Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 556 (2007) 1–8
www.elsevier.com/locate/ejphar

Gas6/Axl-PI3K/Akt pathway plays a central role in the effect of statins on inorganic phosphate-induced calcification of vascular smooth muscle cells

Bo-Kyung Son [a], Koichi Kozaki [b], Katsuya Iijima [a], Masato Eto [a], Toru Nakano [c], Masahiro Akishita [a], Yasuyoshi Ouchi [a,*]

[a] *Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan*
[b] *Department of Geriatric Medicine, Kyorin University School of Medicine, Tokyo, Japan*
[c] *Discovery Research Laboratory, Shionogi and Co., Ltd., Osaka, Japan*

Received 19 May 2006; received in revised form 22 September 2006; accepted 27 September 2006
Available online 18 October 2006

Abstract

Apoptosis is essential for the initiation and progression of vascular calcification. Recently, we showed that *3-hydroxy-3-methylglutaryl* (HMG) CoA reductase inhibitors (statins) have a protective effect against vascular smooth muscle cell calcification by inhibiting apoptosis, where growth arrest-specific gene 6 (Gas6) plays a pivotal role. In the present study, we clarified the downstream targets of Gas6-mediated survival signaling in inorganic phosphate (Pi)-induced apoptosis and examined the effect of statins. We found that fluvastatin and pravastatin significantly inhibited Pi-induced apoptosis and calcification in a concentration-dependent manner in human aortic smooth muscle cells (HASMC), as was found with atorvastatin previously. Gas6 and its receptor, Axl, expression were downregulated in the presence of Pi, and recombinant human Gas6 (rhGas6) significantly inhibited apoptosis and calcification in a concentration-dependent manner. During apoptosis, Pi suppressed Akt phosphorylation, which was reversed by rhGas6. Wortmannin, a specific *phosphatidylinositol 3-OH kinase* (PI3K) inhibitor, abolished the increase in Akt phosphorylation by rhGas6 and eliminated the inhibitory effect of rhGas6 on both Pi-induced apoptosis and calcification, suggesting that PI3K-Akt is a downstream signal of the Gas6-mediated survival pathway. Pi reduced phosphorylation of Bcl2 and Bad, and activated caspase 3, all of which were reversed by rhGas6. The inhibitory effect of statins on Pi-induced apoptosis was accompanied by restoration of the Gas6-mediated survival signal pathway: upregulation of Gas6 and Axl expression, increased phosphorylation of Akt and Bcl2, and inhibition of Bad and caspase 3 activation. These findings indicate that the Gas6-mediated survival pathway is the target of statins' effect to prevent vascular calcification.

Keywords: Calcification; Apoptosis; Gas6; Axl; Akt; Bcl2

1. Introduction

Vascular calcification, such as coronary and aortic calcification, is clinically important in the development of cardiovascular disease (Eggen, 1968). Two distinct forms of vascular calcification are well recognized. One is medial calcification, which occurs between the cell layers of smooth muscle cells and is related to aging, diabetes and chronic renal failure (Neubauer, 1971; Goodman et al., 2000). The other is atherosclerotic calcification, which occurs in the intima during the development of atheromatous disease (Wexler et al., 1996). In diabetic patients, medial calcification has been shown to be a strong independent predictor of cardiovascular mortality (Everhart et al., 1988).

We recently demonstrated that atorvastatin prevented inorganic phosphate (Pi)-induced calcification by inhibiting apoptosis, one of the important processes regulating calcification. This was mediated by growth arrest-specific gene 6 (Gas6), a vitamin K-dependent protein (Son et al., 2006). Gas6 binds to Axl, the predominant receptor for Gas6, on the cell surface and transduces the signal by Axl autophosphorylation (Mark et al., 1996). Gas6-Axl interaction has been shown to be implicated in the regulation of multiple cellular functions (Yanagita et al., 2001; Goruppi et al., 1996; Nakano et al., 1997; Fridell et al., 1998). Especially, they are known to protect a range of cell types

* Corresponding author. Tel.: +81 3 5800 8652; fax: +81 3 5800 6530.
E-mail address: youchi-tky@umin.ac.jp (Y. Ouchi).

0014-2999/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2006.09.070

from apoptotic death (Goruppi et al., 1996, 1999; Healy et al., 2001). However, the downstream targets of Gas6-mediated signaling in Pi-induced apoptosis and the effect of statins on this pathway are poorly understood.

With respect to the targets of Gas6-Axl interaction, Lee et al. (2002) showed that activation of Akt is necessary for Gas6-dependent cell survival. Akt is an important mediator of metabolic and survival responses after growth factor stimulation. Akt is activated by phosphorylation, which is performed by phosphatidylinositol 3-OH kinase (PI3K), a kinase that is activated by Gas6-Axl interaction (Lee et al., 2002; Ming Cao et al., 2001). Activation of Akt leads to downstream signaling events including those associated with mitochondrial regulators of apoptosis such as Bcl2 and Bad.

In the present study, we examined the effect of statins using two different types: lipophilic fluvastatin and hydrophilic pravastatin. We investigated the effect of statins on Pi-induced apoptosis and calcification as well as on signaling components in this process. Consequently, we found that both statins restored the Gas6-mediated survival pathway, with upregulation of the expression of Gas6 and Axl, increased phosphorylation of Akt, Bcl2 and Bad; and finally inhibition of caspase 3 activation, resulting in the prevention of apoptosis and subsequent calcification in human aortic smooth muscle cells (HASMC).

2. Materials and methods

2.1. Materials

Pravastatin and fluvastatin were supplied by Sankyo Co. Ltd. and Tanabe Seiyaku Co., Ltd., respectively. Recombinant human Gas6 (rhGas6) was prepared as described previously (Ming et al., 2001). Wortmannin was purchased from Calbiochem. All other reagents were of analytical grade.

2.2. Cell culture

HASMC were obtained from Clonetics. They were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% *fetal bovine serum* (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.

2.3. 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. After the indicated incubation period, cells were decalcified with 0.6 M HCl, and Ca content in the supernatant was determined by the *o*-cresolphthalein complexone method (C-Test, WAKO). The remaining cells were solubilized in 0.1 M NaOH/0.1% *sodium dodecyl sulfate* (SDS), and cell protein content was measured by Bio-Rad protein assay. Calcification was visualized by von Kossa's method. Briefly, the cells were fixed with 4% formaldehyde and exposed to 5% aqueous $AgNO_3$.

2.4. Induction and determination of apoptosis

Two different time courses were tested to investigate Pi-induced apoptosis and examine the effect of statins, under short-term (within 24 h) and long-term (up to 10 days) conditions (Son et al., 2006).

2.4.1. TdT-mediated dUTP nick end-labeling (TUNEL) assay

TUNEL assay to detect DNA fragmentation was performed using a commercially available kit (ApopTag Plus, Chemicon). Briefly, the samples were preincubated with equilibration buffer for 10 min, and subsequently incubated with terminal deoxyribonucleotidyl transferase in the presence of digoxigenin-conjugated dUTP for 1 h at 37 °C. The reaction was terminated by incubating the samples in stopping buffer for 30 min. After 3 rinses with *phosphate-buffered saline* (PBS), a fluorescein-labeled anti-digoxigenin antibody was applied for 30 min, and the samples were rinsed 4 times with PBS. The samples were then stained, mounted with DAPI (*4′,6-diamino-2-phenylindole*)/antifade, and examined by fluorescence microscopy.

2.4.2. Detection of DNA fragmentation by ELISA

Cytoplasmic histone-associated DNA fragments were determined with a cell-death detection ELISA^{plus} kit (Roche) as a quantitative index of apoptosis. Briefly, after the cells were incubated in lysis buffer for 30 min, 20 μl of the cell lysates was used for the assay. Following addition of substrate, colorimetric change was determined as the absorbance value measured at 405 nm.

2.5. Immunoblotting

The effect of Pi and statins on the expression of Gas6 and Axl, phosphorylation of Akt, Bcl2 and Bad, and activation of caspase 3 was examined at 12 h. The collected cell lysates were applied to SDS-polyacrylamide gels under reducing conditions, and transferred to a polyvinylidene difluoride (PVDF) membrane. Immunoblot analysis was performed using specific primary antibodies: anti-Axl, anti-Gas6 (Santa Cruz Biotechnology), anti-caspase 3, anti-Akt, anti-Bcl2, anti-phospho-Akt, anti-phospho-Bcl2, anti-phospho-Bad (Cell Signaling Technology), and anti-Bad (Transduction Laboratories). After incubation with horseradish peroxidase-conjugated secondary antibodies (Amersham Pharmacia), blots were visualized by enhanced chemiluminescence and autoradiography (ECL Plus, Amersham Pharmacia). Experiments were performed with at least three different cell populations.

2.6. Statistical analysis

All results are presented as mean±S.E.M. Statistical comparisons were made by ANOVA, unless otherwise stated. A value of $P<0.05$ was considered to be significant.

3. Results

3.1. Statins inhibit Pi-induced apoptosis and calcification in HASMC

In HASMC, a high Pi level (≥2.6 mM), comparable to that of hyperphosphatemia in end-stage renal disease, significantly induced calcification. Fluvastatin showed an inhibitory effect on Pi-induced calcification at as high a concentration as 0.1 μM (26.1±2.3% of control), while pravastatin showed the degree of effect at 50 μM (27.4±3.1% of control) (Fig. 1A). An inhibitory effect on Ca deposition was also found by von Kossa's staining (Fig. 1B). Both statins prevented Pi-induced apoptosis at the same concentrations as those at which they prevented calcification (Fig. 1C). An antiapoptotic effect of statins was also observed by TUNEL assay on day 6 (Fig. 1D).

3.2. Gas6 plays an important role in Pi-induced apoptosis

In the presence of 2.6 mM Pi, the expression of Gas6 and Axl was markedly downregulated (Fig. 2A). To investigate the role of Gas6 in Pi-induced apoptosis and calcification, first, we tested whether supplementation of rhGas6 could prevent Pi-induced apoptosis. In HASMC, rhGas6 significantly inhibited Pi-induced apoptosis in a concentration-dependent manner (Fig. 2B). Furthermore, during apoptosis, activated products of caspase 3 (17 and 19 kDa) were significantly increased by 2.6 mM Pi, which was reversed by rhGas6 (Fig. 2C). Next, we examined the effect of rhGas6 on calcification. Recombinant human Gas6 significantly inhibited Pi-induced calcification on day 6 in a concentration-dependent manner (Fig. 2D), suggesting that Gas6 plays an important role in Pi-induced apoptosis and calcification.

