, 105 ms), and fluid-attenuated inversion-recovery (FLAIR)-weighted images (TR, 8000 ms; TE, 105 ms; 5-mm slice thickness) were obtained in the axial plane. MRI images were examined to differentiate between WML, characterized by isointense signals on T1-weighted images and hyperintense signals on T2-weighted and FLAIR images, and cerebral infarction, characterized by hypointense signals on T1-weighted images and hyperintense signals on T2-weighted and FLAIR images.

White matter lesions were classified as periventricular hyperintensities (PVH), which adjoined the lateral ventricle, and deep white matter hyperintensities (DWMH), located in the deep white matter apart from the lateral ventricles.

Periventricular and deep white matter hyperintensity scores

Periventricular hyperintensities were evaluated in six regions in three slices: adjacent to the frontal horns, lateral ventricular body, occipital horns, frontal central semiovale in the parietal region, and occipital centrum semiovale in the parietal region in both hemispheres (Fig. 2). Each area was rated as five grades according to the systematic quantification method developed by Junque *et al.*: (0) no hyperintensities; (1) less than 25% of the brain area; (2) 25–50%; (3) 50–75%; and (4) more than 75%.[11] The sum of all grades in the six regions was defined as the PVH score (range, 0–24).

Deep white matter hyperintensities were evaluated in the frontal, temporal, parietal and occipital lobes, and in the basal ganglia in both hemispheres (Fig. 3). Each lesion was rated as three grades according to the diameter by the study of de Groot *et al.*: (1) 1–3 mm; (2) 3–10 mm; and (3) more than 10 mm. The sum of all grades in five regions in both hemispheres was defined as the DWMH score.[4] Analysis was performed assuming that the white matter scores of PVH and DWMH were quantitative interval scales.

Statistical analysis

The relationship between two continuous variables such as MMSE, GDS-15 or vitality index, and WML (PVH or DWMH) score was analyzed by univariate linear regression analysis, and the correlation was analyzed by means of Pearson's simple correlation coefficients. Statistical significance was set at $P < 0.05$.

The relation of cognitive impairment or low vitality with PVH score or DWMH score was assessed by means of multivariate logistic regression analysis with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. Cognitive impairment and low vitality were defined as an MMSE score of 23 or less[19] and a vitality index of 9 or less, respectively. Odds ratios and 95% confidence interval were calculated from the, coefficients and their standard errors.

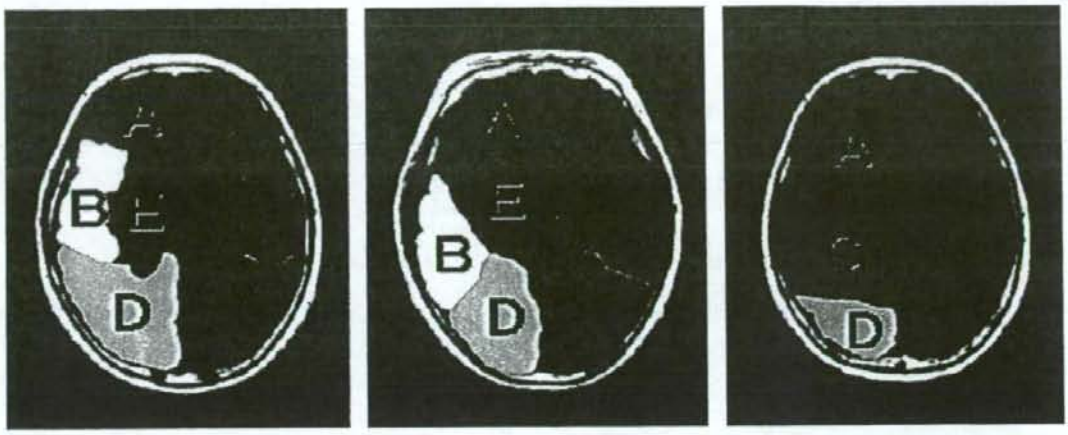

Figure 3 Evaluation of deep white matter hyperintensities (DWMH). DWMH were evaluated in the (A) frontal, (B) temporal, (C) parietal and (D) occipital lobes, and (E) in the basal ganglia in both hemispheres. Each lesion was rated as three grades according to diameter by the method of de Groot *et al.*: (1) 1–3 mm; (2) 3–10 mm; and (3) >10 mm.[4] The sum of all grades in five regions in both hemispheres was defined as the DWMH score.

