

To determine whether increased expression of LOX-1 protein by HB-EGF depends upon induced expression of LOX-1 mRNA, Northern blot analyses were performed. As shown in Fig. 2A, HB-EGF increased the amount of LOX-1 mRNA concentration-dependently. Time-course experiments showed that increased levels of LOX-1 mRNA were detectable similarly to the protein expression (Fig. 2B).

3.2. HB-EGF increased the uptake of DiI-labeled Ox-LDL

To determine whether upregulated expression of LOX-1 by HB-EGF is correlated with enhanced uptake of Ox-LDL,

uptake of DiI-labeled Ox-LDL into BSMCs were measured. After treatment with or without HB-EGF for 12 h, BSMCs were incubated with DiI-labeled Ox-LDL for additional 2 h. As shown in Fig. 3A, HB-EGF increased the internalization of DiI-Ox-LDL into BSMCs. The internalization of DiI-Ox-LDL increased by HB-EGF was inhibited by the 100-fold excess amount of unlabeled Ox-LDL. Increased internalization of DiI-Ox-LDL by HB-EGF was also inhibited by anti-LOX-1 monoclonal antibody (Fig. 3B). These results demonstrated that increases in LOX-1 expression by HB-EGF were associated with enhanced specific Ox-LDL uptake in BSMCs.

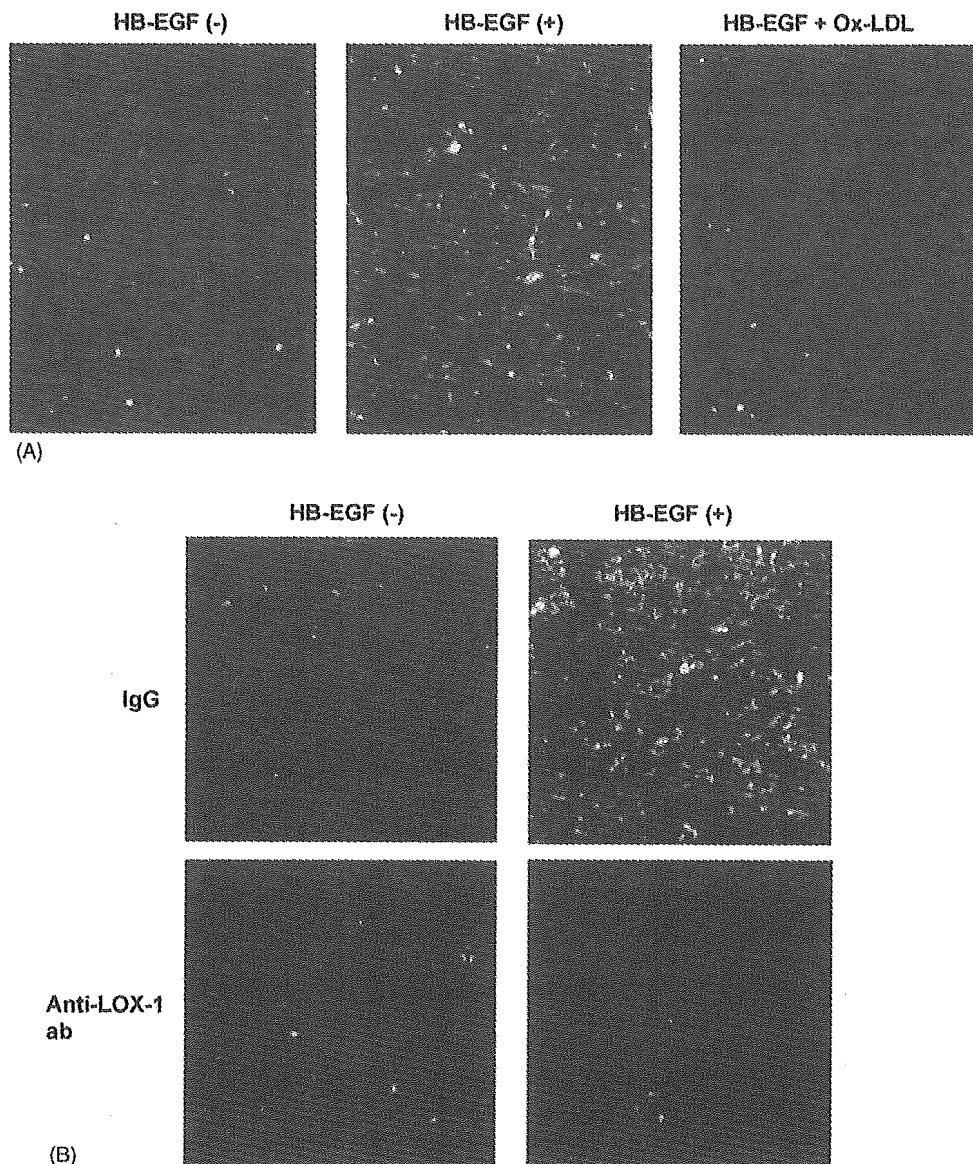


Fig. 3. HB-EGF increases the specific uptake of Ox-LDL in BSMCs. After treatment with or without 10 ng/ml of HB-EGF for 12 h, BSMCs were incubated with 5 µg/ml of DiI-labeled Ox-LDL for additional 2 h, in the presence or absence of 500 µg/ml of unlabeled Ox-LDL (A), and in the presence of a rat anti-LOX-1 monoclonal antibody or an irrelevant rat IgG (B). Representative pictures under fluorescence microscopy are shown.

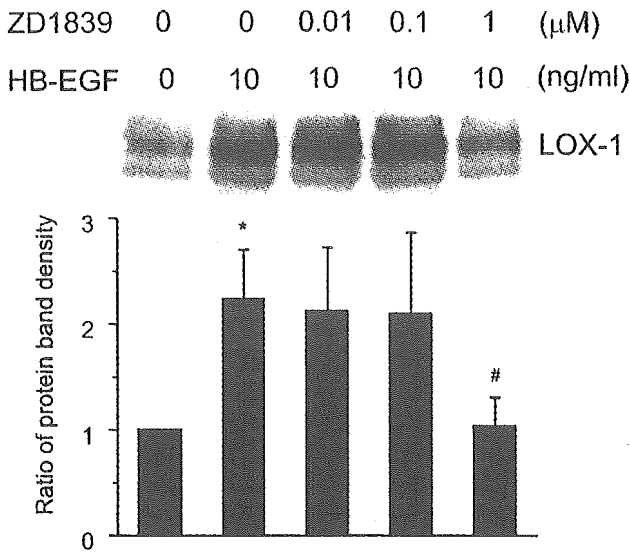


Fig. 4. HB-EGF-induced LOX-1 expression depends upon EGF receptor phosphorylation. After pretreatment with the indicated concentrations of ZD1839 for 1 h, BSMCs were treated with HB-EGF in the presence of ZD1839 for 10 h and then total cell lysates were subjected to Western blotting with a mouse anti-LOX-1 monoclonal antibody. Bar graphs indicate the mean \pm S.D. of three independent experiments. * $P < 0.001$, vs. 0 ng/ml of HB-EGF and # $P < 0.005$ vs. 10 ng/ml of HB-EGF.