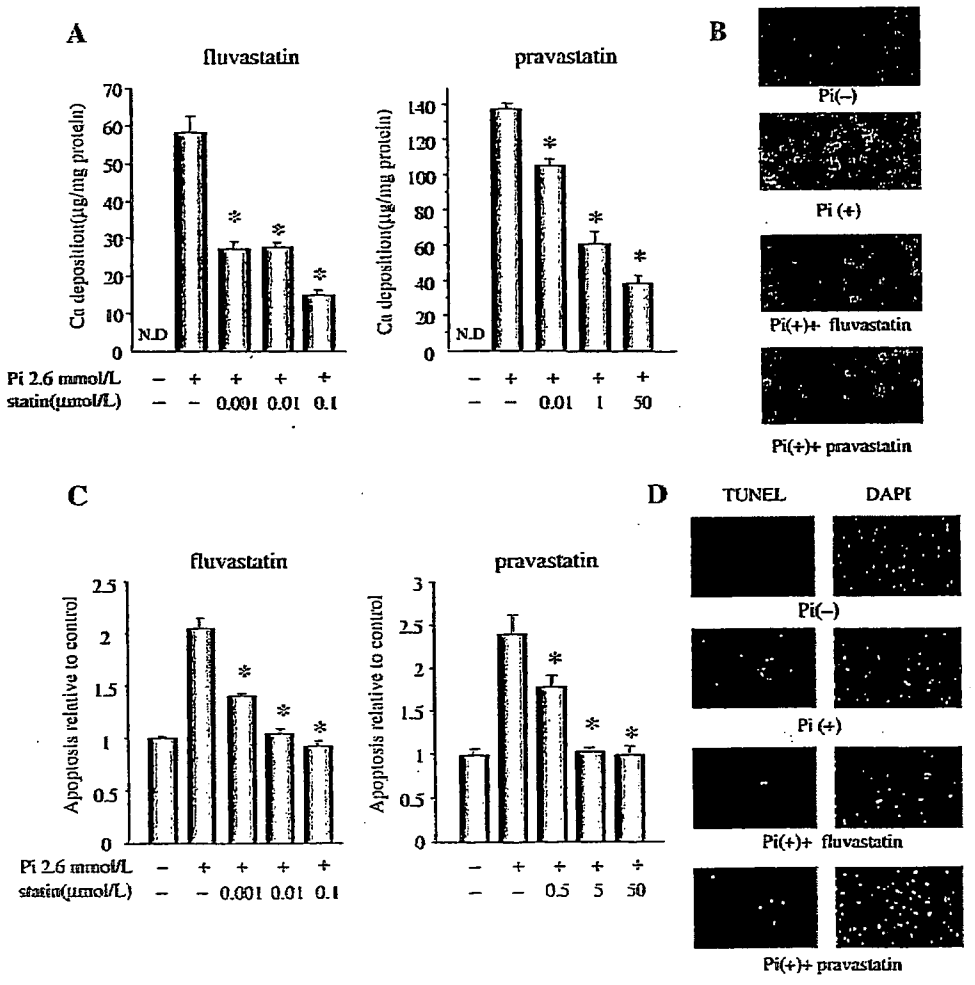


Fig. 1. Statins prevent Pi-induced apoptosis and calcification. HASMC were cultured with the indicated concentrations of fluvastatin and pravastatin in the presence of 2.6 mM Pi for 6 days. Ca deposition was measured by *o*-cresolphthalein complexone method, and normalized by cell protein content. All values are presented as mean± S.E.M. (n=6). *P<0.05 vs. statin (−) by Fisher's test. N.D. stands for "not detected" (A). On day 6, the inhibitory effect of fluvastatin (0.1 μM) and pravastatin (50 μM) on 2.6 mM Pi [Pi(+)]-induced Ca deposition was evaluated at the light microscopic level with von Kossa's staining (B). Serum-starved HASMC were cultured with the indicated concentrations of fluvastatin and pravastatin for 12 h and then incubated with 2.6 mM Pi for an additional 24 h. A quantitative index of apoptosis, determined by ELISA, is presented as the relative value to that without statins and 2.6 mM Pi. All values are presented as mean±S.E.M. (n=3). *P<0.05 vs. 2.6 mM Pi, statin (−) by Fisher's test (C). The antiapoptotic effect of fluvastatin (0.1 μM) and pravastatin (50 μM) was evaluated by TUNEL staining (green) on day 6. Nuclei were counterstained with DAPI (4′,6-diamino-2-phenylindole, blue) (D).

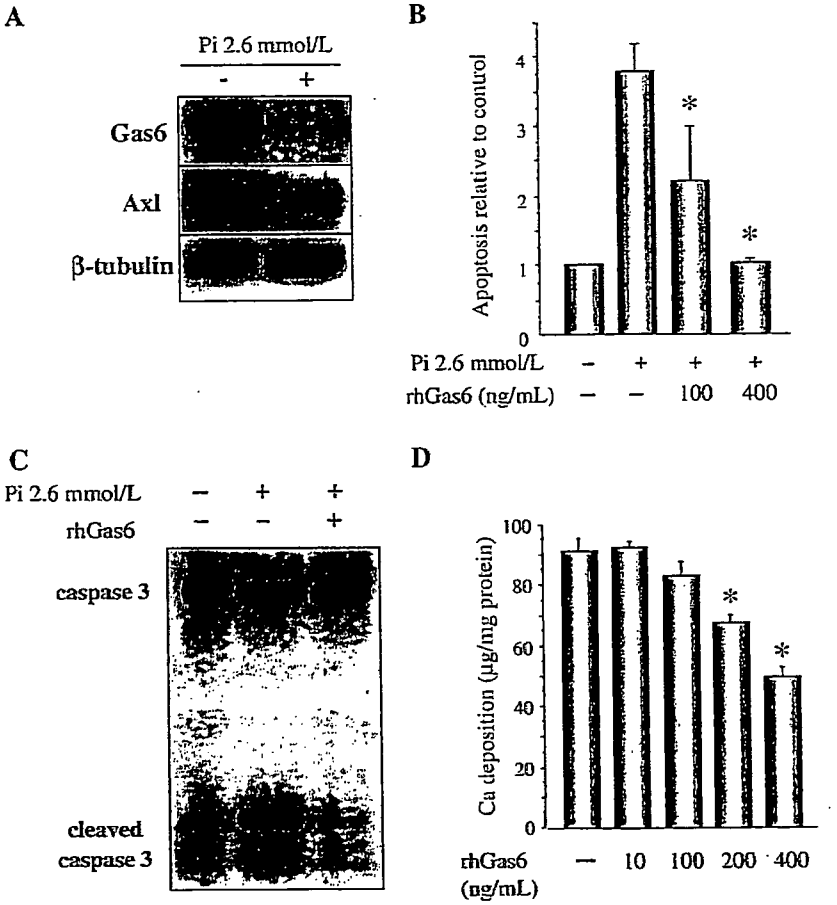


Fig. 2. Pi suppresses Gas6 and Axl expression, and rhGas6 inhibits caspase-dependent apoptosis and calcification. HASMC were cultured in the presence or absence of 2.6 mM Pi for 12 h. Cell lysates were collected and subjected to SDS-PAGE followed by immunoblotting with antibodies to Gas6, Axl or β-tubulin (A). After pretreatment with the indicated concentrations of rhGas6, apoptosis was induced by 2.6 mM Pi. All values are presented as mean±S.E.M. (n=3). *P<0.05 vs. 2.6 mM Pi, rhGas6 (−) by Fisher's test (B). HASMC were pretreated with rhGas6 (400 ng/ml) for 1 h, then cultured with 2.6 mM Pi for 12 h. Cell lysates were immunoblotted with an antibody that recognizes caspase-3 (35 kDa) and the cleaved forms of caspase-3 (17 and 19 kDa) (C). For measurement of Ca deposition, HASMC were cultured with the indicated concentrations of rhGas6 in the presence of 2.6 mM Pi for 6 days. All values are presented as mean±S.E.M. (n=6). *P<0.05 by Fisher's test (D). Experiments were performed with at least three different cell populations.

3.3. Downregulation of phospho-Akt participates in Pi-induced apoptosis

Since in NIH-3T3 fibroblasts, the antiapoptotic effect of Gas6-Axl interaction has been shown to be mediated by Akt phosphorylation (Goruppi et al., 1999), we examined whether Akt participates in the signaling of downregulation of the Gas6-Axl interaction during Pi-induced apoptosis. In the presence of 2.6 mM Pi, Akt phosphorylation was downregulated in a time-dependent manner, whereas the expression of total Akt was not changed (Fig. 3A). In addition, rhGas6 abrogated the Pi-induced decrease in Akt phosphorylation, implying that subsequent downregulation of Akt phosphorylation is the pathway of Pi-induced apoptosis (Fig. 3B).

Because Akt phosphorylation is regulated by PI3K, we examined the effect of wortmannin, a specific PI3K inhibitor, on rhGas6-mediated phosphorylation of Akt. As shown in Fig. 3B, wortmannin abrogated the rhGas6-induced phosphorylation of Akt and further eliminated the inhibitory effect of rhGas6 on Pi-induced apoptosis and calcification (Fig. 3C, D). These results indicate that the preventive effect of rhGas6 on Pi-induced apoptosis and calcification was mediated by the PI3K-Akt pathway.

3.4. Pi suppresses Bcl2 phosphorylation and activates Bad

To establish the downstream components of Pi-induced apoptosis, two key apoptosis-regulating proteins, Bcl2 and Bad, were analyzed. During apoptosis, phosphorylation of Bcl2 (active form) and Bad (inactive form) was markedly reduced by 2.6 mM Pi in a time-dependent manner. The expression level of their total protein was not changed in this period (Fig. 4A, B). By supplementation of the medium with rhGas6, the decrease in phosphorylation of Bcl2 and Bad by Pi was reversed to almost the basal level (Fig. 4C, D). These results indicate that Pi promotes apoptosis by inactivating Bcl2 and activating Bad via a Gas6-dependent pathway.