Periventricular hyperintensity score or DWMH score was compared between subjects who did or did not exhibit each symptom of geriatric syndrome and analyzed by Student's *t*-test. When the difference was considered to be significant ($P < 0.05$), the difference was further assessed by means of multivariate logistic regression analysis with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease.

Ethical considerations

This study was approved by the ethical committees of the institutes involved in this project. We explained this study clearly, and obtained written consent from all participants and their guardians (mainly family members). All the data were stored and analyzed carefully to preserve the subjects' anonymity and protect their privacy.

Results

Clinical data

The clinical characteristics of the study subjects are shown in Table 1. The mean age of subjects was 74.5 ± 7.8 years (mean ± SD), and subjects aged 65 or older comprised 88.1%. The mean body mass index was 21.8 ± 3.3 kg/m^2 and none of the subjects were obese. Of the subjects, 10.1% had experienced stroke or other cerebrovascular disease and 22.7% were smokers. Hypertension, diabetes and hyperlipidemia were present in 50.7%, 27.3% and 50.0% of the subjects, respectively.

White matter lesions

Periventricular hyperintensities and DWMH were observed in 77.7% and 96.7% of the total subjects, respectively. The mean score of PVH and DWMH was 5.5 ± 4.8 and 35.5 ± 39.8, respectively (Table 1). Pearson's correlation analysis showed a strong positive correlation between PVH score and DWHM score ($r = 0.56$, $P < 0.0001$). In relation to aging, a positive correlation was found between PVH score and age ($r = 0.34$, $P < 0.0001$), and between DWMH score and age ($r = 0.28$, $P < 0.0001$).

Cognitive and psychological assessment

The mean score of MMSE, GDS-15 and vitality index was 23.1 ± 5.3, 5.0 ± 3.5 and 9.4 ± 1.2 points, respectively, indicating that the subjects showed cognitive decline, depression and decreased vitality, all to a mild extent. Given that a score of 23 or below on MMSE is regarded as the presence of cognitive impairment,[19] 47.5% of the subjects fell into this category. The causes of cognitive impairment were Alzheimer disease (AD; 53.3%), vascular dementia (VaD; 16.4%), combined dementia of AD and VaD (9.0%) and other types of dementia (21.3%). Pearson's correlation analysis revealed a negative correlation between PVH score and MMSE, PVH score and vitality index, DWMH score and MMSE, and DWMH score and vitality index,

Table 1 Clinical characteristics of study subjects

	Prevalence ($n = 286$)	Mean ± standard deviation
Clinical characteristics		
Age (years)		74.5 ± 7.8
Women (%)	74.0	
Height (m)		1.55 ± 0.08
Bodyweight (kg)		52.4 ± 10.6
Body mass index (kg/m^2)		21.8 ± 3.3
Systolic blood pressure (mmHg)		135.3 ± 20.2
Diastolic blood pressure (mmHg)		76.3 ± 11.8
Prevalence of complications		
Hypertension (%)	50.7	
Diabetes (%)	27.3	
Hyperlipidemia (%)	50.0	
Past history of cerebrovascular disease (%)	10.1	
Smoking (%)	22.7	
Cognitive and psychological assessment		
Mini-Mental State Examination (0–30 points)		23.1 ± 5.3
Geriatric depression scale (0–15 points)		5.0 ± 3.5
Vitality index (0–10 points)		9.4 ± 1.2
White matter lesions		
Periventricular hyperintensities (points)	5.5 ± 4.8	
Deep white matter hyperintensities (points)	35.5 ± 39.8	

Table 2 Relationship between white matter lesions and global cognition (MMSE), depressive state (GDS-15) and vitality (vitality index)

	Linear regression PVH score	DWMH score
MMSE	−0.380**	−0.272**
GDS-15	0.022	−0.066
Vitality index	−0.432**	−0.184*

Univariate linear regression analysis: *$P < 0.01$, **$P < 0.0001$. DVMH, deep white matter hyperintensity; GDS-15, 15-item Geriatric Depression Scale; MMSE, Mini-Mental State Examination; PVH, periventricular hyperintensity.

respectively (Table 2). It was also found that calculation (serial subtraction of 7 from 100) was negatively correlated with PVH score ($r = -0.156$, $P = 0.04$, data not shown), and verbal fluency (naming as many vegetables as possible) was negatively correlated with PVH score ($r = -0.216$, $P < 0.01$, data not shown). On the other hand, no significant correlation was found between PVH score and GDS-15, or between DWMH score and GDS-15. Multiple logistic analysis revealed that PVH score and DWMH score remained significant determinants of cognitive impairment (MMSE, ≤23) and low vitality (vitality index, ≤9) after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 3).

