Tacrolimus but not cyclosporine A enhances FGF-2-induced VEGF release in osteoblasts

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Abstract. We previously reported that basic fibroblast growth factor (FGF-2) stimulates the release of vascular endothelial growth factor (VEGF) via p44/p42 mitogen-activated protein (MAP) kinase and stress-activated protein kinase/c-*Jun* N-terminal kinase (SAPK/JNK) in osteoblast-like MC3T3-E1 cells and that FGF-2-activated p38 MAP kinase negatively regulates VEGF release. In addition, p70 S6 kinase activated by FGF-2 negatively regulates VEGF release via SAPK/JNK. In the present study, we investigated the effects of tacrolimus (FK506) and cyclosporine A, well-known immunosuppressants, on the FGF-2-induced VEGF release in these cells. Tacrolimus, but not cyclosporine A which alone had no effect on VEGF basal levels, significantly enhanced FGF-2-stimulated VEGF release. Tacrolimus markedly enhanced FGF-2-induced phosphorylation of SAPK/JNK without affecting the phosphorylation of p44/p42 MAP or p38 MAP kinases. SP600125, a specific inhibitor of SAPK/JNK, reduced the amplification by tacrolimus of the FGF-2-induced VEGF release. The FGF-2-induced phosphorylation of p70 S6 kinase was suppressed by tacrolimus. These results strongly suggest that tacrolimus enhances FGF-2-stimulated VEGF release via up-regulation of SAPK/JNK through modulating p70 S6 kinase in osteoblasts.

Introduction

Vascular endothelial growth factor (VEGF) is an angiogenic growth factor displaying high specificity for vascular endothelial cells (1). VEGF, which is synthesized and secreted by a variety of cell types, stimulates proliferation of endothelial cells and increases capillary permeability (1). Bone metabolism is strictly regulated by osteoblasts and osteoclasts, responsible for bone formation and bone resorption, respectively (2). In addition, it is currently recognized that bone remodeling carried out by osteoblasts and osteoclasts is accompanied by new capillary extending (3,4). Capillary endothelial cells provide the microvasculature during bone remodeling. It is speculated that the activities of osteoblasts, osteoclasts, and capillary endothelial cells are closely coordinated and regulate bone metabolism (5). It is considered that these functional cells influence one another via humoral factors, as well as by direct cell-to-cell contact. As for bone metabolism, it has been reported that an inactivation of VEGF causes complete suppression of blood vessel invasion concomitant with impaired trabecular bone formation and expansion of the hypertrophic chondrocyte zone in the mouse tibial epiphyseal growth plate (6). It has been reported that osteoblasts among bone cells produce and secrete VEGF in response to various physiological agents (1,7-9). Based on these findings, it is well recognized that VEGF secreted from osteoblasts may play a crucial role in regulating bone metabolism (5,10). However, the exact mechanism underlying VEGF synthesis in osteoblasts and its release from these cells remains to be clarified.

Basic fibroblast growth factor (FGF-2) is produced in osteoblasts and FGF-2 is embedded in the bone matrix (11,12). FGF-2 expression in osteoblasts is detected during fracture repair (13). Therefore, it is considered that FGF-2 plays a pivotal role in fracture healing, bone remodeling and osteogenesis (14). We demonstrated that FGF-2 auto-phosphorylates FGF receptors 1 and 2 among four structurally related high affinity receptors in osteoblast-like MC3T3-E1 cells (15). As for VEGF release, we previously reported (16,17) that FGF-2 stimulates VEGF release in MC3T3-E1 cells, and that release is positively regulated by p44/p42 mitogen-activated protein (MAP) kinase and stress-activated protein kinase/c-*Jun* N-terminal kinase (SAPK/JNK) among the MAP kinase superfamily (18), while FGF-2-activated p38 MAP kinase

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negatively regulates VEGF release. In addition, we recently demonstrated that p70 S6 kinase functions at a point upstream from SAPK/JNK and limits FGF-2-stimulated VEGF release via down-regulating SAPK/JNK (19).

Tacrolimus (FK506) and cyclosporine A are potent immunosuppressive agents which are used clinically to prevent tissue rejection in organ transplants and to treat autoimmune diseases (20,21). As for bone metabolism, it has been shown that immunosuppressants cause bone loss when systemically administered at high doses over the long term (22,23). Recently, though, these agents reportedly up-regulate alkaline phosphatase activity and osteocalcin levels, and promote bone formation (24,25). Therefore, it is speculated that immunosuppressants induce osteoblast differentiation. However, the exact role of immunosuppressive agents in bone metabolism and osteoblasts is not fully understood.

In the present study, we investigated the effects of tacrolimus and cyclosporine A on the FGF-2-stimulated VEGF release in osteoblast-like MC3T3-E1 cells. We showed that tacrolimus but not cyclosporine A amplifies VEGF release via up-regulation of SAPK/JNK in these cells.

Materials and methods

Materials. The FGF-2 and mouse VEGF enzyme immunoassay kits were purchased from R&D Systems, Inc. (Minneapolis, MN). Tacrolimus hydrate (FK506) was purchased from Sigma Chemical Co. (St. Louis, MO). Cyclosporine A and SP600125 were obtained from Calbiochem-Novabiochem Co. (La Jolla, CA). Phospho-specific p44/p42 MAP kinase antibodies, p44/p42 MAP kinase antibodies, phospho-specific p38 MAP kinase antibodies, p38 MAP kinase antibodies, phospho-specific SAPK/JNK antibodies, SAPK/JNK antibodies, phospho-specific p70 S6 kinase antibodies and p70 S6 kinase antibodies were purchased from Cell Signaling, Inc. (Beverly, MA). The ECL Western blotting detection system was purchased from Amersham Biosciences (Piscataway, NJ). Other materials and chemicals were obtained from commercial sources. Tacrolimus, cyclosporine A and SP600125 were dissolved in dimethyl sulfoxide (DMSO). The maximum concentration of DMSO was 0.1%, which did not affect the assay for VEGF or the Western blot analysis.