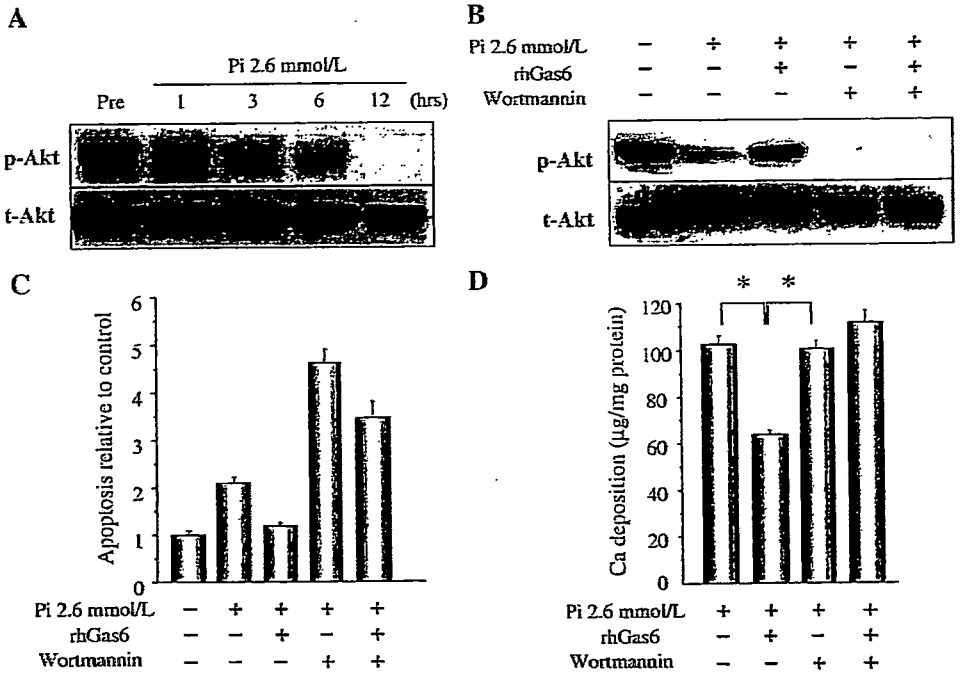


Fig. 3. Pi decreases Akt phosphorylation, and wortmannin abrogates the inhibitory effect of rhGas6 on Akt phosphorylation, apoptosis and calcification. HASMC were cultured in the presence of 2.6 mM Pi for the indicated periods. Cell lysates were immunoblotted with anti-phospho-Akt (p-Akt) antibody and total Akt (t-Akt) antibody (A). HASMC were pretreated with rhGas6 (400 ng/ml), wortmannin (1 μM), or both for 1 h, and then treated with 2.6 mM Pi for 12 h. Cell lysates were immunoblotted with p-Akt and t-Akt antibody (B). After pretreatment with rhGas6 (400 ng/ml) and wortmannin (1 μM), apoptosis was induced by 2.6 mM Pi. All values are presented as mean±S.E.M. (n=3). *P<0.05 vs. 2.6 mM Pi, rhGas6 (−), wortmannin (−) by Fisher's test (C). HASMC were cultured with rhGas6 (400 ng/ml) and with or without wortmannin (1 μM) in the presence of 2.6 mM Pi for 6 days. Ca content was measured and normalized by cell protein content. All values are presented as mean±S.E.M. (n=6). *P<0.05 by Fisher's test (D).

3.5. Gas6-mediated survival pathway is the target of statins' effect on apoptosis

To investigate whether the antiapoptotic effect of statins is associated with the Gas6-mediated survival pathway, first, we examined the effect of statins on the expression of Gas6 and Axl. As shown in Fig. 5A and B, both fluvastatin and pravastatin restored the expression of Gas6 and Axl, which was downregulated by 2.6 mM Pi. Because we have shown that the Gas6-mediated survival pathway is Akt-dependent, the effect of statins on Akt phosphorylation was examined. The Pi-induced decrease in Akt phosphorylation was restored by both statins, while total Akt expression was not changed. In addition, we found that both statins stimulated phosphorylation of Bcl2 and

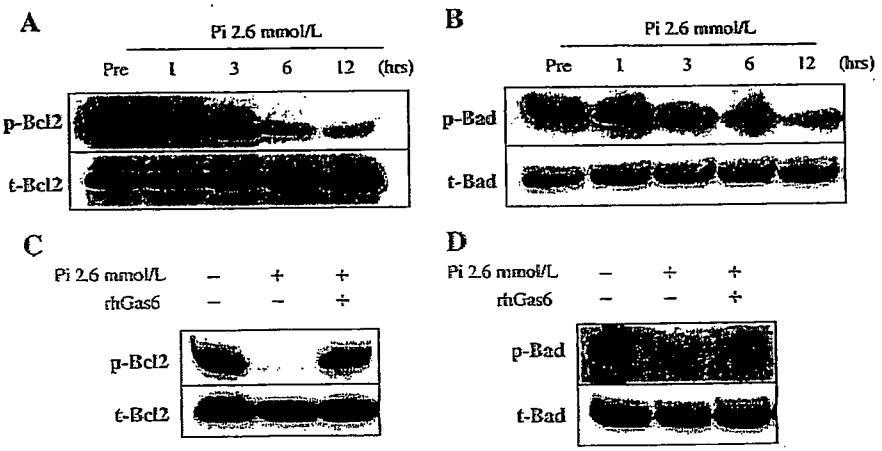


Fig. 4. RhGas6 restores Pi-induced decrease in phosphorylation of Bcl2 and Bad. HASMC were exposed to 2.6 mM Pi for the indicated periods, and cell lysates were subjected to immunoblotting with anti-phospho-Bcl2 (p-Bcl2) antibody and total Bcl2 (t-Bcl2) antibody (A), or with anti-phospho-Bad (p-Bad) antibody and total Bad (t-Bad) antibody (B). HASMC were pretreated with rhGas6 (400 ng/ml) for 1 h, and then treated with 2.6 mM Pi for 12 h. Cell lysates were subjected to immunoblotting with p-Bcl2 and t-Bcl2 antibody (C), or with p-Bad and t-Bad antibody (D).

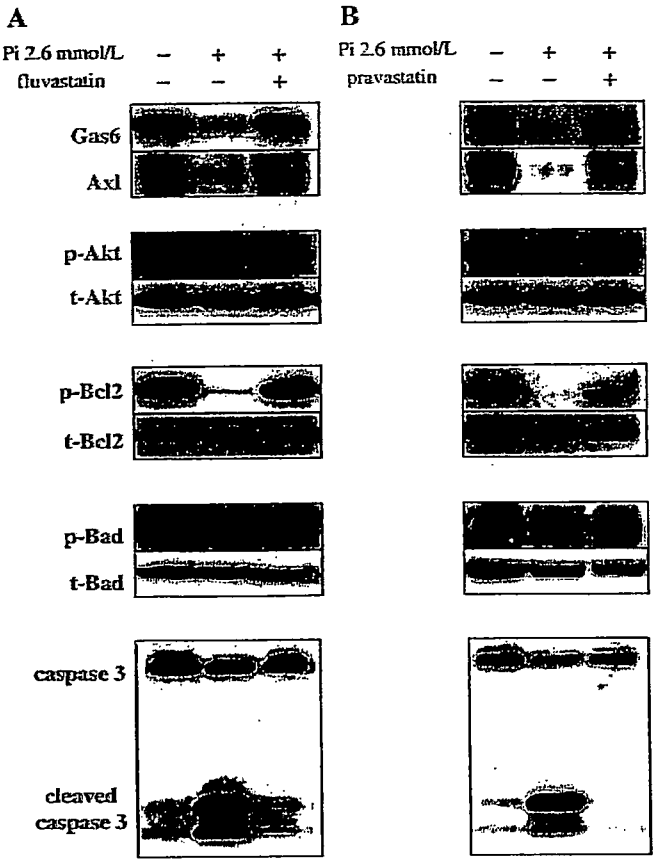


Fig. 5. Antiapoptotic effect of statins is associated with upregulation of Gas6-Axl survival pathway. After pretreatment with 0.1 μM fluvastatin (A) and 50 μM pravastatin (B) for 12 h, apoptosis was induced by 2.6 mM Pi. After 12 h, cell lysates were collected and subjected to SDS-PAGE followed by immunoblotting with antibodies that recognize Gas6 and Axl, with phospho-specific Akt (p-Akt) and total Akt (t-Akt) antibody, with phospho-specific Bcl2 (p-Bcl2) and total Bcl2 (t-Bcl2) antibody, or with phospho-specific Bad (p-Bad) and total Bad (t-Bad) antibody. Cell lysates were immunoblotted with an antibody that recognizes uncleaved caspase-3 (35 kDa) and the cleaved forms of caspase-3 (17 and 19 kDa).

Bad, with total expression unchanged. Pi-induced caspase 3 activation was also prevented by both statins. Taken together, these findings suggest that the inhibitory effect of statins on Pi-induced apoptosis is mediated by restoration of the Gas6-mediated survival pathway; PI3K-induced Akt phosphorylation, Bcl2 activation, Bad inactivation, and caspase 3 inactivation.

4. Discussion

In the present study, we found that both lipophilic fluvastatin and hydrophilic pravastatin protected against Pi-induced apoptosis and calcification in HASMC, as we found with atorvastatin previously. With regard to the different potency of statins, we found that the inhibitory effect of pravastatin was inferior to those of fluvastatin and atorvastatin, which exerted similar effects on calcification and apoptosis. This might relate to our previous finding that the inhibition of calcification by statins was not dependent on the mevalonate pathway (Son et al., 2006). Consequently, the inhibitory effect on calcification was not parallel to the cholesterol-lowering effect. We speculate that the difference between statins was derived from their affinity to *vascular smooth muscle cells* (VSMC), that is, lipophilic statins have stronger effects on VSMC calcification than hydrophilic statins.

The antiapoptotic effect of statins was induced by restoration of the Gas6-mediated survival pathway: PI3K-induced Akt phosphorylation, Bcl2 and Bad phosphorylation, and caspase 3 inactivation. Gas6 plays a crucial role in the effect of statins on Pi-induced apoptosis. Gas6, a secreted vitamin K-dependent protein, binds to the receptors of the mammalian Axl protein-tyrosine kinase family; Axl, Sky, and Mer, with different affinities (Nagata et al., 1996). Gas6 and Axl have been shown to localize in the neointima of the artery after balloon injury, in which they presumably modulate several cell functions such as differentiation, adhesion, migration, proliferation, and survival in a cell-specific manner (Melaragno et al., 1998). The Gas6-Axl interaction is also shown to upregulate scavenger receptor A expression in VSMC (Ming et al., 2001), and facilitates the clearance of apoptotic cells by macrophages (Ishimoto et al., 2000). Of the above functions, protection against apoptotic cell death has been most studied (Goruppi et al., 1996; Healy et al., 2001; Lee et al., 2002; Nakano et al., 1996). Consistently, the expression of Gas6 and Axl was downregulated by Pi, leading to apoptosis and subsequent calcification.

Several intracellular signaling pathways mediated by Gas6-Axl interaction have been shown previously (Goruppi et al., 1999; Lee et al., 2002; Ming et al., 2001). Akt, which is necessary for Gas6-dependent survival, is a critical downstream effector of the PI3K-dependent antiapoptotic pathway. In VSMC, it has been reported that the PI3K-Akt pathway mediates Gas6 induction of scavenger receptor A (Ming et al., 2001). Consistent with these reports, our study provides evidence that the PI3K-Akt pathway is a target of Gas6-Axl interaction, and downregulation of Akt phosphorylation is associated with Pi-induced apoptosis and calcification. Moreover, it is known that PI3K-Akt affects the cell death program through the Bcl2 family of proteins. This protein family is a critical regulator of apoptosis in a variety of cell types, and the balance of antiapoptotic members, such as Bcl2, versus proapoptotic mediators, such as Bad, determines cell fate (Reed, 1997). Bcl2, whose phosphorylation is required for its anti-apoptotic activity (Ruvolo et al., 2001), inhibits programmed cell death by several mechanisms: It binds to caspase CED-4 (Apaf-1) and prevents the cell execution cascade; Bcl2 alters mitochondrial membrane potential and inhibits the release of cytochrome c. On the other hand, Bad plays a proapoptotic role in its dephosphorylated form by binding to Bcl2 and reversing its anti-apoptotic effect; phosphorylation of Bad results in its cytosolic sequestration by 14-3-3 and hampers its binding to Bcl2 (Zha et al., 1996). It was also reported that Bad is directly phosphorylated by PI3K-Akt (del Peso et al., 1997). In the present study, Bcl2 was inactivated and Bad was activated (both proteins were dephosphorylated) by Pi, directing the cells to apoptosis, and rhGas6 restored phosphorylation of Bcl2 and Bad. During apoptosis, one of the final biochemical events leading to programmed

cell death is activation of the caspase cascade. Activation of caspase 3 is required for internucleosomal DNA degradation (Woo et al., 1998), and caspase inhibition prevents the release of apoptotic bodies from cells (Zhang et al., 1999). In the present study, supplementation of the medium with rhGas6 prevented Pi-induced caspase 3 activation. These results clearly show that Pi downregulates Gas6-Axl, decreases PI3K-mediated Akt phosphorylation, inactivates Bcl2, activates Bad, and activates caspase 3, leading to apoptosis.