One hundred and ninety subjects reported symptoms of geriatric syndrome. The frequency is shown in Table 4. Frequent symptoms (>20%) were tripping (32.1%), constipation (26.3%), gait disturbance (23.2%) and pollakiuria (22.1%). Student's *t*-test showed that PVH score was significantly greater in subjects who exhibited the following symptoms of geriatric syndrome: hallucinations, delusions, gait disturbance, tripping, falls, pollakiuria, urinary incontinence, weight loss, apathy, swallowing difficulty, tremor and muscle stiffness. Multiple logistic analysis revealed that PVH score remained a significant determinant of hallucinations, tripping, pollakiuria, urinary incontinence, weight loss, apathy and swallowing difficulty after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 5). By the same method, DWMH score was

Table 3 Periventricular hyperintensity and deep white matter hyperintensity scores as determinants of cognitive impairment and low vitality

	PVH score			DWMH score		
	OR	95% CI	*P*-value	OR	95% CI	*P*-value
Cognitive impairment	1.185	1.084–1.295	<0.001	1.010	1.001–1.021	<0.05
Low vitality	1.260	1.133–1.401	<0.0001	1.025	1.012–1.039	<0.001

Cognitive impairment and low vitality were defined as MMSE ≤23 and vitality index ≤9, respectively. Multiple logistic analysis was performed after adjustment for age, sex, hypertension, diabetes, hyperlipidemia, and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; DVMH, deep white matter hyperintensity; OR, odds ratio; PVH, periventricular hyperintensity.

significantly greater in subjects who exhibited the following symptoms of geriatric syndrome: hallucinations, delusions, gait disturbance, tripping, falls, pollakiuria, urinary incontinence and constipation. Multiple logistic analysis revealed that DWMH score remained a significant determinant of hallucinations, delusions, tripping, urinary incontinence and constipation after adjustment for age, sex, presence of hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease (Table 6).

Discussion

Elderly persons are affected by multiple chronic diseases. Once they are affected by serious illness, full recovery cannot be expected with medical treatment, because elderly patients are often trapped in a vicious circle of illness and poor quality of life (QOL). This is the reason why care and welfare contribute to the total well-being of the elderly. Physicians need to pay great attention to improving QOL as well as treating illness. Thus, it is important to comprehend the whole picture of their life by means of comprehensive geriatric assessment, which evaluates multiple aspects of an elderly person's life, such as activities of daily living, cognition, mood, vitality, communication and social environment.

The present study confirmed a negative correlation between the severity of WML and MMSE score. Multivariate analysis showed that the presence of WML was a significant risk factor for cognitive impairment, even after adjustment for confounding factors of age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease. The mechanism and the size and location of WML that impair cognitive function are not yet clear. However, from previous studies, it seems convincing that a reduction of blood flow in the frontal lobe plays an important role in cognitive impairment in elderly people who exhibit WML.[20,21] Clinical manifestations of WML include attention deficit and a decline in information-processing ability.[4,13,22] Junque *et al.* reported the reappearance of primitive reflexes, one of the symptoms of frontal lobe dysfunction, in patients with WML.[11] In this study, patients with PVH showed attention deficit (incapability of calculation) and verbal inarticulacy (naming less vegetables), implying the impairment of frontal lobe function. WML, as reported previously,[6,23] were negatively correlated with vitality. Multiple logistic regression analysis, using potential risk factors including advanced age as confounding variables, found that the presence of WML was an independent risk factor for low vitality. Additionally, a relation between PVH score and apathy, a significant symptom of geriatric syndrome, was also found. From previous studies showing the importance of frontal lobe function in vitality,[24–26] we assume that blood flow reduction in the frontal lobe may account for the apathy and low vitality in patients with WML. More precisely, WML disrupting the frontal-subcortical circuit may result in dysfunction in the anterior cingulate and dorsolateral prefrontal circuits, thereby leading to apathy and decreased vitality.[5,6,20] Increase in PVH score or DWMH score was not apparently correlated with depression, probably because depression is associated with many factors such as aging, female sex, hyperlipidemia and medication.[27–29] The subjects in this study were mostly elderly (88.1%) and female (74.0%). We assume that these confounding conditions made it difficult to prove a true relation between WML and depression. From analysis of the association of WML with geriatric syndrome, it appears that WML have a relation to psychiatric symptoms (hallucinations and delusions), gait abnormalities (gait disturbance, tripping and falls), urinary symptoms (pollakiuria and urinary incontinence) and possibly with parkinsonism (swallowing difficulty, tremor and muscle stiffness). It was reported that WML were related to gait abnormalities,[5–7] presumably caused by disruption of the frontal-subcortical circuit.[30] Some other studies suggested that parkinsonism is also a contributing factor to gait disturbance in patients with WML.[6,31] Interestingly, we found that both gait abnormalities and symptoms of parkinsonism were associated with WML.