3.3. HB-EGF-induced expression of LOX-1 depends upon EGF receptor phosphorylation

Previous studies have shown that EGF receptor phosphorylation mediates HB-EGF-dependent intracellular signal transduction. Therefore, we sought to determine if HB-EGF-induced LOX-1 expression depends upon EGF receptor phosphorylation. As shown in Fig. 4, ZD1839 (1 μM), an EGF receptor tyrosine kinase inhibitor that blocks EGF receptor phosphorylation [33], inhibited LOX-1 expression induced by HB-EGF to the basal level.

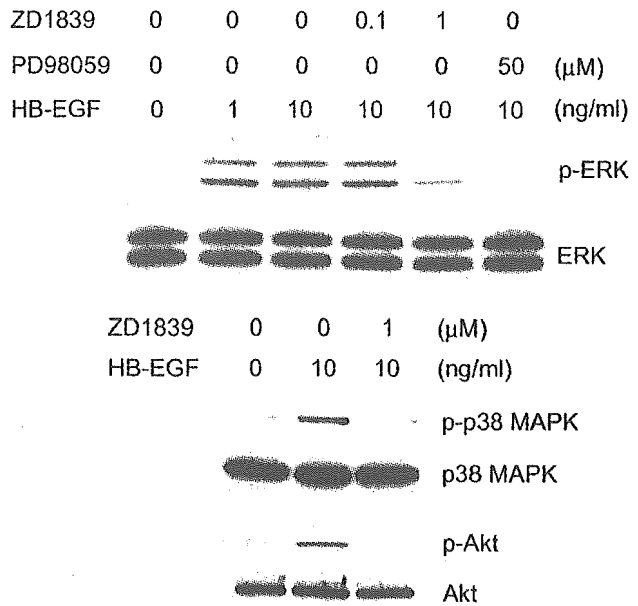


Fig. 5. HB-EGF induces phosphorylation of ERK, p38 MAPK and Akt. After pretreatment with the indicated concentrations of ZD1839 or PD98059 for 1 h, BSMCs were treated with HB-EGF in the presence of each reagent for 10 h and then total cell lysates were subjected to Western blotting with an anti-ERK, anti-phosphorylated ERK, anti-p38 MAPK, anti-phosphorylated p38 MAPK, anti-Akt or anti-phosphorylated Akt polyclonal antibody. A representative result from three independent experiments is shown.

3.4. HB-EGF induces phosphorylation of ERK, p38 MAPK and Akt depending upon EGF receptor phosphorylation

To determine whether HB-EGF, in fact, activates mitogen-activated protein kinases, such as ERK and p38 MAPK, or phosphatidylinositol 3-kinase (PI3K) in BSMCs, phosphorylation of ERK, p38 MAPK and Akt elicited by HB-EGF was measured. HB-EGF dose-dependently activated phosphorylation of ERK, as shown in Fig. 5. HB-EGF-induced

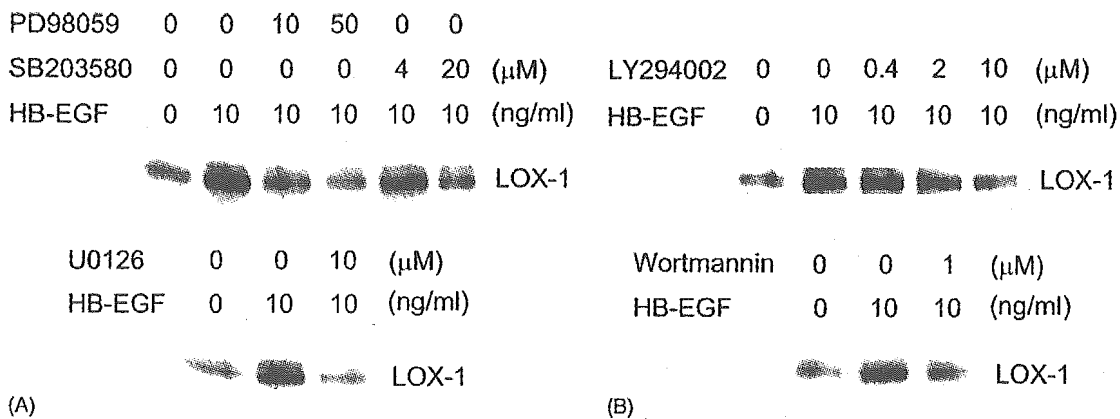


Fig. 6. HB-EGF-induced LOX-1 expression depends upon activation of MAPKs and PI3K. After pretreatment with the indicated concentrations of PD98059, SB203580, or U0126 (A), and LY294002 or wortmannin (B) for 1 h, BSMCs were treated with HB-EGF in the presence of each reagent for 10 h and then total cell lysates were subjected to Western blotting with a mouse anti-LOX-1 monoclonal antibody. A representative result from three independent experiments is shown.

ERK phosphorylation was suppressed by ZD1839, as well as PD98059, a MEK1 inhibitor, indicating the dependency upon EGF receptor phosphorylation. HB-EGF also slightly activated phosphorylation of p38 MAPK and Akt, which were suppressed by the EGF receptor phosphorylation inhibitor.

3.5. HB-EGF-induced expression of LOX-1 depends upon ERK, p38 MAPK and PI3K activation

We have further examined the dependency of HB-EGF-induced LOX-1 expression upon ERK and p38 MAPK as well as PI3K. As shown in Fig. 6A, both PD98059 and SB203580, inhibitors of MEK1 and p38 MAPK, respectively, significantly suppressed LOX-1 expression induced by HB-EGF. U0126, a strong and specific inhibitor of MEK1/2, also suppressed HB-EGF-induced LOX-1 expression. PI3K inhibitors, such as LY294002 and wortmannin, also suppressed HB-EGF-induced LOX-1 expression (Fig. 6B).

4. Discussion

Ox-LDL appears to play key roles in atherosclerotic progression and atherosclerotic plaque rupture. Effects of Ox-LDL on vascular cells appear to be mediated, at least in part, by Ox-LDL receptors, including LOX-1. In fact, LOX-1-mediated Ox-LDL uptake has been shown to induce cellular oxidative stress and activation of the proinflammatory transcription factor NF- κ B [34] in vascular endothelial cells. In vascular smooth muscle cells, LOX-1-mediated uptake of Ox-LDL induces apoptosis [10], which may potentially stimulate rupture of atheromatous plaques in concert with other proinflammatory responses [35–37].

On the other hand, HB-EGF is a potent mitogen for smooth muscle cells, which is produced by vascular endothelial cells, smooth muscle cells, macrophages, and T lymphocytes [21–23]. HB-EGF has been shown to modulate smooth muscle phenotype, including induction of a macrophage colony-stimulating factor receptor (c-fms) [38]. Expression of HB-EGF can be induced by a variety of biological stimuli, including proinflammatory cytokines [24] and a phospholipid component in Ox-LDL [25–27]. Upregulated expression of HB-EGF and EGF receptors has been demonstrated in human atherosclerotic lesions [29–31], as well as animal models of vascular injury [39], thus suggesting a pivotal role in atherogenesis.