The present study demonstrated that statins restored the Gas6-mediated survival pathway. Consistent with these results, Akt phosphorylation has been reported to be an antiapoptotic mechanism of statins: pravastatin inhibited hypoxia-induced apoptosis through activation of Akt in cardiomyocytes (Bergmann et al., 2004), and simvastatin and pravastatin enhanced phosphorylation of Akt and promoted angiogenesis in endothelial cells (Kureishi et al., 2000). Recently, it was reported that statins inhibit caspase 3 activation driven by protein kinase C inhibitors in the process of apoptosis, suggesting that caspase 3 is also under the control of statins during apoptosis (Tanaka et al., 2004).

In this study, we performed experiments under both short-term (within 24 h) and long-term (up to 10 days) conditions. In general, short-term experiments are able to examine acute cell behavior, such as signaling and transcription. However, because obvious HASMC calcification takes at least 3 days, we also performed long-term experiments. Downregulation of Gas6, Axl expression and reduced phosphorylation of Akt, Bcl2, and Bad, and a beneficial effect of statins were consistently found in the long-term condition. This confirms that the Gas6-Axl survival signal is the key mechanism for Pi-induced calcification.

It is concluded that statins inhibit Pi-induced apoptosis via the Gas6/Axl-PI3K-Akt signal pathway, which has a crucial role in the prevention of HASMC calcification. This study adds further evidence of the pleiotropic effects of statins, suggesting a therapeutic strategy for the prevention of vascular calcification.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan (No. 15390239), Mitsui Sumitomo Insurance Welfare Foundation, Ono Medical Research Foundation, Kanzawa Medical Research Foundation, Novartis Foundation for Gerontological Research, and Takeda Research Foundation. We thank Yuki Ito for technical assistance.

References

Bergmann, M.W., Rechner, C., Freund, C., Baurand, A., Jamali, A., Dietz, R., 2004. Statins inhibit reoxygenation-induced cardiomyocyte apoptosis: role for glycogen synthase kinase 3β and transcription factor β-catenin. J. Mol. Cell. Cardiol. 37, 681–690.

del Peso, L., Gonzalez-Garcia, M., Page, C., Herrera, R., Nunez, G., 1997. Interleukin-3-induced phosphorylation of Bad through protein kinase Akt. Science 278, 687–689.

Eggen, D.A., 1968. Relationship of calcified lesions to clinically significant atherosclerotic lesions. Ann. N. Y. Acad. Sci. 149, 752–767.

Everhart, J.E., Pettitt, D.J., Knowler, W.C., Rose, F.A., Bennett, P.H., 1988. Medial artery calcification and its association with mortality and complications of diabetes. Diabetologia 31, 16–23.

Fridell, Y.W., Villa Jr., J., Attar, E.C., Liu, E.T., 1998. Gas6 induces Axl-mediated chemotaxis of vascular smooth muscle cells. J. Biol. Chem. 273, 7123–7126.

Goodman, W.G., Goldin, J., Kuizon, B.D., Yoon, C., Gales, B., Sider, D., Wang, Y., Chung, J., Emerick, A., Greaser, L., Elashoff, R.M., Salusky, I.B., 2000. Coronary-artery calcification in young adults with end-stage renal disease who are undergoing dialysis. N. Engl. J. Med. 342, 1478–1483.

Goruppi, S., Ruaro, E., Schneider, C., 1996. Gas6, the ligand of Axl tyrosine kinase receptor, has mitogenic and survival activities for serum starved NIH3T3 fibroblasts. Oncogene 12, 471–480.

Goruppi, S., Ruaro, E., Varnum, B., Schneider, C., 1999. Gas6-mediated survival in NIH3T3 cells activates stress signaling cascade and is independent of Ras. Oncogene 18, 4224–4236.

Healy, A.M., Schwartz, J.J., Zhu, X., Herrick, B.E., Varnum, B., Farber, H.W., 2001. Gas6 promotes Axl-mediated survival in pulmonary endothelial cells. Am. J. Physiol., Lung Cell. Mol. Physiol 280, L1273–L1281.

Ishimoto, Y., Ohashi, K., Mizuno, K., Nakano, T., 2000. Promotion of the uptake of PS liposomes and apoptotic cells by a product of growth arrest-specific gene, gas6. J. Biochem. (Tokyo) 127, 411–417.

Kureishi, Y., Luo, Z., Shiojima, I., Bialik, A., Fulton, D., Lefer, D.J., Sessa, W.C., Walsh, K., 2000. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. Nat. Med. 6, 1004–1010.

Lee, W.P., Wen, Y., Varnum, B., Hung, M.C., 2002. Akt is required for Axl-Gas6 signaling to protect cells from E1A-mediated apoptosis. Oncogene 21, 329–336.

Mark, M.R., Chen, J., Hammonds, R.G., Sadick, M., Godowski, P.J., 1996. Characterization of Gas6, a member of the superfamily of G domain-containing proteins, as a ligand for Rse and Axl. J. Biol. Chem. 271, 9785–9789.

Melaragno, M.G., Wuthrich, D.A., Poppa, V., Gill, D., Lindner, V., Berk, B.C., Corson, M.A., 1998. Increased expression of Axl tyrosine kinase after vascular injury and regulation by G protein-coupled receptor agonists in rats. Circ. Res. 83, 697–704.

Ming Cao, W., Murao, K., Imachi, H., Sato, M., Nakano, T., Kodama, T., Sasaguri, Y., Wong, N.C., Takahara, J., Ishida, T., 2001. Phosphatidylinositol 3-OH kinase-Akt/protein kinase B pathway mediates Gas6 induction of scavenger receptor a in immortalized human vascular smooth muscle cell line. Arterioscler. Thromb. Vasc. Biol. 21, 1592–1597.

Nagata, K., Ohashi, K., Nakano, T., Arita, H., Zong, C., Hanafusa, H., Mizuno, K., 1996. Identification of the product of growth arrest-specific gene 6 as a common ligand for Axl, Sky, and Mer receptor tyrosine kinases. J. Biol. Chem. 271, 30022–30027.

Nakano, T., Kawamoto, K., Higashino, K., Arita, H., 1996. Prevention of growth-arrest induced cell death of vascular smooth muscle cells by a product of growth arrest-specific gene, gas6. FEBS Lett. 387, 78–80.

Nakano, T., Ishimoto, Y., Kishino, J., Umeda, M., Inoue, K., Nagata, K., Ohashi, K., Mizuno, K., Arita, H., 1997. Cell adhesion to phosphatidylserine mediated by a product of growth arrest-specific gene 6. J. Biol. Chem. 272, 29411–29414.

Neubauer, B., 1971. A quantitative study of peripheral arterial calcification and glucose tolerance in elderly diabetics and non-diabetics. Diabetologia 7, 409–413.

Reed, J.C., 1997. Double identity for proteins of the Bcl-2 family. Nature 387, 773–776.

Ruvolo, P.P., Deng, X., May, W.S., 2001. Phosphorylation of Bcl2 and regulation of apoptosis. Leukemia 515–522.

Son, B.K., Kozaki, K., Iijima, K., Eto, M., Kojima, T., Ota, H., Senda, Y., Maemura, K., Nakano, T., Akishita, M., Ouchi, Y., 2006. Statins protect human aortic smooth muscle cells from inorganic phosphate-induced calcification by restoring Gas6-Axl survival pathway. Circ. Res. 98, 1024–1031.

Tanaka, K., Honda, M., Takabatake, T., 2004. Anti-apoptotic effect of atorvastatin, a 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitor, on cardiac myocytes through protein kinase C activation. Clin. Exp. Pharmacol. Physiol. 31, 360–364.

Wexler, L., Brundage, B., Crouse, J., Detrano, R., Fuster, V., Maddahi, J., Rumberger, J., Stanford, W., White, R., Taubert, K., 1996. Coronary artery calcification: pathophysiology, epidemiology, imaging methods, and clinical

implications. A statement for health professionals from the American Heart Association. Writing Group. Circulation 94, 1175–1192.

Woo, M., Hakem, R., Soengas, M.S., Duncan, G.S., Shahinian, A., Kagi, D., Hakem, A., McCurrach, M., Khoo, W., Kaufman, S.A., Senaldi, G., Howard, T., Lowe, S.W., Mak, T.W., 1998. Essential contribution of caspase3/CPP32 to apoptosis and its associated nuclear changes. Genes Dev. 12, 806–819.

Yanagita, M., Arai, H., Ishii, K., Nakano, T., Ohashi, K., Mizuno, K., Varnum, B., Fukatsu, A., Doi, T., Kita, T., 2001. Gas6 regulates mesangial cell proliferation through Axl in experimental glomerulonephritis. Am. J. Pathol. 158, 1423–1432.

Zha, J., Harada, H., Yang, E., Jockel, J., Korsmeyer, S.J., 1996. Serine phosphorylation of death agonist BAD in response to survival factor results in binding to 14-3-3 not BCL-X(L). Cell 87, 619–628.

Zhang, J., Reedy, M.C., Hannun, Y.A., Obeid, L.M., 1999. Inhibition of caspases inhibits the release of apoptotic bodies: Bcl2 inhibits the initiation of formation of apoptotic bodies in chemotherapeutic agent-induced apoptosis. J. Cell Biol. 145, 99–108.