The present study confirmed an association between WML and voiding dysfunction (pollakiuria and incontinence). It was reported that urinary dysfunction was derived from damage to the frontal-subcortical

Table 4 Comparison of periventricular hyperintensity and deep white matter hyperintensity scores between subjects who did or did not exhibit each symptom of geriatric syndrome

Geriatric syndrome	Prevalence (%)	PVH score Symptom Present	Absent	*P*-value	DWMH score Symptom Present	Absent	*P*-value
Hallucination	6.8	**8.5 ± 5.9**	**4.4 ± 4.7**	**<0.01**	**59.8 ± 43.9**	**28.6 ± 35.4**	**<0.01**
Delusion	9.5	**7.6 ± 5.2**	**4.4 ± 4.8**	**0.01**	**56.1 ± 37.6**	**28.2 ± 35.9**	**<0.01**
Insomnia	18.9	4.2 ± 3.6	4.7 ± 4.9	0.56	31.4 ± 36.0	31.3 ± 37.6	0.98
Vertigo	18.9	6.1 ± 6.5	4.4 ± 4.4	0.06	33.4 ± 38.1	30.7 ± 37.0	0.70
Paralysis	2.1	8.5 ± 4.8	4.6 ± 4.9	0.12	59.5 ± 47.2	30.1 ± 36.3	0.11
Numbness	16.6	5.1 ± 4.6	4.6 ± 4.8	0.62	34.6 ± 40.0	29.9 ± 36,0	0.52
Gait disturbance	23.2	**6.7 ± 5.1**	**4.2 ± 4.7**	**<0.01**	**43.3 ± 41.7**	**27.5 ± 34.9**	**0.01**
Tripping	32.1	**6.4 ± 4.5**	**3.9 ± 4.9**	**<0.01**	**42.1 ± 43.7**	**25.9 ± 32.4**	**<0.01**
Falls	17.9	**6.6 ± 4.9**	**4.3 ± 4.8**	**0.01**	**45.8 ± 43.1**	**28.0 ± 35.0**	**0.01**
Pollakiuria	22.1	**8.0 ± 5.8**	**3.8 ± 4.2**	**<0.01**	**41.5 ± 41.0**	**41.5 ± 41.0**	**0.04**
Urinary incontinence	13.8	**7.5 ± 5.1**	**4.3 ± 4.8**	**<0.01**	**52.4 ± 44.9**	**52.4 ± 44.9**	**<0.01**
Constipation	26.3	5.8 ± 4.3	4.4 ± 5.1	0.08	**44.5 ± 45.1**	**44.5 ± 45.1**	**<0.01**
Decreased appetite	14.7	6.1 ± 4.4	4.5 ± 5.0	0.12	42.1 ± 42.6	42.1 ± 42.6	0.11
Weight loss	14.2	**6.9 ± 4.1**	**4.4 ± 5.0**	**0.01**	40.7 ± 41.3	40.7 ± 41.3	0.15
Apathy	7.6	**7.4 ± 3.6**	**4.4 ± 5.0**	**0.03**	30.7 ± 28.1	30.7 ± 28.1	0.97
Speech impairment	2.7	5.6 ± 5.2	4.5 ± 4.7	0.62	35.3 ± 48.0	35.3 ± 48.0	0.80
Swallowing difficulty	14.7	**12.2 ± 4.4**	**4.5 ± 4.8**	**<0.01**	44.6 ± 34.6	44.6 ± 34.6	0.40
Tremor	5.3	**9.1 ± 6.5**	**4.4 ± 4.7**	**<0.01**	45.0 ± 38.1	45.0 ± 38.1	0.24
Muscle stiffness	3.2	**9.2 ± 4.8**	**4.5 ± 4.9**	**0.02**	48.7 ± 43.4	48.7 ± 43.4	0.23

PVH and DWMH score are shown as mean ± SD. Boldface values are statistically significant ($P < 0.05$ by Student's *t*-test). DVMH, deep white matter hyperintensity; PVH, periventricular hyperintensity.