In the present study, we have shown that LOX-1, a receptor for atherogenic Ox-LDL, can be induced by HB-EGF in cultured vascular smooth muscle cells. Enhanced expression of LOX-1 in intimal smooth muscle cells, as well as endothelial cells, in atherosclerotic lesions has also been demonstrated previously [19,20]. Therefore, HB-EGF produced in the intima of atherosclerotic lesions may induce expression of LOX-1, and thereby enhance Ox-LDL-induced

smooth muscle cell apoptosis, if abundant Ox-LDL is present, and thus may destabilize the atherosclerotic plaque and make it prone to rupture, although HB-EGF itself can also directly stimulate smooth muscle cell proliferation and inhibit apoptosis. This mechanism may represent one of the links between smooth muscle proliferation and lipid accumulation in atherogenesis, in addition to induced expression of HB-EGF by a lipid component of Ox-LDL [25–27].

Effects of HB-EGF on LOX-1 expression appear to be mediated by EGF receptor, because ZD1938, which blocks EGF receptor phosphorylation through inhibition of the receptor tyrosine kinase, suppressed HB-EGF-induced LOX-1 expression. In addition, both ERK and p38 MAPK, which are shown to be phosphorylated by HB-EGF, are involved in HB-EGF-induced LOX-1 expression, since PD98059, U0126, and SB203580 inhibited HB-EGF-induced LOX-1 expression. Furthermore, ERK and p38 MAPK phosphorylation by HB-EGF was suppressed by ZD1839, an inhibitor of EGF receptor tyrosine kinase. Moreover, PI3K is also involved in this process, because LY294002 and wortmannin inhibited HB-EGF-induced LOX-1 expression and HB-EGF, in fact, stimulated Akt phosphorylation. These results are consistent with previous reports showing that HB-EGF induces phosphorylation of EGF receptor and activates MAPK, and that HB-EGF-induced DNA synthesis is suppressed by PD98059 and LY294002 [40]. Although transcriptional regulatory mechanisms of LOX-1 gene have not been fully understood, oxidative stress has been implicated in LOX-1 gene induction [41,42]. Therefore, reactive oxygen species or redox-sensitive transcription factors may be upstream or downstream of MAPKs.

HB-EGF is a potent stimulus for smooth muscle cell migration and proliferation, and thus may modify atherosclerotic plaques into smooth muscle-rich stable ones. On the other hand, HB-EGF also induces LOX-1 which is a receptor for Ox-LDL and involved in Ox-LDL-induced apoptosis. Therefore, Ox-LDL might be a key factor to determine the stability of atherosclerotic plaques by modulating the function of smooth muscle cells, in addition to its actions on foam cell transformation and the production of matrix metalloproteinases.

In summary, the present report provides evidence, for the first time, that LOX-1 expression can be induced by HB-EGF in vascular smooth muscle cells. Further studies would elucidate the pathophysiological relevance of HB-EGF-induced smooth muscle LOX-1 expression in atherogenesis in vivo.

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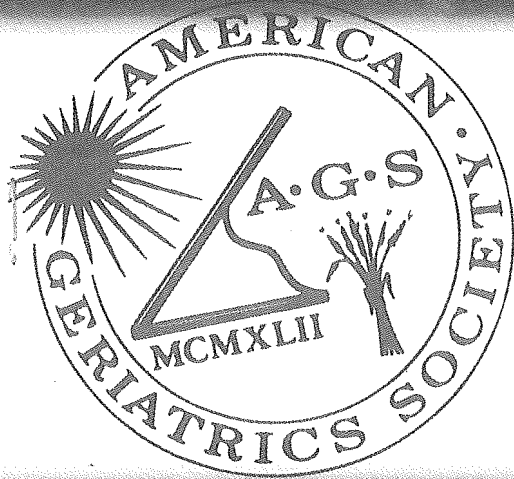
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Feasible Model for Prevention of Functional Decline in Older People: Municipality-Randomized, Controlled Trial

The Effect of a Task-Oriented Walking Intervention in Improving Balance Self-Efficacy Poststroke: A Randomized, Controlled Trial

Comparison of Brain Natriuretic Peptide and Probrain Natriuretic Peptide, in the Diagnosis of Cardiogenic Pulmonary Edema, in Patients Aged 65 and Older

People Aged Over 75 in Atrial Fibrillation On Warfarin: The Rate of Major Hemorrhage and Stroke in More Than 500 Patient-Years of Follow-Up

The Influence of Spiritual Beliefs and Practices on the Treatment Preferences of African Americans: A Review of the Literature

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ical Performance and Muscle Strength in the Elderly” by Penninx et al.¹ Anemia in older people may result from diverse causes associated with economic situation and lifestyle as well as underlying diseases such as malignancy, subclinical infection, and malnutrition. To confirm the findings of Penninx et al. we compared quantitative scores in activities of daily living (ADLs) and depression of community-dwelling elderly with and without anemia living in three towns in Japan. The study population consisted of 411 community-dwelling older people (174 men, 237 women, mean age = 71.7) living in Kyoto, 138 living in Hokkaido (54 men, 84 women, mean age = 79.8), and 379 living in Kochi (147 men, 232 women, mean age = 78.6) in Japan. According to the World Health Organization’s criteria for anemia (hemoglobin < 13 g/dL in men and < 12 g/dL in women),² the percentage of elderly subjects with anemia in Kyoto, Hokkaido, and Kochi was 11.9%, 26.0%, and 27.0%, respectively. Although reports of the prevalence of anemia varied from 2.9% to 61.0%,³ the higher prevalence of elderly subjects with anemia in Hokkaido and in Kochi than in Kyoto was presumed to be due to the higher mean age of the population in Hokkaido and Kochi. Seven basic ADL items (walking, ascending and descending stairs, feeding, dressing, going to the toilet, bathing, grooming) were assessed. Each basic ADL item was evaluated on a four-level scale (3 = completely independent; 2 = needing some help; 1 = needing much help; 0 = completely dependent). Then each item’s score was summed to generate a total basic ADL score ranging from 0 to 21.⁴ For higher-level daily activities, using the Tokyo Metropolitan Institute of Gerontology (TMIG) index of competence, a 13-item index including three sublevels of competence, each rated on a yes/no basis, was assessed: instrumental self-maintenance (5 items: the ability to use public transport, buy daily necessities, prepare a meal, pay bills, and manage banking matters), intellectual activities (4 items: the ability to fill out forms, read newspapers, read books or magazines, and take interest in television programs or news articles on health-related matters), and social roles (4 items: the ability to visit

NO POSITIVE CORRELATION BETWEEN ANEMIA AND DISABILITY IN OLDER PEOPLE IN JAPAN

To the Editor: We have read with interest the article entitled “Anemia Is Associated with Disability and Decreased Phys-

Table 1. Comparison of Activities of Daily Living (ADLs) of Community-Dwelling Elderly with and without Anemia in Kyoto, Japan

Characteristic	Without Anemia	With Anemia	ANOVA P-value
	(n = 360)	(n = 49)	
Age, mean ± SD	71.3 ± 4.5	74.1 ± 5.9	<.001
Female, %	56.5	57	.04
Body mass index, mean ± SD	22.7 ± 2.8	21.7 ± 2.4	.02
ADL score, mean ± SD (range 0–21)	20.9 ± 0.7	20.7 ± 0.6	NS
Information-related function, mean ± SD (range 0–12)	11.7 ± 0.7	11.7 ± 0.5	NS
Instrumental ADL score, mean ± SD (range 0–5)	4.9 ± 0.5	4.8 ± 0.8	NS
Intellectual ADL score, mean ± SD (range 0–4)	3.7 ± 0.6	3.7 ± 0.7	NS
Social roles, mean ± SD (range 0–4)	3.4 ± 1.0	3.5 ± 1.0	NS
Tokyo Metropolitan Institute of Gerontology total score, mean ± SD (range 0–13)	12.1 ± 1.5	12.0 ± 2.0	NS
GDS, mean ± SD (range 0–15)	3.3 ± 3.2	3.9 ± 3.0	NS
GDS ≥ 6, %	22.2	27.7	NS
GDS ≥ 10, %	4.9	6.3	NS