Geriatr Gerontol Int 2007; 7: 305–309

CASE REPORT

Improved cognitive function, mood and brain blood flow in single photon emission computed tomography following individual reminiscence therapy in an elderly patient with Alzheimer's disease

Katsuaki Tanaka, Yukiko Yamada, Yoshio Kobayashi, Kazuki Sonohara, Ayako Machida, Ryuhei Nakai, Koichi Kozaki and Kenji Toba

Department of Geriatric Medicine, Kyorin University, School of Medicine, Mitaka, Tokyo, Japan

An 88-year-old man who was suffering from chronic renal failure and hypertension visited our memory clinic because of recent cognitive decline and a gradual decrease in his vitality and volition. His Mini-Mental State Examination (MMSE) score was 22, his 15-item Geriatric Depression Scale (GDS-15) score was 10, and his Vitality Index (VI; full score, 10) was 6. We diagnosed Alzheimer's disease with depressive mood, and this was supported by findings of global brain atrophy by magnetic resonance imaging and decrease in brain blood flow in the posterior cingulated gyrus and frontal association area by single photon emission computed tomography (SPECT). After completion of a life review of the patient, individual reminiscence therapy was performed once a week for 2 months. After the therapy, a comprehensive geriatric assessment showed that cognitive function, depressive mood and decreased vitality had all markedly improved (MMSE, 29; GDS, 7; VI, 9). Moreover, SPECT showed improved brain blood flow, especially in the frontal lobe. We believe that this is the first case in which reminiscence therapy alone not only improved cognitive function and mood but also reduced neuroimaging abnormalities.

Keywords: Alzheimer's disease, cognitive function, life review, reminiscence, single photon emission computed tomography easy Z-score imaging system (SPECT eZIS).

Introduction

Reminiscence is a psychophysiological therapy proposed by an American geriatric psychiatrist, Robert N. Butler, in 1963,[1] and recommended as a grade D' psychological approach to the management of neuropsychiatric symptoms of dementia by Livingstone *et al.*[2] There are two methods of reminiscence therapy: group reminiscence and individual reminiscence. The former is very common and widely performed in public welfare facilities;[3] in contrast, very few facilities perform individual reminiscence[4] and related documents and references are limited in Japan.[5,6]

Herein, we report the case of an 88-year-old man who was treated by individual reminiscence therapy at our outpatient memory clinic. We show that cognitive function, depressive condition and volition were all

Accepted for publication 9 January 2007.

Correspondence: Kenji Toba MD, Department of Geriatric Medicine, Kyorin University, School of Medicine, 6-20-2 Shinkawa, Mitaka, Tokyo 181-8611, Japan. Email: toba@kyorin-u.ac.jp

An abstract of this report was presented at the 43rd meeting of the Japan Geriatrics Society Kanto-Koshinetsu regional meeting (Tokyo, 11 March 2006).

doi: 10.1111/j.1447-0594.2007.00418.x

improved in a comprehensive geriatric assessment performed after therapy. Objective changes supporting these outcomes were noted in imaging after completion of the individual reminiscence program.

Case report

The patient was an 88-year-old man suffering from chronic renal failure, hypertension and hyperuricemia. He was taking Nifedipine CR tablet 20 mg/day and allopurinol tablet 50 mg/day. He had been born in Asakusa, Tokyo, and had lived at his mother's home in Gifu Prefecture while he was a primary schoolboy. His surviving family members were his wife, a second son and his wife, two grandsons and one granddaughter. His occupation had been as a private primary school teacher, and after retirement he was engaged in editing and publishing biographies of great persons at an educational book publishing company.

Based on a family interview, the patient had shown temporal disorientation, derangement of the capacity to register and decreased activities of daily living (ADL) for several years; for these reasons he visited our outpatient memory clinic. His present illness was not specific, his neurological findings were normal, and no other psychological or psychiatric symptoms were noted. In radiological imaging, global cerebral atrophy was noted in brain magnetic resonance imaging (MRI), and relative decreases in blood flow in the posterior cingulated gyrus and right median plane of the frontal lobe were noted on single photon emission computed tomography (SPECT; ethylene cystine dimer [ECD]).

Although his Geriatric Depression Scale score suggested depression, his symptoms did not satisfy major depression criteria of the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV). Dementia with Lewy bodies (DLB) was suspected based on decreased blood flow in the occipital lobe on SPECT; however, because neurological symptoms such as parkinsonism, hallucination and visual hallucination were absent, and reduction of cognitive function was mild, Alzheimer's-type dementia was diagnosed. Based on this diagnosis, administration of donepezil hydrochloride (Aricept) was considered, but the patient had chronic renal failure and we were concerned about the risk of donepezil-induced rhabdomyolysis. Considering the risk, the family requested that the patient should not receive this drug, so individual reminiscence therapy was initiated instead.

The individual reminiscence procedure was performed as follows:

1 The person with the main responsibility for taking care of the patient (the wife of his second son) first visited the hospital alone, and were interviewed about the patient's profile (shown below) before therapy. This allowed discussion of episodes that she could not mention in the presence of the patient, and allowed a prior understanding of things that should be avoided when talking to the patient. The interview clarified: (i) details of the interviewee and her relationship with the patient; (ii) the patient's diagnosis and medications; (iii) the patient's past medical history, lifestyle and other diseases; (iv) the patient's family members (i.e. marital status and presence or absence of spouse), nickname, parents, siblings, children, grandchildren, close relatives and friends, and other people; (v) native town, places of importance to the patient other than his native town, history of moving; (vi) final level of school education; (vii) professional career (i.e. thoughts on work and feelings of accomplishment); (viii) hobbies (including interests and matters of concern); (ix) likes and dislikes; (x) religion and beliefs; and (xi) particulars regarding medical care and mental state.
2 The above items were discussed in the interview, and the first session of reminiscence therapy was performed 1 week later. Subsequently, sessions of approximately 1 h were performed weekly for 8 weeks (one set of therapy).
3 The person taking care of the patient attended all the sessions to correct paramnesia, and to insert words related to the date and weather to correct temporal disorientation.
4 In the first session, the patient was allowed to talk freely about the times that he had held strong feelings. Later sessions progressed through a chronological life review from boyhood to late middle age, including background, life and manners, and social conditions of the times.
5 The following items were used to jog the memory: a cup and ball, ohajiki (small discs of glass), menko (cardboard), a five-bead abacus, an antique watch, an empty lemonade bottle, primary and junior high school text books from approximately 1910 until the mid-1920s, and a collection of old photographs from the mid-1920s until approximately 1960.
6 Before the fifth session, the effect of the first four sessions was evaluated using a Comprehensive Geriatric Assessment (CGA). The CGA was also performed after completion of all sessions before initiation of the next outpatient treatment, and the final outcome of the individual reminiscence therapy was determined. Only the Mini-Mental State Examination (MMSE), Hasegawa's Dementia Scale – Revised (HDS-R), 15-item Geriatric Depression Scale (GDS-15) and Vitality Index (VI) were evaluated after completion of the fourth session. The CGA was comprised of: (i) Barthel Index (BI) as an index of ADL (full score, 100 points); (ii) MMSE and HDS-R for evaluation of cognitive function; (iii) Dementia Behavior Disturbance Scale (DBD) for behavioral and psychological symptoms of dementia; (iv) GDS-15 as

an index of depression (full score, 15 points); (v) VI for evaluation of vitality and volition (full score, 10 points); and (vi) Zarit's Burden Interview (ZBI) for evaluation of carer's load (full score, 88 points).

There are two approaches to reminiscence therapy: one in which the patient recalls memories from historical news and events, old articles regarding every-day issues, old toys, and printed material such as old books; and a second in which a life review is held, in which the patient looks back on their own history and life and compares this with their current condition. In our case, individual reminiscence therapy was performed using the latter procedure, but the former procedure was employed concomitantly as needed for introductory purposes in the first session and as idle talk when the conversation halted during a session.

On MRI, the entire brain was seen to be markedly atrophied, and some ischemic lesions, such as periventricular high intensity lesions, were also noted. Almost no changes were noted on MRI performed 6 months after completion of the reminiscence program.

On SPECT (^{99m}Tc-ECD) Relative reduction of blood flow was noted in the frontal and occipital lobes, posterior cingulated gyrus and precuneus in easy Z-score imaging system (eZIS) analysis. In eZIS images after completion of the program, improvements were noted in regions that had previously shown reduced blood flow, with a particularly marked increase in blood flow in the frontal lobe, compared to that before therapy (Fig. 1).

Results of the CGA are given in Table 1 and as follows. At the first examination, the MMSE and HDS-R scores were 22 and 14, respectively. These scores increased to 25 and 24, respectively, after completion of four sessions of reminiscence therapy, and to 29 and 21, respectively, after completion of the program, showing a marked improvement of cognitive function compared to that before therapy. The patient showed temporal disorientation and delayed recall of three words, and was unable to enumerate a list of 10 vegetables before therapy, and these characteristics were also markedly improved by the therapy. The MMSE score was still 29 on re-evaluation 6 months after completion of the program.

The GDS-15 score was 10 on the first examination and showed no change after four sessions; however, the score decreased to 7 at completion of all sessions, indicating a slight improvement of depression, and we saw the patient smile more often than before treatment.

The VI and BI scores were 6 and 85, respectively, at the first examination, and increased to 10 and 90, respectively, after four sessions. After completion of all sessions, these scores were 9 and 95, respectively. The patient started to do things that he previously left to others, and started to read ancient documents again. The VI and BI scores remained at 9 and 95, respectively, in tests 6 months after completion of all sessions.

For the DBD and ZBI, the patient had no behavioral or psychological symptoms of dementia, and no numerical changes were noted after the therapy.

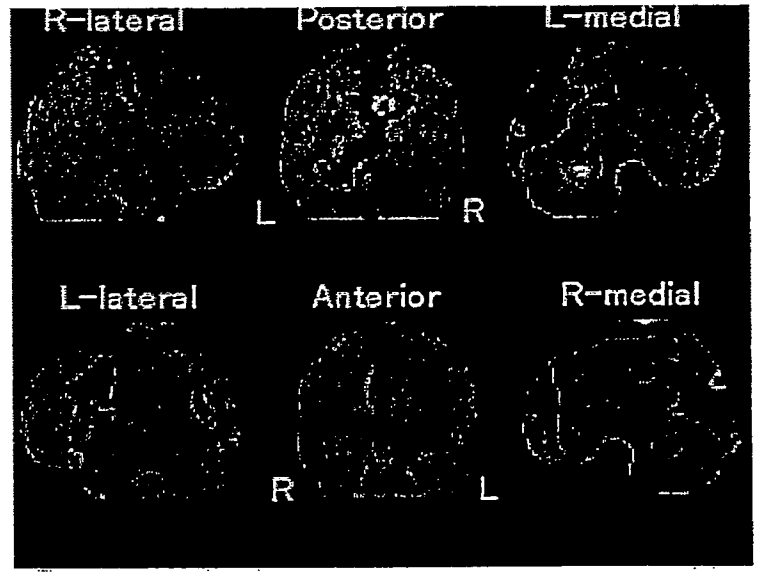
Discussion

Treatment with individual reminiscence therapy alone markedly improved attention, volition and depression in the patient. According to Butler, the pioneer of reminiscence therapy, life review is a healthy psychological behavior in which past events are re-evaluated, and this process brings about improvements in physical as well as mental and social activities,[7] thereby showing an effect on volition. Bohlmeijer *et al.* also reported that reminiscence was effective for senile depression;[8] however, there have been no reports of an objective effect on cognitive function. Several Japanese studies have suggested that reminiscence is effective mainly for psychological depressive tendency and decreased volition, but a marked effect on cognitive function has only been noted in a few reports. Kurokawa *et al.* reported that reminiscence was more effective for vascular dementia than for Alzheimer's-type dementia with regard to improvement of cognitive function,[9] and Urabe *et al.* showed that individual reminiscence improved cognitive function only in a few patients with Alzheimer's-type dementia.[5]

What was the cause of the marked improvement in cognitive function in our patient? One characteristic of the patient was an interest in ancient documents, history, education, politics and economics. He remembered some details of interesting events in his childhood and adolescence, and his memories became clearer as the sessions progressed. Furthermore, his family very cooperatively[10] attended all sessions, understood his condition in detail at each time point, and provided information to the physician, as reflected by the abundant information in the personal chart (life review) obtained before therapy. This background suggests that the following factors contributed to the effectiveness of individual reminiscence therapy for this patient: (i) a personal chart that provided extensive information prior to therapy; (ii) cooperation of the patient's family, not only in taking care of the patient but also in visiting the hospital and attending the therapy sessions; (iii) the patient's retention of memories of childhood and adolescence; (iv) the patient's interest in certain fields, although not very active; and (v) the patient had been solitary, and the sessions provided company and the chance for conversation. The effectiveness of individual reminiscence therapy may be greater in cases that follow this pattern.