Table 5 Periventricular hyperintensity score as determinant of geriatric syndrome

	OR	*P*-value	95% CI
Hallucination	1.12	0.043	1.004–1.248
Tripping	1.11	0.005	1.032–1.194
Pollakiuria	1.17	0.001	1.067–1.278
Urinary incontinence	1.11	0.022	1.015–1.207
Weight loss	1.14	0.007	1.036–1.246
Apathy	1.14	0.027	1.015–1.276
Swallowing difficulty	1.35	0.019	1.050–1.741

Multiple logistic analysis was performed to analyze each symptom of geriatric syndrome, with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; OR, odds ratio.

Table 6 Deep white matter hyperintensity score as determinant of geriatric syndrome

	OR	*P*-value	95% CI
Hallucination	1.017	0.020	1.003–1.032
Delusion	1.016	0.024	1.002–1.030
Tripping	1.011	0.020	1.002–1.020
Urinary incontinence	1.016	0.008	1.004–1.028
Constipation	1.011	0.025	1.001–1.021

Multiple logistic analysis was performed to analyze each symptom of geriatric syndrome, with adjustment for age, sex, hypertension, diabetes, hyperlipidemia and past history of cerebrovascular disease, of which all variables other than age were treated as categorical data. CI, confidence interval; OR, odds ratio.

circuit.[5,20] In relation to the symptoms of parkinsonism (swallowing difficulty, tremor and muscle stiffness), this association was previously explained by dysfunction of the frontal-subcortical circuit.[6,31] The importance of this lesion was also suggested by a study showing that swallowing difficulty occurs with dysfunction of internuncial neurons that link the brainstem to the cerebral cortex.[32]

Considering the cause of manifestation of geriatric syndrome in patients with WML, it appears that damage to associative pathways in the frontal and subcortical regions due to ischemic hypoperfusion is an important mechanism.[5,20,21] It is necessary to localize the responsible connecting pathway for each symptom by a sophisticated approach in the future.

In conclusion, we showed that WML were associated with cognitive impairment, low vitality and geriatric syndrome of psychological disorders, gait disturbance,

urinary problems and parkinsonism. Evaluating WML in relation to geriatric syndrome and building a preventive measure against WML is an important future task for maintaining the independence of elderly people.

Acknowledgments

This study was supported by a Longevity Science Research Grant from the Ministry of Health, Labor and Welfare of Japan (H15-Choju-013) and by Mitsui Sumitomo Insurance Welfare Foundation (2004, 2006), and by the Japan Health Foundation. We thank Yukiko Yamada and Ayako Machida for their technical assistance.

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Geriatr Gerontol Int 2008; 8: 278–283

ORIGINAL ARTICLE: EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Stress-induced blood pressure elevation in subjects with mild cognitive impairment: Effects of the dual-type calcium channel blocker, cilnidipine

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Aim: We investigated whether mental stress-induced blood pressure elevation was related to cognitive function in the elderly, and further examined the effects of the dual-type calcium channel blocker, cilnidipine, on stress induced hypertension in subjects with mild cognitive impairment.

Methods: In study I, 39 consecutive outpatients (mean age ± standard deviation, 77 ± 8 years), who were referred to our memory clinic and were not taking any medications, were studied. They were divided into three groups according to cognitive function on the Hasegawa Dementia Scale-Revised (HDSR): group 1 ($n = 8$), 28 points or more; group 2 ($n = 18$), 21–27 points; and group 3 ($n = 13$), 20 points or less. In study II, 14 outpatients with hypertension and mild cognitive impairment (aged 79 ± 8 years; HDSR score, 24 ± 4) were assigned to receive cilnidipine (10–20 mg/day). The control group ($n = 10$) matched for age, HDSR and blood pressure was followed without cilnidipine.

Results: In study I, although age and basal blood pressure were similar among the three groups, the blood pressure response to a mental arithmetic test was twice as large in group 2 (26 ± 12 mmHg in systolic pressure and 11 ± 8 mmHg in diastolic pressure) as those in groups 1 and 3. In study II, after 4 weeks, cilnidipine treatment significantly decreased the blood pressure responses to the mental arithmetic test compared to the baseline as well as to those of the control group.

Conclusions: Stress-induced blood pressure elevations are exaggerated in subjects with mild cognitive impairment. Cilnidipine may have inhibitory effects on stress-induced hypertension.

Keywords: calcium antagonists, dementia, hypertension, mental stress.

Introduction

Mental stress-induced increases in blood pressure (BP) and heart rate are often experienced during daily living. Psychophysiological cardiovascular reactivity is caused by autonomic nervous system activation via the hypothalamus–pituitary–adrenal axis,[1–3] and is

Accepted for publication 1 July 2008.