SD = standard deviation; ANOVA = analysis of variance; NS = nonsignificant; GDS = Geriatric Depression Scale.

friends, give advice to relatives and friends, visit someone at the hospital, and initiate a conversation with younger people). The Geriatric Depression Scale-15 (GDS-15) was used to screen older people for depression. Table 1 compares the mean age, sex ratio, body mass index, ADL scores, mean GDS-15 score, and the prevalence of depression (GDS cut-off = 6 (mild depression) and 10 (severe depression) of the groups with and without anemia in Kyoto. The nonanemic group was significantly younger than the anemic group, there were proportionally more women in the anemic group than in the nonanemic group, and body mass index was lower in the anemic group than in the nonanemic group, but there were no significant differences in basic and advanced ADLs, depression score, and prevalence of depression between the two groups. These findings were common to the other two towns (Hokkaido and Kochi) in Japan. The findings⁵ concluding that anemia in elderly residents in a nursing home in Japan might decrease the survival rate but that anemia itself did not lead to lower ADL scores coincided with our findings in Japan. Our findings suggest that the relationship between anemia and disability in older people in Italy that Penninx et al. reported is not necessarily a universal phenomenon, at least in community-dwelling elderly population in Japan. Anemia in older people may be associated with long-standing dietary habits and economic status as well as underlying diseases. Although anemia is an important issue in geriatric medicine, diverse relationships between anemia and other health conditions in older people require further study.

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ORIGINAL ARTICLE

Comprehensive geriatric assessment for community-dwelling elderly in Asia compared with those in Japan: VI. Maubin in Myanmar

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Background: The objective of the present study is to compare the findings of comprehensive geriatric assessments of community-dwelling elderly in Maubin township, Myanmar with those in Japan.

Methods: A cross-sectional, study was undertaken of community-dwelling people aged 60 years and over who were living in downtown Maubin and two rural villages near Maubin city, and 411 people aged 65 years and over who were living in Sonobe, Kyoto, Japan. They were examined using a common comprehensive geriatric assessment tool, which included interviews regarding activities of daily living (ADL), medical and social history, quality of life (QOL) and the 15-item Geriatric Depression Scale. Anthropometric, neurobehavioral and blood chemical examinations were also conducted. Using ANOVA and Post Hoc Scheffe's *F*-test, findings from the three groups were compared.

Results: Scores of basic ADL, instrumental self-maintenance, intellectual activities, social roles, QOL, Tokyo Metropolitan Institute of Gerontology Index of Competence, body mass index, total cholesterol levels, blood hemoglobin levels and HDL levels were lower in Myanmar's elderly subjects than in Japanese ones. There was no significant difference in prevalence of depression. Mean blood pressure measurements and rates of subjects with systolic pressure > 140 mmHg or diastolic pressure > 90 mmHg and prevalence of stroke were higher in downtown Maubin than in Japan. The atherogenic index was higher in Myanmar's elderly than in Japanese.

Conclusion: In Myanmar subjects had lower ADL and QOL scores than Japanese elderly. Of particular note is the higher prevalence of anemia and subjects with history of stroke in Myanmar than in Japan. Further study is needed to detect the cause of high prevalence of stroke in Myanmar.

Keywords: ADL, community-dwelling elderly, comprehensive geriatric assessment, Myanmar, QOL.

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Introduction

Since 1990, we have carried out comprehensive geriatric assessments of the functioning of community-dwelling elderly and providing efficient education

promote the health of the elderly populations living several towns in Japan.¹⁻¹³ The findings suggest that diseases and frailty in community-dwelling old subjects are diverse and are influenced by ecologic differences such as natural environments, historical backgrounds, the lifestyle, habits, religion and health promotion policies in the areas.

In this paper, as the sixth of a consecutive series of geriatric comparisons between Asian countries and Japan, we addressed the elderly living in Myanmar, one of the poorest countries in Asia. Comparisons of the general and geographic differences between Myanmar and Japan are shown in Table 1. (GDP/capita: Myanmar, US\$1800; Japan US\$28 200)¹⁴ Myanmar is sandwiched between Thailand and Laos to the east and Bangladesh to the west with India and China bordering it to the north. The country extends from approximately N28° to N10° latitude and undergoes an annual three-season cycle that follows the classic 'dry and wet monsoon climate' pattern common to other parts of mainland South-East Asia. In April and May, it can be unpleasantly hot. In the study area, temperatures often exceed 40° and most of the women use a traditional sunscreen called 'Thanaka' for protection from the strong ultra violet light (Fig. 1). Most of the country's agriculture is centered along the floodplains of the 2000 km-long Ayeyarwady (Irrawaddy) River. This study was completed in Maubin township in the Irrawaddy delta about 80 km west of the capital, Yangon.

In order to develop the appropriate policies to detect ecology-related risk factors for frailty in the elderly and to prevent disabilities in the elderly population, and also to provide useful care services to the frail elderly, we conducted comprehensive geriatric assessments in Myanmar.

Methods

Subjects

Myanmar

The study population consisted 209 elderly subjects living in downtown Maubin city (male : female, 119 : 94; mean age, 71.2 ± 7.4 years) and 127 subjects living in two rural villages situated 20 km south of city; A-LanGy village and Kyon-Sot village. Subjects examined were 100% of all eligible subjects aged 60 years or older in the two villages, and those in downtown Maubin were 35.9% of eligible subjects who were randomly sampled. This research was carried out in November 2004 at Maubin township in the Irrawaddy delta, 80 km southeast from Yangon (Fig. 2). Downtown Maubin and the two villages have populations of 49 400 and 2400, respectively, and the proportion aged 60 years or older was estimated at the average level for Myanmar (5.8%). Most people in the two villages were employed in farming and fisheries, while most of those in downtown Maubin were employed as merchants, shopkeepers, government workers, as well as farmers. In Myanmar there are several minorities throughout the country, but 82.1% of the study population was Burmese followed by Kayin (12.4%), Indian (1.4%), Chinese (1.1%), Chin (0.6%) and Shan (0.3%). All the elderly living in those areas were informed that they could have a health check-up including questionnaire, blood chemical examination, blood pressure measurement and consultation with a physician. Finally, 336 elderly subjects participated in our survey after giving informed consent. All subjects were interviewed and interpreted for our Japanese researchers by eight medical students from Yangon Medical Institute I.

Table 1 Comparison of the general and geographic factors between Myanmar and Japan

	Myanmar	Japan
Ethnicity	Burmar	Japanese
Total population in 2004	43 million	127 million
% 65 years or older	4.9%	19%
Religion	Buddhism (Hinayana)	Buddhism (Mahayana) or Shintoism
Economics		
GDP/capita in 2003	US\$1800	US\$28 200
Population bellow poverty line	25%	NA
Official Language	Burmese, English	Japanese
Adults literacy		
Total, per cent (male : female)	85.3 (89.2 : 81.4)	99 (99 : 99)
Life expectancy at birth in 2004		
Total (male : female)	56.0 (54.2 : 57.9)	81.0 (77.7 : 84.5)
Climate	Tropical wet climate	Humid subtropical climate

NA, not applicable.