An important characteristic of this case was the increased blood flow in the frontal association area, which is considered to be the center of volition, in

Before therapy



After the therapy

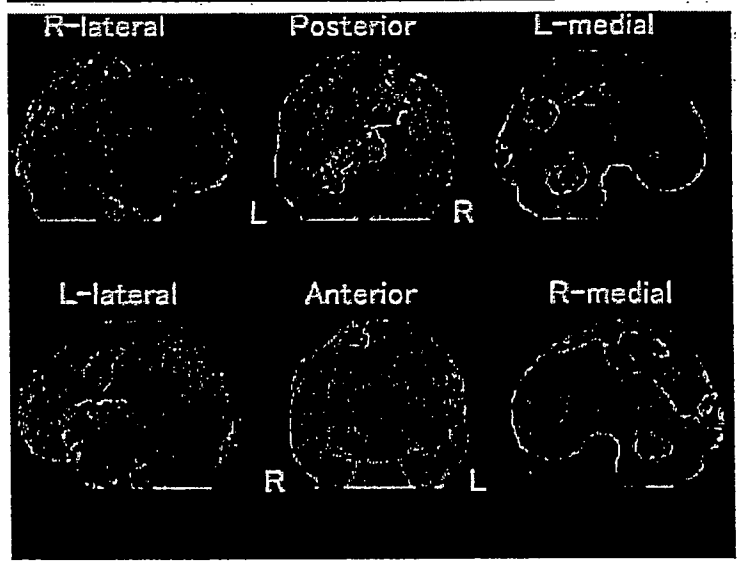


Figure 1 Easy Z-score imaging system (eZIS) image of single photon emission computed tomography (SPECT) before and after individual reminiscence therapy. Reduction of blood flow in the right frontal, and both left temporal, median-parietal and occipital lobes, posterior cingulated gyrus, and precuneus (upper panel). Comparing the SPECT findings before therapy, marked increase in the frontal lobe is noted (bottom panel).

SPECT (eZIS) performed after reminiscence therapy. Ushijima *et al.* performed SPECT and MMSE in 59 patients with Alzheimer's-type dementia and in 12 normal volunteers to find areas of reduced blood flow, and investigated the relationship between cognitive function and blood flow;[11] the results showed that attentiveness and calculation ability were associated with reduced blood flow in the frontal cortex. Migneco *et al.* reported that decreased volition (apathy) in Alzheimer's disease is related to reduced blood flow in the anterior cingulated gyrus in SPECT,[12] and Holthoff *et al.* also investigated decreased volition in early stage Alzheimer's disease by positron emission tomography, and found that decreased blood flow in the left orbitofrontal region had an influence.[13] Although association of decreased cognitive function and volition with a reduction of regional blood flow in the brain has been reported,[14] there has been no previous report of a marked increase in cerebral blood flow caused by individual reminiscence therapy. Therefore, this case provides an important demonstration of the relationship between blood flow in the frontal lobe and volition and cognitive function.

Table 1 Effect of reminiscence therapy on the score of Comprehensive Geriatric Assessment

	Before 13 May	After 2 months 5 August	End of session 16 September
Barthel Index (0–100)	85	90	95
MMSE (0–30)	22	25	29
HDS-R (0–30)	14	24	21
GDS (0–15)	10	10	7
Vitality Index (0–10)	6	10	9
Zarit Burden Interview (0–88)	21		19

GDS-15, 15-item Geriatric Depression Scale; HDS-R, Hasegawa's Dementia Scale – Revised; Mini-Mental State Examination (MMSE).

References

1 Butler RN. The life review; an interpretation of reminiscence in aged. *Psychiatry* 1963; 26: 65–76.
2 Livingstone G, Johnston K, Katona C, Paton J, Lyketsos CG. Systematic review of psychological approaches to the management of neuropsychiatric symptoms of dementia. *Am J Psychiatry* 2005; 162: 1996–2021.
3 Okumura Y, Tanimukai T, Kuse J. Assessment and number of sessions of reminiscence for elderly dementia patients. *J Jpn Soc Dement Care* 2005; 4: 24–31. (In Japanese with English abstract.)
4 Haight BK. The therapeutic role of a structured life review process in homebound elderly subject. *J Gerontol Psychol Sci* 1988; 43: 40–44.
5 Urabe M, Ogomori K. [Individual reminiscence therapy for dementia patients by interview in the presence of a person taking care of the patient.] *Rinsho Seishin Igaku* 2004; 33: 445–452. (In Japanese.)
6 Tanaka Y, Oyamada T. [Study of individual reminiscence for the elderly: (I) Gifu University Department of Education Study Report.] *Jinbunkagaku* 1997; 45: 77–89. (In Japanese.)
7 Butler RN. Successful aging and the role of the life review. *J Am Geriatr Soc* 1974; 22: 529–535.
8 Bohlmeijer E, Smit F, Cuijpers P. Effects of reminiscence and life review on life review on late-life depression: a meta-analysis. *Int J Geriatr Psychiatry* 2003; 18: 1088–1094.
9 Kurokawa Y (ed.). [Psychotherapy for the elderly: reminiscence.] Tokyo: Seisinshobou, 2005. (In Japanese.)
10 Nomura T. [Life reviews of dementia patients and their families.] *J Jpn Soc Dement Care* 2002; 1: 9–12. (In Japanese.)
11 Ushijima Y, Okuyama C, Mori S, Nakamura T, Kubota T, Nishimura T. Relationship between cognitive function and regional cerebral blood flow in Alzheimer's disease. *Nud Med Commun* 2002; 23: 779–784.
12 Migneco O, Benoit M, Koulibaly PM *et al.* statistical parametric mapping analysis indicate that apathy is a cingulate syndrome: a study of Alzheimer's disease and non-demented patients. *Neurol Image* 2001; 13: 896–902.
13 Holthoff VA, Beuthien-Baumann B, Kalbe E *et al.* Regional cerebral metabolism in early Alzheimer's disease with clinically significant apathy or depression. *Biol Psychiatry* 2005; 57: 412–421.
14 Benoit M, Clairet S, Koulibaly PM, Darcourt J, Robert PH. Brain perfusion correlates of the Apathy Inventory dimensions of Alzheimer's disease. *Int J Geriatr Psychiatry* 2004; 19: 864–869.

超高齢者におけるクレアチニンクリアランス推定式の比較検討

平山　俊一　　菊池　令子　　井上慎一郎　　塚原　大輔　　末光　有美
小林　義雄　　杉山　陽一　　長谷川　浩　　神崎　恒一　　井上　剛輔
鳥羽　研二

日本老年医学会雑誌　第44巻　第1号　別刷

〈原　著〉

超高齢者におけるクレアチニンクリアランス推定式の比較検討

平山　俊一[1)]　菊池　令子[2)]　井上慎一郎[2)]　塚原　大輔[2)]　末光　有美[2)]
小林　義雄[2)]　杉山　陽一[2)]　長谷川　浩[2)]　神崎　恒一[2)]　井上　剛輔[3)]
鳥羽　研二[2)]

要　約　目的：高齢患者は外来では24時間クレアチニンクリアランスの測定が困難であり，服用薬物数も多いため，クレアチニンクリアランス実測値をできるだけ正確に反映する推定式を利用することは臨床上重要である．対象：各種基礎疾患を有する85歳以上の超高齢者67名を含む入院高齢者143名（男性73名　女性70名　平均年齢82.9±8.6歳）．方法：4種のクレアチニンクリアランス推定式から得られた推定値と24時間クレアチニンクリアランスの実測値との相関を比較検討した．結果と結論：全体として今回の検討では超高齢者においてもCockcroft and Gaultの式による推定値が最もよい相関を示した．85歳以上の女性超高齢者において実測値と推定式の相関が低く，推定式の改定についても今後の検討課題と思われる．

Key words：超高齢者，クレアチニンクリアランス，推定式，Cockcroft and Gaultの式，安田の式

（日老医誌　2007；44：90-94）

緒　言

高齢社会の到来により，外来入院を問わず，高齢患者が増加の一途をたどっている．厚生労働省の推計によると，2004年度において85歳以上の超高齢者は273.4万人と報告されている[1)]．高齢者に腎排泄型薬剤を投与する際，適正な用量を設定するため腎機能を正確に評価する必要がある．腎機能を表す指標として，糸球体濾過量には一般的に内因性クレアチニンクリアランス（以下Ccrと略す）が使われている．クリアランス試験には24時間蓄尿が必要であるが，時間を要することや被験者に排尿，蓄尿という負担があり繁雑であることから外来で測定することは容易ではない．このため血清クレアチニン値（以下Scrと略す）からCcrを推定するいくつかの数式が提案されている．しかしこれらの数式は実際に投薬の必要な諸疾患を有する高齢者に当てはめる際，筋肉量の減少などのためScrによるCcr推定値と実測したCcrがかけ離れた値を取ることがある．外来の超高齢患者においても適切な薬物療法を行うためには腎機能を正確に評価する必要がある．このため種々の推定式による相関を調べどの推定式が最もよく超高齢者に適合するか検討を行った．

対象及び方法

杏林大学病院高齢医学科に2004年9月から2006年1月の間に入院した60歳以上の症例のうち，短期入院や，蓄尿不可能症例を除外し，尿道留置カテーテルを使用している患者や蓄尿が可能と判断された症例全例を対象にした．疾患や治療による除外は設けず，脳血管障害，感染症，経口摂取不良，利尿剤，補液などの様々な基礎疾患，治療を有する高齢者（平均年齢82.9±8.6歳（男性82.0±8.8歳　女性83.8±8.3歳））例を対象に行った．男女比及び84歳以下と85歳以上の症例数に偏りはなかった（表1）．対象高齢者全体の平均Scrは1.31±0.87mg/dlであった．身体測定，血液検査，尿検査などを測定し24時間蓄尿によるCcrを計算した．なお，Ccrは未補正のものを使用した．安田の式[2)]，Cockcroft and Gaultの式[3)]（以下C&G式と略す），折田の式[4)]，Walserの式[5)]の推定値を算出し，それぞれ推定値と実測値の相関を回帰分析，相関係数の差の検定により解析し比較検討した．さらに，層別解析として，84歳までの前期及び後期高齢者群76名と，85歳以上の超高齢者67名について男女別に層別解析を行った．