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 doi: 10.1111/j.1447-0594.2008.00482.x

modulated by individual characteristics, environmental exposures, interpersonal and social contexts and genetic factors.[4–7] It has been reported that the subjects with exaggerated cardiovascular reactivity to mental stress are predisposed to hypertension,[8,9] atherosclerosis[10–12] and cerebrovascular disease such as stroke and silent cerebral infarction.[13,14]

Although the central nervous system plays an important role in mental stress-induced cardiovascular reactivity,[1–3] little is known about the relationship between the reactivity and cognitive function or dementia. Association of increased short-time BP variability with cognitive impairment[15] suggests but does not directly demonstrate the link between BP responses to mental stress and cognitive function. Because the patients with cognitive impairment cannot easily perform cognitive tasks, we hypothesized that exaggerated mental stress responses would result in BP elevation in such patients.

To test this hypothesis, we conducted a cross-sectional study examining the relationship between cognitive function and BP responses to a mental arithmetic test using the subjects who were referred to our memory clinic and not taking any medications. Furthermore, we examined the effects of cilnidipine, an N- and L-type calcium channel blocker, on BP responses to a mental arithmetic test in hypertensive patients with mild cognitive impairment.

Methods

Subjects

The subjects who were referred to our memory clinic and were suspected to have hypertension on the first visit were enrolled. Depressive patients (15-item Geriatric Depression Scale score of ≥10 points) and post-stroke patients were excluded from the study. Each subject gave written informed consent before enrollment in this study. The study protocol was approved by the ethics committee of Kyorin University School of Medicine.

In study I, 39 consecutive patients (20 men and 19 women, aged 77 ± 8 years), who showed high-normal or higher BP (>130 mmHg in systolic or >85 mmHg in diastolic) and were not taking any medications, were enrolled. They underwent mental stress tests and were divided into three groups according to cognitive function on the Hasegawa Dementia Scale-Revised (HDSR): group 1 ($n = 8$), 28 points or more; group 2 ($n = 18$), 21–27 points; and group 3 ($n = 13$), 20 points or less. All the patients in group 3 and five patients in group 2 were clinically diagnosed to have Alzheimer's disease, but none were so in group 1.

In study II, 14 patients with hypertension (>140 mmHg in systolic or 90 mmHg in diastolic BP, or taking antihypertensive agents) and mild cognitive impairment (aged 79 ± 8 years; HDSR score, 24 ± 4 points; HDSR range, 21–27) were assigned to receive cilnidipine. Nine patients of group 2 in study I, who showed more than 140 mmHg in systolic or 90 mmHg in diastolic BP, were included in study II. Fifteen treated ($n = 11$) or untreated ($n = 4$) hypertensive patients were additionally included in study II. The dose of cilnidipine was initiated at 10 mg/day, and was increased to 20 mg/day if systolic BP was more than 150 mmHg or diastolic BP was more than 90 mmHg 2 weeks later. The patients were followed for an additional 2 weeks. Separately, the control group ($n = 10$) matched for age, HDSR and baseline BP were followed for 4 weeks. Mental stress tests were performed before and after the 4-week study period. Any medications except for cilnidipine were not changed throughout the study period.

Mental arithmetic test

After resting for 5 min in a quiet room, baseline BP and pulse rate (PR) were measured using an automated, digital electro sphygmomanometer (HEM-727IC; Omron Healthcare, Kyoto, Japan) on the non-dominant arm in the sitting position. Then, each subject was instructed to continuously subtract 7 from 213 as accurately as possible. BP and PR were measured again after 1 min of subtraction to evaluate the response to mental arithmetic. Measurements of BP and PR were repeated twice at each step, and the average values were used in the analyses. This test was modified for patients with cognitive impairment from the original version.[16] The correlation coefficients between the two repeated measurements of a 4-week interval were 0.971 for systolic BP and 0.850 for diastolic BP ($n = 15$, $P < 0.01$) after mental arithmetic.

Data analysis

The values are expressed as mean ± standard deviation in the text, tables and figures unless otherwise specified. Differences between the groups were analyzed using one-factor ANOVA, followed by a Newman–Keuls test. Changes in BP and PR during the study period were analyzed using a paired Student's *t*-test. $P < 0.05$ was considered statistically significant.