Figure 1 Upper left and right, a child and frail older person with 'Thanaka' roughly applied to their faces; lower left, a woman with Thanaka' applied to her face.

Japan

Sonobe town is situated in Kyoto prefecture, in the middle portion of Japan (Fig. 2) and has a population of 16 700. The demographic data, scores of ADLs, QOLs, and medical data, such as blood pressure, were similar to the other Japanese towns, which we studied previously.^{3-13,15} The proportion of the population aged 65 years or older in Sonobe was 20.0%. All the elderly living in this town were given a self-rating questionnaire by mail, and then they were invited to check their health status, including blood chemical examination, blood pressure measurement and consultation with a physician in several community-houses called 'Kominkan'. Finally, the 411 elderly (male : female = 174 : 237; mean age, 71.7 years old; 12.3% of eligible subjects) participated in this survey at October in 2003.

Items of comprehensive geriatric assessment

Items of comprehensive geriatric assessment included activities of daily living (ADL), assessment of depression and quantitative assessment of quality of life (QOL) as well as medical and anthropometric indicators.

Activities of daily living

For basic-ADL assessment, each subject rated his/her independence in seven activities (walking, ascending and descending stairs, feeding, dressing, making his/her toilet, bathing, grooming) based on a scale from 3 to 0: 3, completely independent; 2, need some help; 1, need help much; 0, completely dependent). The items were added to give scores ranging from 0 to 21, with low scores indicating disability. Information-related function was assessed by summing the scores for four functions (visual acuity, hearing acuity, conversation and memory in one day) using a rating scale from 0 (cannot at all) to 3 (completely independent) to produce a total in the range of 0-12. For higher-level functional capacity, each subject rated his/her independence based on the Tokyo Metropolitan Institute of Gerontology (TMIG) index of competence.^{16,17} This assessment consists of a 13-item index including three sublevels of competence: (i) instrumental ADLs (five items: the ability to use public transport, buy daily necessities, prepare a meal, pay bills, handle banking matters; all rated on a yes/no basis); (ii) intellectual activities (four items: the ability to fill in forms, read newspapers, read books or magazines and interest in television programs or news articles on health-related

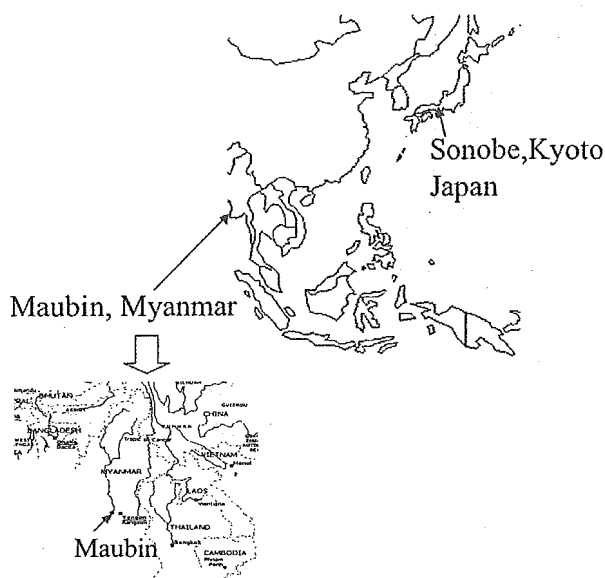


Figure 2 The study areas; Maubin township is situated in Irrawaddy delta, 80 km southeast of Yangon.

matters; all rated on a yes/no basis); and (iii) social role (four items, the ability to visit friends, give advice to relatives and friends who confide, visit someone at the hospital and initiate conversation with younger people; rated on a yes/no basis).

Depression and quality of life scores

We screened for depressive symptoms using the Japanese version of the 15-item Geriatric Depression Scale (GDS-15).^{18–20} We defined depression as a GDS-15 score of 6 or more, with a score of 6–9 indicating 'mild depression', and a score of 10 or more indicating 'severe depression'. QOLs were assessed using a 100 mm visual analog scale (VAS), with the worst QOL on the left end of the scale and best on the right, in the following five items; subjective sense of health, relationships with family, relationships with friends, financial status and subjective happiness.^{21–23} An explanation in Burmese was added for better understanding of the VAS.

Social, anthropometrical and medical assessments

Living conditions, lifestyle (drinking alcohol and smoking), and medical histories (histories of stroke, heart diseases and osteoarthropathies) were also assessed. Two blood pressure measurements in a sitting position were taken using an auto-sphygmomanometer (HEM 757, Omron, Japan) were averaged into blood pressure level of the subjects. In Myanmar, blood chemical analysis was conducted in the study area using a portable blood chemical analyzer (SP-4420, Arkray, Japan), and in Japan, it was done by a laboratory company, SRL. Blood sugar was measured casually both in Myanmar and in Japan.

Statistical analysis

Statistical analysis was performed using StatView ver.5 for Macintosh (SAS institute, Inc., Cary, NC). The one-factor ANOVA was used for continuous variables and the χ^2 test was used for categorical variables. *P*-values less than 0.05 were used to indicate statistical significance. Where *P*-values were less than 0.05 by one-factor ANOVA, Post Hoc Scheffe's *F*-test were performed to compare the scores between the two groups.

Results

Table 2 shows the comparison of baseline characteristics among the three elderly groups living in downtown Maubin, those in rural villages near in Myanmar Maubin and those in Sonobe, Kyoto, in Japan. The elderly subjects in rural villages in Myanmar were significantly younger (68.7 years old) than those in Sonobe (71.7 years old) and those in downtown Maubin (71.2 years old). There were differences in the ratio of men to women between downtown Maubin and Sonobe (55.2% and 42.3% male respectively). The percentage of the widowed was higher in downtown Maubin and rural villages in Myanmar (41.6% and 36.2%) than in Sonobe (18.8%). The proportions of elderly living alone in downtown Maubin and rural villages in Myanmar (13.9% and 13.4%) were higher than those in Sonobe (7.6%). The rates of the elderly working or do gardening every day in the two groups in Myanmar (56.0% and 59.1%) were higher than those in Sonobe (15.1%). More elderly in the rural villages were engaged in agriculture than in downtown Maubin, while the percentage of merchants and other workers was higher in downtown Maubin than the other. The proportions of subjects in the two groups in Myanmar who walked and exercised every day were higher (63.2% and 67.7%) than those in Sonobe (8.5%). The proportions of subjects who drank alcohol 'every day' in the two groups in Myanmar (1.4% and 0.8%) were lower than those in Sonobe (22.0%), while the percentages of subjects who were 'past' smokers were higher in the two areas in Myanmar (32.4% and 46.5%) than in Sonobe (14.1%).