また実測値と推定式からの値との一致を箱ヒゲ図で求

1) S. Hirayama：東京薬科大学
2) R. Kikuchi, S. Inoue, D. Tsukahara, Y. Suemitsu, Y. Kobayashi, Y. Sugiyama, H. Hasegawa, K. Kouzaki, K. Toba：杏林大学病院高齢医学科
3) G. Inoue：都東村山老人ホーム診療所内科
受付日：2006.4.18，採用日：2006.7.12

表1　対象年齢分布

Age（歳）	n		
	男性	女性	全体
～84	42	34	76
85～	31	36	67
全体	73	70	143

め，値が外れ値となった症例については，患者の疾患や治療の背景，測定時の問題点について調査した．

本研究は，杏林大学高齢医学の入院に際して，CCr測定値を臨床研究に使用することを口頭で説明し同意を得て試行した．

(1) 安田の式

男性：Ccr（ml/min）＝（176－年齢）×体重（kg）÷（100×Scr（mg/100 ml））

女性：Ccr（ml/min）＝（158－年齢）×体重（kg）÷（100×Scr（mg/100 ml））

(2) Cockcroft and Gault の式

男性：Ccr（ml/min）＝（140－年齢）×体重（kg）÷（72×Scr（mg/100 ml））

女性：Ccr（ml/min）＝｛(140－年齢）×体重（kg）÷（72×Scr（mg/100 ml））｝×0.85

(3) 折田の式

男性：Ccr（ml/min）＝（－0.065×年齢－0.493×BMI＋33）÷（体重（kg）×Scr（mg/100 ml））×14.4

女性：Ccr（ml/min）＝（－0.052×年齢－0.202×BMI＋21）÷（体重（kg）×Scr（mg/100 ml））×14.4

(4) Walser の式

男性：Ccr（ml/min）＝7.57÷Scr（mM）－0.103×年齢＋0.096×体重（kg）－6.66

女性：Ccr（ml/min）＝6.06÷Scr（mM）－0.08×年齢＋0.08×体重（kg）－4.81

成　績

85歳未満の前期及び後期高齢者群において，安田，C&G，折田，Walser の推定値と24時間蓄尿による実測値の相関係数（r）は安田 r＝0.761，C&G　r＝0.761，折田 r＝0.693，Walser　r＝0.553 と安田の式，C&G 式で強い傾向があった．超高齢者群において，各々の推定式による推定値と実測値の相関係数は安田 r＝0.718，C&G r＝0.739，折田 r＝0.697，Walser r＝0.645 と，安田の式，C&G 式で相関が強い傾向があった（図1，図2）．超高齢者を男女に分け両群で各々の推定値と実測値の相関係数 r を比較したところ，男性で安田 r＝0.840，C&G r＝0.841，折田 r＝0.791，Walser　r＝0.736，女性で安田 r＝0.678，C&G　r＝0.690，折田 r＝0.667，Walser　r＝0.582 となり，男性に強い相関傾向があり，女性の相関係数は低かった（図3，図4）．また，超高齢者群において回帰係数を比較したところ，男性で安田＝0.796，C&G＝0.988，折田＝0.577，Walser＝0.375　女性で安田＝1.088，C&G＝1.262，折田＝0.776，Walser＝0.395 となった．

図5は超高齢者を男女で比較したものである．縦軸は実測値と推定値のずれの割合を示したもの（(実測値－推定値）×100/実測値）である．折田，Walser の式では，男女共に推定値が高く評価される傾向がある．

85歳以上の超高齢者での箱ひげ図における外れ値を検討し，実測値が高値となる6例の患者背景を調べた．輸液4例，利尿剤や Ca 拮抗薬など腎血流量を増加させる薬剤4例，腎不全2例，Scr 高値2例，心不全2例，CRP 高値2例であった．また，推定値が高値となる7例の患者背景を調べた．輸液5例，蓄尿不全または蓄尿少量4例，腎不全4例，癌3例，コントロール不良の糖尿病1例，胸水貯留，腹水貯留1例，肥満1例であった．

考　察

服用薬物数が多いほど薬剤有害作用の発現率は増加する傾向にある．また，加齢によってもその傾向は増加する[6]．その原因には加齢に伴う薬物動態学的・薬力学的な変化，多剤併用による相互作用，日常生活活動度（ADL）・認知機能の低下などが考えられるが，特に重大な原因として，腎機能の低下による相対的過量投与が挙げられる．Scr による腎機能の推定にはいくつか方法があるが高齢者，特に超高齢者になると筋肉量の低下により Scr が腎機能の低下と不相応な低値を示すことがしばしば見られる．Ccr 測定上の更なる問題点として正確な蓄尿の可否がある．加齢に伴う残尿，失禁の増加や患者自身による蓄尿もれなどにより，正確な24時間蓄尿が困難なことがある．1日尿量が少ないとき，Ccr 実測値と推定値のばらつきが大きいとの報告もある．今回は尿道留置カテーテルを使用している患者や蓄尿が可能と判断された患者の症例を対象とし，努めて正確な採尿を試みた．しかしながら，本来行うべきクリアランス法の実施には正確な蓄尿と安静を要し，判定に時間がかかるため実際の外来診療では実施困難なことが多い．従って Scr より Ccr を推定する種々の方法が提案されてきた．今回検討した安田の式，Cockcroft and Gault の式，折田の式，Walser の式は代表的な推定式であり Scr 値，性別，年齢，体重より Ccr を推定できる．C&G 式は欧米で最も広く用いられており欧米人によい相関を示して

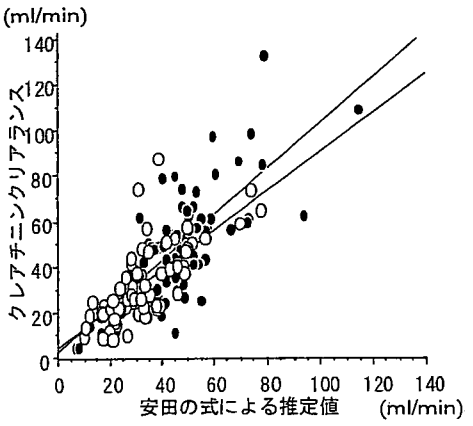


図1　安田の式　84歳以下と85歳以上の比較
○85歳以上；Y = 4.57 + 0.860X（r = 0.718）
●84歳以下；Y = 1.85 + 1.007X（r = 0.761）

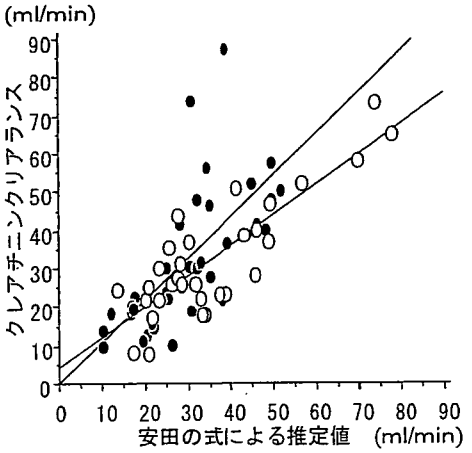


図3　安田の式　85歳以上の性差
○男性；回帰式 Y = 4.09 + 0.796X（r = 0.840）
●女性；回帰式 Y = 0.21 + 1.088X（r = 0.678）

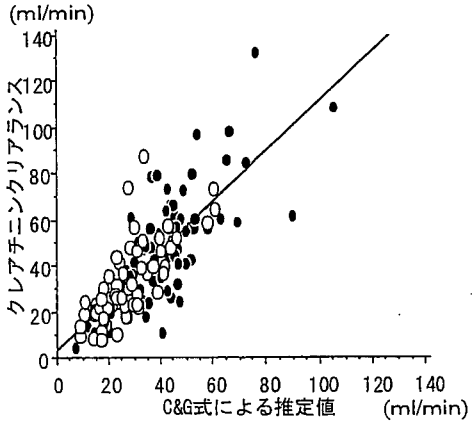


図2　C&G式　84歳以下と85歳以上の比較
○85歳以上；Y = 3.20 + 1.078X（r = 0.739）
●84歳以下；Y = 3.33 + 1.082X（r = 0.761）

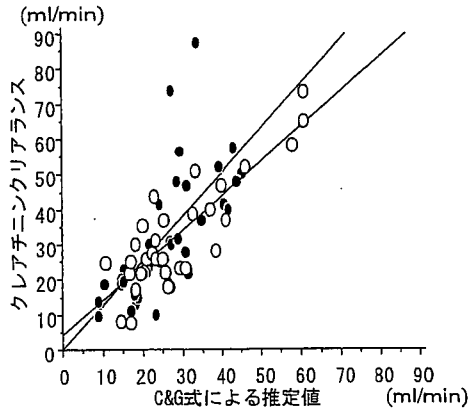


図4　C&G式　85歳以上の性差
○男性；回帰式 Y = 4.07 + 0.988X（r = 0.841）
●女性；回帰式 Y = − 0.09 + 1.262X（r = 0.690）

いる．今回の検討でも超高齢者における相関が0.739と最もよい相関を示した．この原因として日本人の体格が欧米化してきたことやC&G式作成時の対象年齢が18〜92歳と超高齢者も含まれていること，作成時の対象症例数が多いことが考えられる．C&Gの式に対して他の3式はいずれもその後に発表されたもので，安田の式は1.4mg/dl以下の血清クレアチニン値を示す高齢者に限定して式を求めたもので，腎不全患者は含めずに高齢者の腎機能を推定しようとしたものである[2]．一方，Walserの式は血清クレアチニン値を2.0mg/dl以上におき，腎不全患者のみを対象としている[5]．堀尾らの式は腎疾患患者を対象として，推定式にBMIの項を加えて肥満の特徴加味して作成された[4]．したがって，今回の対象のように腎機能が広範囲に亘る場合，C-Gの式以外では，いずれもずれが出てしまう結果となったのは，式の作成経緯による要素も大きいと考えられる．

今回，臨床の現場では安定した時期より外来や急性期での腎機能評価を必要とするため，疾患による除外は設けず，脳血管障害，感染症，経口摂取不良，利尿剤，補液などの様々な基礎疾患，治療を有する高齢者を対象に行った．推定式と実測値の乖離に関して，実測値が大きい場合は，輸液や降圧剤など腎血流量を増加させる治療が関与していた場合が多かった．この場合は臨床的には大きな実害は考えられない．一方，実測値が推定式より小さい場合は，相対的な薬物の過量投与など安全管理上，