Results

Study I: Stress-induced BP elevation in the subjects as categorized by cognitive function

The characteristics of the subjects in the three groups are shown in Table 1. There were no significant differences

Table 1 Characteristics of subjects in study I

	Group 1 (HDSR, ≥28 points)	Group 2 (HDSR, 21–27 points)	Group 3 (HDSR, ≤20 points)
No. of subjects (men/women)	8 (4/4)	18 (10/8)	13 (6/7)
HDSR, points	29.0 ± 1.0	24.6 ± 1.9§	14.7 ± 3.8‡
Age, years	75 ± 8	78 ± 7	78 ± 7
SBP, mmHg	150 ± 14	148 ± 20	145 ± 20
DBP, mmHg	84 ± 12	77 ± 10	74 ± 11
Pulse rate (b.p.m.)	71 ± 11	73 ± 11	73 ± 11

Values are expressed as mean ± standard deviation. §$P < 0.01$ vs group 1; ‡$P < 0.01$ vs group 2. All other variables are not significantly different among the groups. DBP, diastolic blood pressure; HDSR, Hasegawa Dementia Scale-Revised; SBP, systolic blood pressure.

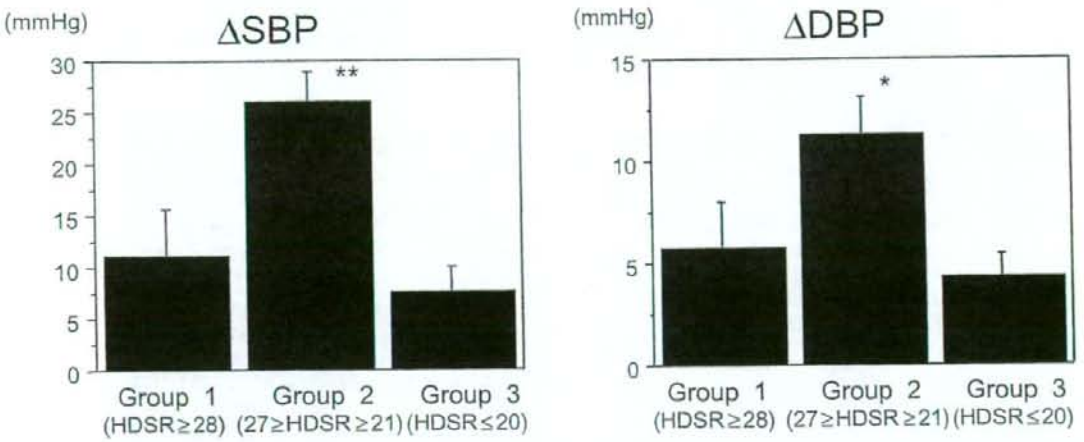

Figure 1 Influence of cognitive function on stress-induced blood pressure elevation. The changes of systolic (SBP) and diastolic blood pressure (DBP) during the mental arithmetic test in study I are shown. Values are expressed as mean ± standard error of the mean. Group 1, Hasegawa Dementia Scale-Revised (HDSR) score of ≥28 points; group 2, 21–27 points; and group 3, ≤20 points. *$P < 0.05$, **$P < 0.01$ vs groups 1 and 3.

in sex, age and baseline BP between the groups. As shown in Figure 1, the responses of both systolic and diastolic BP to the mental arithmetic test were twice as large in group 2 as those in groups 1 and 3.

Study II: Effects of cilnidipine on stress-induced BP elevation

All the subjects completed the protocol of study II. The characteristics of the subjects are shown in Table 2. The average dose of cilnidipine used in the cilnidipine group was 13 ± 5 mg/day. There were no significant differences in cognitive function, age and resting BP between the control group and the cilnidipine group, although resting systolic and diastolic BP fell significantly by the treatment with cilnidipine for 4 weeks. Figure 2 shows the BP responses to the mental arithmetic test. In the control group, BP responses did not change during the study period. In the cilnidipine group, however, BP responses to the mental arithmetic test were significantly decreased after 4 weeks. As a result, there were significant differences in the responses of systolic and diastolic BP to the mental arithmetic test between the control group and the cilnidipine group at the end of the study. We attempted to calibrate the reactivity by baseline BP. The percent changes of systolic BP during the mental arithmetic test were significantly smaller in the cilnidipine group than in the control group after treatment (12 ± 5% cilnidipine vs 18 ± 6% control, $P < 0.05$), although they were comparable in both groups before treatment (18 ± 6% cilnidipine vs 20 ± 9% control).