The rates of elderly subjects taking any medication 'every day' were lower in downtown Maubin and in the rural villages in Myanmar (27.6% and 4.7%) than in Sonobe (47.2%), while the rates of elderly subjects taking any medication, most of which were traditional ones 'occasionally' were higher in Myanmar (40.9% and 48.7%) than in Sonobe (9.8%). The proportions of elderly subjects taking antihypertensive medication every day were lower in downtown Maubin and rural villages in Myanmar (13.9% and 2.4%) than in Sonobe (31.1%), but there were more people taking such medication 'sometimes' in Myanmar (28.7% and 29.9%)

Table 2 Baseline Characteristics between the community-dwelling elderly living in Myanmar and in Japan

	Downtown Maubin, Myanmar (<i>n</i> = 209)	Villages near Maubin, Myanmar (<i>n</i> = 127)	Sonobe, Kyoto Japan (<i>n</i> = 411)	<i>P</i>
Age	71.2 ± 7.4	68.7 ± 6.3	71.7 ± 4.8	
Male/female	116/94*	60/67	174/237	< 0.01
Marital status				< 0.0001
Widowed (%)	41.6*	36.2*	18.8	
Unmarried (%)	2.9	2.4	0.2	
Divorced (%)	2.4	2.4	0.7	
Lifestyle				
Living (%)				< 0.0001
With children or parents	48.8	55.9	53.4	
With spouse only	32.5	23.6	38.2	
Alone	13.9*	13.4*	7.6	
Others	4.8	7.1	0.7	
Work or do gardening (%)				< 0.0001
Yes (daily)	56.0*	59.1*	15.1	
Yes (sometimes)	6.2		0.7	
No	37.8	33.1	84.1	
Occupation				< 0.0001
Agriculture	10.4	57.6**	NA	
Merchant	20.4	3	NA	
Industry	6.2	1.5	NA	
Others	24.6	9.9	NA	
Retired	38.4	28	NA	
Do walking and exercise				< 0.0001
Yes (daily)	63.2*	67.7*	8.5	
Yes (sometimes)	17.7	15.8	4.2	
No	19.1	16.5	87.3	
Habits				
Drinking alcohol (%)				< 0.0001
Daily	1.4	0.8	22	
Sometimes	5.2	7.9	26.7	
No	93.3	90.5	51.4	
Smoking (%)				< 0.0001
Current	12.4	11	13.8	
Past	32.4	46.5	14.1	
No	55.2	42.5	72.0	
Medical				
Taking any medication (%)				< 0.0001
Daily	27.6	4.7	47.2	
Occasionally	40.9	48.7	9.8	
No	31.4	46.5	43	
Taking anti-hypertensive medication (%)			< 0.0001	
Daily	13.9	2.4	31.1	
Taking sometimes	28.7	29.9	2.9	
No	54.1	55.9	65	
Do not know	3.3	11.8	1	

Table 2 Continued

	Downtown Maubin, Myanmar (n = 209)	Villages near Maubin, Myanmar (n = 127)	Sonobe, Kyoto Japan (n = 411)	P
Recognition of blood pressure (%)				< 0.0001
Normal	30.8	21.2	60.6	
High (usually)	6.3	6.3	12.2	
High (occasionally)	38.0	29.9	24.8	
Do not know	25.0	42.5	2.4	
History of Stroke (%)				< 0.0001
Yes (male : female)	15.7* (18.1 : 12.8)	7.1** (6.7 : 7.4)	4 (5.2 : 3.1)	
No	83.8	92.9	96.0	
Do not know	0.5	0.0	0.0	
History of heart disease (%)				< 0.0001
Yes	11.4	2.4	21.2	
No	49	60.3	78.8	
Do not know	39.5	37.3	0.0	
History of osteoarthritis (%)				< 0.0001
Yes	22.6*	22.0*	59.4	
No	76.9	77.2	40.6	
Do not know	0.5	0.8	0.0	
History of fall (%)	26.2*	22*	12.8	0.0001

* $P < 0.05$ by Fisher's PLSD and by χ^2 test (comparison between downtown Maubin and Sonobe or the two rural villages near Maubin city and Sonobe); ** $P < 0.05$ by Fisher's PLSD and by χ^2 test (comparison between downtown Maubin and the two rural villages near Maubin city).

P-values were determined by ANOVA and χ^2 test (comparison among three groups).

NA, data is not available.

than in Sonobe (2.9%). Actually more people in downtown Maubin and rural villages in Myanmar (25.0% and 42.5%) did not know their blood pressure history. The proportion of subjects who recognized their own apparent history of stroke was higher in downtown Maubin (15.7%) than in rural villages (7.1%) and than those in Sonobe (4.0%). In downtown Maubin and Sonobe, more men than women had a histories of stroke. Many elderly in the two groups in Myanmar (39.5% and 37.3%) did not know if they had suffered heart disease or not. The rates of osteoarthritis were much lower in the two areas in Myanmar (22.6% and 22.0%) than in Sonobe (59.4%). A history of falls in the last year was more frequent in the two areas in Myanmar (26.2% and 22.0%) than in Sonobe (12.8%).

Table 3 shows the comparison of scores in ADL, GDS-15 and quantitative QOL among the three community-dwelling elderly groups. Scores in basic ADL, instrumental self-maintenance, intellectual activities, social role and TMIG-IC were higher in Japanese elderly subjects than those in those in Myanmar. In comparison within Myanmar, there were no significant differences in any mean score in ADL, GDS-15 or in the prevalence of depression, defined as cut-point (GDS ≥ 6) between downtown Maubin and the two rural villages near Maubin city. The prevalence of

depression, was higher in women than men in these three groups, but the difference was more obvious in Myanmar than in Japan (downtown Maubin: men 16.4%, women 30.9%; rural villages near Maubin city: men, 13.3%; women, 22.7%; Sonobe: men, 21.9%, women, 23.3%). The elderly in Myanmar had significantly lower scores in all QOL items. Compared with Myanmar, scores for a subjective sense of financial satisfaction was significantly higher in elderly subjects in rural villages near Maubin city than those in Maubin downtown.

Table 4 shows the comparison of anthropometrical indicators among the three elderly groups. Systolic and diastolic blood pressures were higher in subjects in Myanmar than in Japanese subject, although body mass index was significantly higher in elderly subjects in Japan than those in Myanmar. Within Myanmar, the mean value of systolic blood pressure and body mass index (BMI) were significantly higher in subjects in downtown Maubin than those in rural villages near Maubin city. Rates of subjects with systolic pressure > 140 mmHg or diastolic pressure > 90 mmHg based on the measurements of casual blood pressure was a great deal higher in elderly subjects in downtown Maubin than those in the two villages near Maubin city or in Japan.