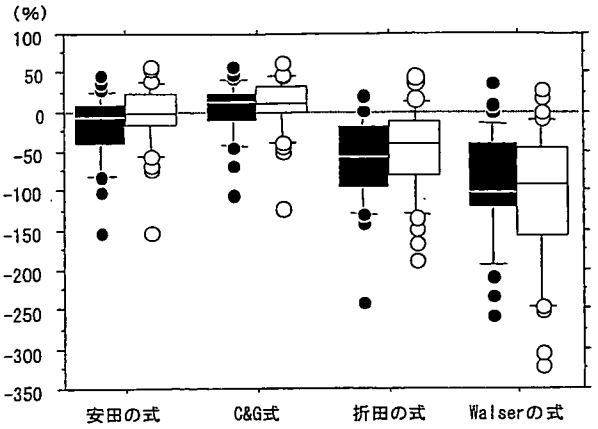


図5　超高齢者男女別において各推定式による推定値と実測値とのずれを箱ひげ図で％表示したもの
縦軸（実測値－推定値）×100/実測値
●男性
○女性

も問題となる．今回の検討では，腎不全，癌，乏尿，コントロール不良の糖尿病，胸水，腹水など複数の病態が重なる重症例で，有効循環血液量も日々変動しうる症例であった．このような症例に救急外来で遭遇した場合，血清クレアチニンから推定されるCcrの精度が低い可能性があることを銘記すべきであろう．Scrについては6.9までの高値も含まれているが，高値を除いた検討を行っても相関に大きな変化は見られなかった．全式において84歳までの前期及び後期高齢者群と85歳以上の超高齢者群に分け，相関を比較したところ，超高齢者群での相関が低い傾向にあり，超高齢者群での合併疾患の増加の影響が示唆される．これらを考慮しても，4種の推定式を比べると相関係数が最も高いC&G式が本邦超高齢者におけるCcr推定式として最適と考えられた．

超高齢者群を男女にわけC&Gの相関係数を比較したところ，男性0.841女性0.690と男性の相関が高い傾向にあった．また，回帰係数を比較したところ男性ではC&G式，女性では安田の式が1に近い値を示した．85歳以上の男性に安田の式を用いると過大評価する可能性があり，85歳以上の女性にC&G式を用いると過小評価する可能性がある．

一方，前期及び後期高齢者群の回帰係数を比較したところ男女ともに安田の式が1に近い値を示した．超高齢者の筋肉量について本邦での正確なデータは少ないが，中島らによれば70歳以降男性では上腕筋周囲，上腕筋面積が急速に減少するが女性ではほとんど変わらない[7]ことから女性の筋肉減少が時代とともに変化し，推定式の再構築が迫られている可能性があり，今後の検討課題と思われた．

本研究の限界として，膀胱留置カテーテルの適応がない蓄尿不可能症例を除外していることがあげられる．具体的には尿失禁症例や，認知症などが含まれるが，これらの症例に対してカテーテル留置を行ってクレアチニンクリアランスを測定し，高齢者全体に対するの推定式の良否を判断する研究は今後の課題であろう．

結　語

超高齢者において，正常値から腎不全を含む範囲の腎機能の判定に，24時間クレアチニンクリアランスの実測値と，すでに発表されている4つの式から求めた推定値とを比較して，超高齢者での推定式の有用性を検討した．4つの推定式のうち，C-Gの式はこの研究の目的にもっとも合致していた．一方，安田の式（高齢者，Scr: 1.4mg/dl以下），Wの式（Scr　2.0mg/dl以上）はいずれもその適用の目的の範囲で，また堀尾の式は腎疾患群内で有用と思われた．

全体として，臨床的に使用するうえでC&G式が最も優れているが，超高齢者への適用に当たっては，10%程度，推定値が低く求まるので，補正が望ましい．

今後超高齢者については，体格，サルコペニアの時代的変遷を考慮して改訂していく必要がある．

謝辞：本研究の一部は，長寿科学総合研究「縦断研究を基礎にした介護予防ガイドライン策定研究（H16痴呆骨折013；班長鳥羽研二），長寿医療研究事業「高齢者の安全な薬物療法ガイドライン策定研究（班長鳥羽研二）によった．

文　献

1) 厚生労働省ホームページ　平成17年度厚生統計要覧　総人口・日本人人口．性×年齢階級別．
2) 安田兵衛：腎機能の年齢的変化に関する研究．医学と生物学　1980; 101: 83-86.
3) Cockcroft DW, Gault MH: Prediction of Creatinine Clearance from Serum Creatinine. Nephron 1976; 16: 31-41.
4) Masaru Horio, Yoshimasa Orita, Shiro Manabe, Mitsuhiko Sakata, Megumu Fukunaga: Formula and Nomogram for Prediction Creatinine Clearance from Serum Creatinine Concentration. Clinical and Experimental Nephrology (1324-1751) 1997; 110-114.
5) Walser M, Drew HH, Guldan JL: Prediction of glomerular filtration rate from serum creatinine concentration in advanced chronic renal failure. Kidney International 1993; 44: 1145-1148.
6) 鳥羽研二，秋下雅弘，水野有三，江頭正人，金　承範，阿古潤哉ほか：薬剤起因性疾患．日老医誌　1999; 36: 181-185.
7) 中島久美子，秦　葭哉：身体組成としての筋肉量のアセスメント．日老医誌　2004; 42: 881-886.

Creatinine clearance estimation in the extremely elderly subjects

Shunichi Hirayama[1], Reiko Kikuchi[2], Shinichiro Inoue[2], Daisuke Tsukahara[2], Yumi Suemitsu[2], Yoshio Kobayashi[2], Yoichi Sugiyama[2], Hiroshi Hasegawa[2], Koichi Kouzaki[2], Gosuke Inoue[3] and Kenji Toba[2]

Abstract

Background: It has been reported that elderly outpatients take at least 6 different kinds of medication.

Purpose: To know which formula will best predict creatinine clearance, because 24-hour urine collection is difficult for elderly outpatients.

Patients and Methods: We compared four types of formulae (Cockcroft & Gault, Yasuda, Orita, Walser) to estimate creatinine clearance using serum creatinine of 143 elderly inpatients (73 men, 70 women, mean age 82.9±8.6 years old) including 67 extremely elderly people with various underlying diseases.

Result: The formula of Cockcroft and Gault showed the best correlation with creatinine clearance in the extremely elderly subjects ($r=0.74$) as well as in people under 85 years ($r=0.76$). However, the estimated values of the extremely elderly women were lower than actual creatinine clearance.

Conclusion: The formula of Cockcroft and Gault is the best predictive equation of creatinine clearance, except in the extremely elderly women.

Key words: *Extremely elderly, Creatinine clearance, Predicting formula, Cockcroft & Gault's formula, Yasuda's formula*
(Nippon Ronen Igakkai Zasshi 2007; 44: 90–94)

1) Tokyo University of Pharmacy and Life Science
2) Department of Geriatric Medicine, Kyorin University, School of Medicine
3) Department of Internal Medicine, Higashimurayama Nursing Home

Effectiveness of Multidimensional Exercises for the Treatment of Stress Urinary Incontinence in Elderly Community-Dwelling Japanese Women: A Randomized, Controlled, Crossover Trial

Hunkyung Kim, PhD, Takao Suzuki, MD, PhD, Yuko Yoshida, PhD, and Hideyo Yoshida, MD, PhD

OBJECTIVES: To evaluate the effectiveness of pelvic floor muscle (PFM) and fitness exercises in reducing urine leakage in elderly women with stress urinary incontinence (UI).

DESIGN: Randomized, crossover, follow-up trial.

SETTING: Urban community in Japan.

PARTICIPANTS: Seventy women aged 70 and older who reported urine leakage one or more times per month; 35 were randomly assigned to intervention and the other 35 to control.

INTERVENTION: The intervention group attended an exercise class aimed at enhancing PFMs and fitness. Duration of the exercise was 60 minutes per session twice a week for 3 months. After 3 months of exercise, the intervention group was followed for 1 year.

MEASUREMENTS: Body mass index (BMI), urine leakage, walking speed, and muscle strength were measured at baseline, after the intervention, and at follow-up.

RESULTS: In the intervention group, maximum walking speed and adductor muscle strength increased significantly after the intervention; there were no significant changes in the control group. After 3 months of exercise, 54.5% of the intervention group and 9.4% of the control group reported being continent. Within the cured group of UI, a significantly higher proportion had decreased their BMI at 3 months ($P = .03$) and increased walking speed at 3 ($P = .04$) and 12 ($P = .047$) months.

CONCLUSION: Decrease in BMI and increase in walking speed may contribute to the treatment of UI, although the data do not support a positive correlation between strengthening of adductor muscle and improvement of UI, which needs more research. J Am Geriatr Soc 55:1932–1939, 2007.

Key words: urinary incontinence; pelvic floor muscle exercise; fitness exercise; Japanese elderly women

From the Research Team for Promoting Independence of the Elderly, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan.

Address correspondence to Hunkyung Kim, PhD, Research Team for Promoting Independence of the Elderly, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakaecho, Itabashi-ku, Tokyo 173-0015, Japan. E-mail: kimhk@tmig.or.jp

DOI: 10.1111/j.1532-5415.2007.01447.x

Urinary incontinence (UI), particularly in elderly people, is considered to be an important factor for admission to a long-term care and has been associated with loss of independence, poorer quality of life, restricted social activities, and greater anxiety and social isolation.[1,2] Thus, prevention and treatment of UI in its early stages are important strategies for maintaining health and independence in elderly people.

Common treatments for UI include surgery, drug therapies, and behavioral interventions. Behavioral interventions such as pelvic floor muscle (PFM) exercises and bladder training have potential benefits with few risks and no side effects. PFM exercises, initiated by Kegel in 1948,[3] have been found to be efficacious for stress incontinence. Many investigators have validated the effectiveness of the short-term effect of PFM exercises in reducing urine leakage,[4–7] with improvement rates reported to range from 17% to 84%.[4,5] One study[6] demonstrated that exercise is more effective in women younger than 55 than in women aged 55 and older, indicating that the effect might be age dependent. Another study[5] reported that the results of PFM exercise is dependent on the degree and duration of treatment and frequent supervision by a therapist. Although the effects of PFM exercise have been observed in a large number of studies, the intervention conditions were different in these studies, and direct comparison of results is difficult. When evaluating the reported effects, the conditions that influence the intervention outcome should be considered carefully.

Several studies have examined the long-term effect of PFM exercise.[8–12] These studies were mainly hospital-based and conducted in subjects with a wide age range[11,12] in young postpartum women,[8] with a small number of participants,[12] or no control group.[10] Few randomized studies have reported the effect of PFM and functional fitness exercise on UI in community-dwelling elderly subjects.

The purposes of the present study were to evaluate the short- and long-term effects of PFM and functional fitness exercise and whether the fitness improvement was correlated with reduction in urine leakage in elderly community-dwelling Japanese women with stress UI.

0002-8614/07/$15.00