Table 2 Characteristics of subjects in study II

	Control	Cilnidipine
No. of subjects (men/women)	10 (5/5)	14 (6/8)
HDSR, points	26.0 ± 3.0	25.0 ± 4.0
Age, years	81 ± 8	79 ± 8
Pretreatment drugs		
ACEI/ARB, *n* (%)	3 (30%)	3 (21%)
CCB, *n* (%)	3 (30%)	3 (21%)
Before treatment		
SBP, mmHg	153 ± 17	161 ± 20
DBP, mmHg	80 ± 7	85 ± 11
Pulse rate, b.p.m.	75 ± 9	74 ± 13
4 weeks after treatment		
SBP, mmHg	151 ± 18	144 ± 16†
DBP, mmHg	79 ± 7	76 ± 9†
Pulse rate, bpm	75 ± 8	77 ± 13

Values are expressed as mean ± standard deviation. †$P < 0.01$ vs baseline. No significant differences were found between the control and cilnidipine groups. ACEI/ARB, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers; CCB, L-type calcium channel blocker; DBP, diastolic blood pressure; HDSR, Hasegawa Dementia Scale-Revised; SBP, systolic blood pressure.

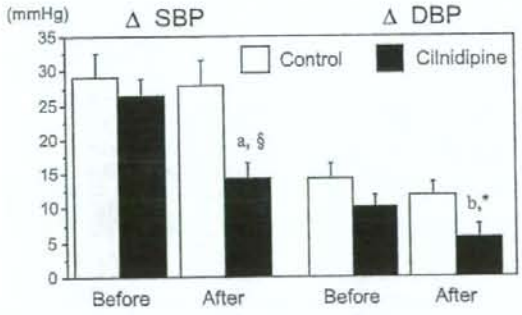

Figure 2 Effects of cilnidipine on stress-induced blood pressure elevation in elderly hypertensives with mild cognitive impairment. The changes of systolic (SBP) and diastolic blood pressure (DBP) during the mental arithmetic test before and after the treatment in study II are shown. Values are expressed as mean ± standard error of the mean. ª$P < 0.01$, ᵇ$P < 0.05$ vs before treatment. §$P < 0.01$, *$P < 0.05$ vs control.

Discussion

In study I, we investigated whether mental stress-induced BP elevation was related to cognitive function in the elderly who were referred to our memory clinic. A few studies have shown the relationship between mental stress-induced BP responses and cognitive function. Pierce *et al.*[17] have reported that BP responses during neuropsychological testing were unrelated to cognitive performance in college-aged subjects. Alternatively, Waldstein *et al.*[18] have reported that higher stress-induced BP reactivity is associated with poorer performance on tests of cognitive function in stroke- and dementia-free middle-aged and older adults (ages 54–79 years). In the present study, using older subjects with or without cognitive impairment, we found that the relation between mental stress-induced BP elevation and cognitive function was inverted U-shaped, and that stress-induced BP elevations were exaggerated in subjects with mild cognitive impairment.

Subjects with mild cognitive impairment can recognize their cognitive decline,[19] thus anxiety and irritation during the mental arithmetic test may arouse the accelerated BP response. By contrast, the mental arithmetic test is not likely to impose a heavy burden on demented subjects, because they display deficits in executive function,[20] often associated with depression and apathy.[21] Recently, greater variability in BP on 24-h ambulatory monitoring has been associated with poorer cognitive performance or cognitive impairment in samples of older adults.[15,22] Murakami *et al.*[23] investigated the relation between pressor responses to mental arithmetic tests and 24-h BP variability in normotensive subjects and hypertensive patients. They reported that the pressor response during the mental arithmetic test was significantly correlated with the value of 24-h BP variability in both subjects. Taken together, it is possible that mental stress-induced BP elevation in daily life is a strong determinant of BP variability in subjects with mild cognitive impairment.

In study II, we found that cilnidipine had inhibitory effects on stress-induced BP elevation in subjects with hypertension and mild cognitive impairment. Cilnidipine is a 1,4-dihydropyridine derivative calcium antagonist with potent inhibitory actions against not only L-type but also N-type voltage-dependent calcium channels.[24] Fujita *et al.*[25] have recently reported that cilnidipine is superior to amlodipine in preventing the progression of proteinuria in hypertensive patients with chronic kidney disease. The N-type voltage-dependent calcium channel plays an important role in sympathetic neurotransmission and regulates the release of norepinephrine from sympathetic nerve endings.[26] Accordingly, several studies have reported the inhibitory effects of cilnidipine on ambulatory BP and "white coat effect" in patients with essential hypertension.[27–30] The present results found in elderly hypertensives with mild cognitive impairment are consistent with these studies, and may provide a therapeutic implication in elderly hypertension.

Controversy exists as to prognostic significance of stress-induced BP elevation, typically known as the white coat effect, clinic-ambulatory BP difference.[31]