Table 3 Comparison of scores for activities of daily living (ADL) and quality of life (QOL) between community-dwelling elderly living in Myanmar and in Japan

	Downtown Maubin in Myanmar (<i>n</i> = 209)	Two villages near Maubin in Myanmar (<i>n</i> = 127)	Sonobe, Kyoto in Japan (<i>n</i> = 411)	<i>P</i>
ADLs				
Scores of basic ADL (range, 0–21) (mean ± SD)	19.7 ± 2.7*	20.0 ± 2.1*	20.8 ± 0.7	< 0.0001
Per cent of independence of basic ADL	60.3	63.7	89.2	< 0.0001
Scores of information ADL (range, 0–12) (mean ± SD)	10.8 ± 1.2*	10.8 ± 1.2*	11.7 ± 0.8	< 0.0001
Per cent off independence of Information ADL	37.7	26	81.1	< 0.0001
Scores of instrumental ADL (range, 0–5) (mean ± SD)	3.5 ± 1.8*	3.8 ± 1.3*	4.9 ± 0.5	< 0.0001
Per cent of independence of instrumental ADL	42.9	40.9	92.6	< 0.0001
Scores of intellectual activity (range, 0–4) (mean ± SD)	2.3 ± 1.6*	2.0 ± 1.6*	3.7 ± 0.6	< 0.0001
Per cent of independence of intellectual ADL	37.6	28.3	80.2	< 0.0001
Scores of social role (range, 0–4) (mean ± SD)	3.3 ± 1.2	3.6 ± 0.8	3.5 ± 1.0	0.06
Per cent of social independence	68.5	76.4	69.2	NS
Scores of TMIG (range, 0–13) (mean ± SD)	9.1 ± 4.0	9.4 ± 3.1*	12.1 ± 1.6	< 0.0001
Per cent of independence of TMIG	33.8	21.3	58.4	< 0.0001
Depression				
Scores of GDS (range, 0–15) (mean ± SD)	3.8 ± 3.3	3.4 ± 2.7	3.4 ± 3.2	NS
Per cent of GDS ≥ 6	22.9	18.3	22.7	NS
Per cent of GDS ≥ 10	7.6	2.4	5.1	NS
QOL factors (range, 0–100)				
Subjective health (mean ± SD)	55.9 ± 19.8*	59.7 ± 18.6*	67.2 ± 17.8	< 0.0001
Family relationship (mean ± SD)	69.2 ± 21.6*	71.3 ± 20.1*	81.5 ± 16.9	< 0.0001
Friend relationship (mean ± SD)	63.8 ± 20.1*	66.0 ± 20.1*	79.8 ± 16.7	< 0.0001
Financial satisfaction (mean ± SD)	44.7 ± 18.4*	51.1 ± 18.6***	64.0 ± 21.5	< 0.0001
Subjective happiness (mean ± SD)	60.7 ± 21.9*	62.5 ± 20.2*	73.2 ± 17.6	< 0.0001

P* < 0.05 between downtown Maubin and Sonobe in Post Hoc Scheffe's *F*-test; *P* < 0.05 between two villages near Maubin city and Sonobe in Post Hoc Scheffe's *F*-test; **P* < 0.05 between downtown Maubin and two villages near Maubin city in Post Hoc Scheffe's *F*-test.

P-value based on ANOVA for continuous variables and χ^2 test for categorical variables.

GDS, geriatric depression scale; SD, standard deviation; TMIG, Tokyo Metropolitan Institute of Gerontology.

Table 5 shows the comparison of blood chemical findings among three elderly groups. Levels of serum total cholesterol (TC), HDL and GOT and hemoglobin concentrations were lower in elderly subjects in Myanmar than those in Japan. Atherogenic index ((TC – HDL)/HDL) and blood glucose levels were significantly higher in subjects in Myanmar than those in Japan. Of particular note are the facts that the rate of subjects with casual blood glucose level > 140 mg/dL was significantly higher in Myanmar and the prevalence of anemia, based on the

WHO criteria (defined as Hb < 13 g/dL in men, < 12 g/dL in women) was substantially higher in subjects in Myanmar than those in Japan. There were differences in total cholesterol levels, hemoglobin concentration and serum uric acid levels between the two groups in Myanmar.

Discussion

We found that the subjects in Myanmar had lower scores in basic ADL and TMIG-IC than Japanese subjects. The

Table 4 Comparison of anthropometrical indicators and blood pressure measurement among the community-dwelling elderly in Myanmar and in Japan

	Downtown Maubin Myanmar (n = 209)	Two villages near Maubin Myanmar (n = 127)	Sonobe, Kyoto Japan (n = 411)	P
Anthropometrical				
Height (mean ± SD), cm	155.7 ± 13.1	155.5 ± 8.3	154.2 ± 8.6	NS
Weight (mean ± SD), kg	48.6 ± 11.6*	45.8 ± 8.9***	53.9 ± 8.7	< 0.0001
Body mass index (mean ± SD)	21.0 ± 13.2*	18.9 ± 3.2***	22.6 ± 2.7	< 0.0001
Blood Pressure				
Systolic blood pressure (mean ± SD) (mmHg)	151 ± 30*	142 ± 29**	141 ± 20	< 0.0001
Diastolic blood pressure (mean ± SD) (mmHg)	85 ± 17*	83 ± 17*	78 ± 11	< 0.0001
Prevalence of hypertension (%) (SBP > 140 or DBP > 90)	61.9	48.0	48.7	0.0042

*P < 0.05 versus Sonobe, in Post Hoc Scheffe's *F*-test; **P < 0.05 between downtown Maubin and two villages near Maubin in Post Hoc Scheffe's *F*-test.

P-value based on ANOVA for continuous variables and χ^2 test for categorical variables.
SD, standard deviation.

Table 5 Comparison of blood chemical findings among the community-dwelling elderly in Myanmar and in Japan

	Downtown Maubin Myanmar (n = 209)	Two villages near Maubin Myanmar (n = 127)	Sonobe, Kyoto Japan (n = 411)	P
Total cholesterol (mean ± SD) (mg/dL)	175.1 ± 39.7*	164.0 ± 36.9***	209.5 ± 35.0	< 0.0001
HDL-cholesterol (mean ± SD) (mg/dL)	33.1 ± 9.2*	30.8 ± 10.2*	65.6 ± 16.7	< 0.0001
Atherogenic index (mean ± SD)	4.7 ± 2.3*	5.1 ± 4.7*	2.4 ± 0.9	< 0.0001
Creatinine (mean ± SD) (mg/dL)	1.03 ± 0.31*	1.00 ± 0.23***	0.89 ± 0.19	< 0.0001
Casual blood sugar (mean ± SD) (mg/dL)	124.6 ± 63.3*	119.4 ± 60.3*	99.3 ± 21.1	0.01
Per cent of impaired glucose tolerance (BS?140 mg/dL)	18.1	15.1	4.4	< 0.0001
Hemoglobin (mean ± SD) (g/dL)	10.9 ± 1.5*	10.5 ± 1.7***	13.6 ± 1.4	< 0.0001
Per cent of anemia (men : Hb < 13 g/dL, women : Hb < 12 g/dL)	86.1	88.8	12.0	< 0.0001
Uric acid (mean ± SD) (mg/dL)	4.6 ± 1.3 *	4.2 ± 1.1***	5.0 ± 1.3	< 0.0001
GOT (mean ± SD) (IU/L)	21.1 ± 9.3*	21.9 ± 6.6*	25.5 ± 16.2	0.0002
GPT (mean ± SD) (IU/L)	18.9 ± 0.9.9	20.0 ± 7.4	20.8 ± 22.3	NS

*P < 0.05 versus Sonobe, in Post Hoc Scheffe's *F*-test; **P < 0.05 between downtown Maubin and two villages near Maubin in Post Hoc Scheffe's *F*-test.

P-value based on ANOVA for continuous variables and χ^2 test for categorical variables.
SD, standard deviation.

difference in scores in ADL between Myanmar and Japan was more obvious in higher functional capacities, such as instrumental ADL and intellectual activities than in the basic one. These higher functions may be more greatly influenced by differences in economic status, infrastructure and educational level between two countries than basic ADL, because instrumental ADL was associated with economic activities, such as public transportation and shopping. But there were no significant differences in social ADL among the subjects. Intellec-

tual activities depend on literacy of old people and the average literacy level was lower in Myanmar (85.3%) than Japan (99%). It was also shown that there is a lower quantitative QOL score in Myanmar elderly subjects than in Japanese ones. Analysis of other contributing factors to lower QOL scores in Myanmar elderly, such as social, economic or spiritual factors remained to be determined due to the limitation of this study, but differences in the subjective sense of financial satisfaction between elderly subjects in downtown Maubin and in

rural villages may be partly due to recent economic development and the changing life. (Electricity was available in Maubin downtown, but that not in rural villages.)

In anthropometric measures, Myanmar elderly people had lower body height, body weight and BMI than Japanese people. Mean systolic blood pressures and rates of subjects with systolic pressure >140 mmHg or diastolic pressure >90 mmHg were higher in Myanmar's elderly subjects than in Japanese ones in spite of lower BMI in Myanmar elderly. This dissociation may be partly due to 'white-coat hypertension', but is supposed mainly to be due to different nutritional factors, including salt intake and to genetic differences between Myanmar elderly and Japanese one.²⁴ Rate of subjects with histories of stroke are extraordinarily higher in downtown Maubin than in Japan or the rural villages. Although this study could not prove a causal relationship, a higher prevalence of hypertension and atherosclerosis may raise the prevalence of stroke. One of the reasons why a higher prevalence of elderly subjects with stroke in Myanmar might be, partly due to over intake of oily and salty food or genetic factors.

In blood chemical examinations, total cholesterol, HDL and hemoglobin concentrations were lower in Myanmar elderly than in Japanese. Low density lipoprotein (LDL) data is not available in this study, but a higher atherogenic index in Myanmar elderly suggests a higher prevalence of high LDL-cholesterolemia in Myanmar. Fasting glucose levels were also not available, but higher rates of elderly subjects with casual glucose levels >140 mg/dL in Myanmar elderly subjects suggests a high prevalence of impaired glucose tolerance. This may be associated with the differences in intake of nutrition, lifestyle, prevalence of infectious diseases or other environmental, economic, and hereditary factors among three groups.

Conclusion

Comparative findings of comprehensive geriatric assessments of community-dwelling elderly between Myanmar and Japan were reported. In the economically developing country, Myanmar, the elderly had lower scores for ADL and QOL than Japanese elderly. Of particular note are the facts that the proportion of elderly with a history of stroke and prevalence of hypertension were significantly higher in Myanmar, and the prevalence of anemia was very much higher in Myanmar than in Japan. Further study is needed to detect the cause of the high prevalence of stroke, which worsens the health of the elderly in those areas.

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[67] Purification and Functional Analysis of a
Rab27 Effector Munc13-4 Using a Semiintact Platelet
Dense-Granule Secretion Assay

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Abstract

We have demonstrated that small GTPase Rab27 regulates dense-granule secretion in platelets. Using Rab27a affinity chromatography, we purified Munc13-4 as a novel Rab27a interacting protein from platelet cytosol. This chapter describes the purification of Munc13-4 and an *in vitro* assay system analyzing the mechanism of dense-granule secretion in platelets. The activity of Munc13-4 is tested in this assay.

Introduction

Rab27a was originally identified as a ras-related gene in a cDNA library of megakaryocytes (Nagata *et al.*, 1990). Subsequent discoveries of Rab27a as the responsible gene for Griscelli syndrome (Menasche *et al.*, 2000), an immunodefective disease with hypopigmentation, and its mouse model *ashen* (Wilson *et al.*, 2000) revealed the essential roles of Rab27a in cytotoxic T lymphocytes (CTLs) and melanocytes. In CTLs and melanocytes, Rab27a regulates lytic granule secretion (Stinchcombe *et al.*, 2001) and peripheral distribution of pigment granules (Hume *et al.*, 2001), respectively.

To date, several Rab27 effector molecules, including Slp1~5 and Slac2-a~c, have been documented (Kuroda *et al.*, 2002a,b). All these effectors interact with the GTP-bound form of Rab27 through their N-terminal conserved region, termed the Slp homology domain (SHD) (Kuroda *et al.*, 2002a,b). Evidence suggests that Rab27 interacts with different effectors in different types of cells to regulate distinct steps of membrane traffic. In melanocytes, for example, Rab27a interacts with its effector Slac2-a/melanophilin to regulate melanosome trafficking to the cell periphery, and another effector Slp2 for the tethering of melanosomes to the plasma membrane (Kuroda and Fukuda, 2004).

Rab27a and its close relative Rab27b are abundant Rab proteins in platelets, and they are shown to be associated with dense granules (Barral *et al.*, 2002; Shirakawa *et al.*, 2004). Since thrombogenic mediators, such as

ADP and serotonin, are contained in these granules, the release of dense granules from activated platelets contributes to hemostasis and thrombosis. In a recent study, we have shown that Rab27 regulates Ca^{2+} -induced dense-granule secretion in permeabilized platelets (Shirakawa *et al.*, 2004). Furthermore, we performed Rab27a affinity chromatography and successfully identified Munc13-4 as a novel GTP-bound Rab27a interacting protein in platelet cytosol (Shirakawa *et al.*, 2004). In contrast to Slps and Slac2s, Munc13-4 lacks the conserved Rab27-binding domain (SHD), indicating that Munc13-4 is a distinct type of Rab27 effector. Munc13-4 is a nonneuronal homologue of Munc13-1, a presynaptic protein essential for neurotransmitter release. This structural similarity implies a critical role of Munc13-4 in platelet granule secretion.

Recently, it has been reported that mutations in Munc13-4 cause familial hemophagocytic lymphohistiocytosis type 3 (FHL3), an immunodefective disease with impaired lymphocyte cytotoxicity (Feldmann *et al.*, 2003; Ishii *et al.*, 2005). In CTLs from FHL3 patients, lytic granule secretion is impaired at the perfusion step as observed in Rab27a-defective *ashen* mice (Feldmann *et al.*, 2003). Taken together with our finding that Munc13-4 is a Rab27-binding protein (Shirakawa *et al.*, 2004), this study indicates that Munc13-4 could act as a Rab27a effector in the secretion in not only platelets but also CTLs.

In this chapter, we describe the purification of Munc13-4 from platelet cytosol using Rab27a affinity chromatography. We also describe an *in vitro* secretion assay we developed to analyze the molecular mechanism of dense-granule secretion. The activity of Munc13-4 was evaluated in this assay using recombinant Munc13-4.

Purification of Munc13-4 from Platelet Cytosol Using Rab27a Affinity Chromatography

To identify Rab27 effector proteins in platelets, we performed Rab27a affinity chromatography using platelet cytosol as the source for interacting proteins. This experiment includes three principal steps: (1) preparation of platelet cytosol, (2) purification of glutathione *S*-transferase (GST)-tagged Rab27a, and (3) purification of interacting proteins from platelet cytosol using Rab27a affinity chromatography.

Solution

Buffer A: 50 mM HEPES/KOH, pH 7.4, 78 mM KCl, 4 mM MgCl_2 , 2 mM EGTA, 0.2 mM CaCl_2 , 1 mM dithiothreitol (DTT)