Cell culture. Cloned osteoblast-like MC3T3-E1 cells derived from newborn mouse calvaria (26) were maintained as previously described (27). Briefly, the cells were cultured in α-minimum essential medium (α-MEM) containing 10% fetal calf serum (FCS) at 37˚C in a humidified atmosphere of 5% CO_2/95% air. The cells were seeded into 35-mm ($5x10^4$) or 90-mm ($5x10^5$) diameter dishes in α-MEM containing 10% FCS. After 5 days, the medium was exchanged for α-MEM containing 0.3% FCS. The cells were then used for experiments after 48 h.

Assay for VEGF. The cultured cells were stimulated by various doses of FGF-2 in 1 ml of α-MEM containing 0.3% FCS for the indicated periods. When indicated, the cells were pretreated with tacrolimus, cyclosporine A or SP600125 for 60 min. The conditioned medium was collected at the end of incubation, and the VEGF concentration was measured by ELISA kit.

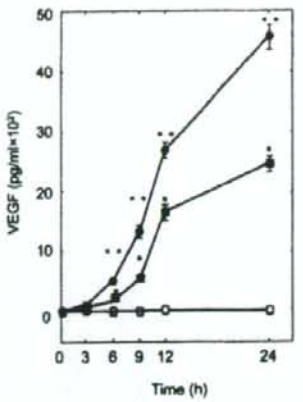

Figure 1. Effect of tacrolimus on the FGF-2-stimulated VEGF release in MC3T3-E1 cells. The cultured cells were pretreated with 300 ng/ml tacrolimus (circle symbols) or vehicle (square symbols) for 60 min, and then stimulated by 70 ng/ml FGF-2 (solid symbols) or vehicle (open symbols) for the indicated periods. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *p<0.05, in comparison to the control. **p<0.05, in comparison to the value of FGF-2 alone.

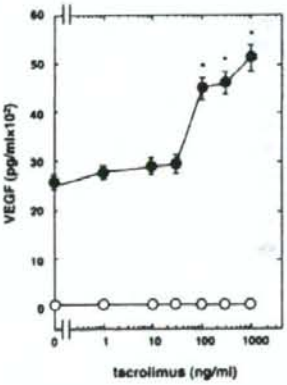

Figure 2. Dose-dependent effect of tacrolimus on the FGF-2-stimulated VEGF release in MC3T3-E1 cells. The cultured cells were pretreated with various doses of tacrolimus for 60 min and then stimulated by 70 ng/ml FGF-2 (●) or vehicle (○) for 24 h. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. *p<0.05, in comparison to the value of FGF-2 alone.

Western blot analysis. The cultured cells were stimulated by FGF-2 in α-MEM containing 0.3% FCS for the indicated periods. The cells were washed twice with phosphate-buffered saline and then lysed, homogenized and sonicated in a lysis buffer containing 62.5 mM Tris/HCl, pH 6.8, 2%

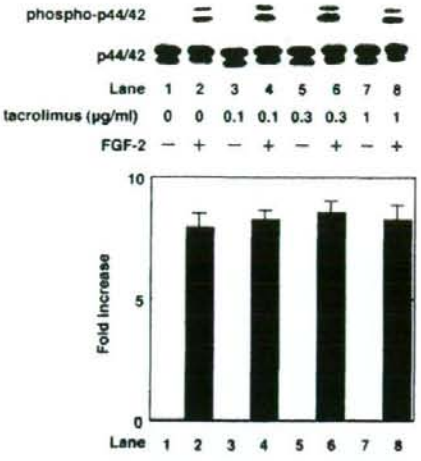

Figure 3. Effects of tacrolimus on the FGF-2-induced phosphorylation of p44/p42 MAP kinase in MC3T3-E1 cells. The cultured cells were pretreated with various doses of tacrolimus for 60 min, and then stimulated by 30 ng/ml FGF-2 or vehicle for 20 min. The extracts of cells were analyzed by Western blotting using antibodies against phospho-specific p44/p42 MAP or p44/p42 MAP kinase. The histogram shows quantitative representations of the phosphorylation level for p44/p42 MAP kinase obtained from a laser densitometric analysis. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

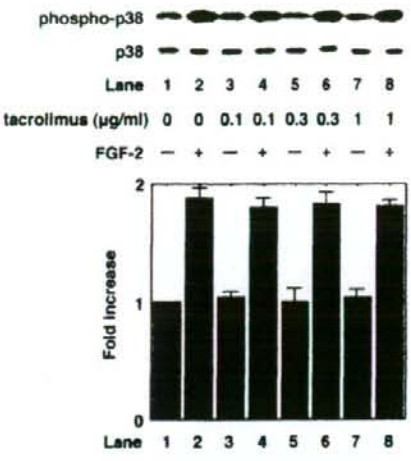

Figure 4. Effects of tacrolimus on the FGF-2-induced phosphorylation of p38 MAP kinase in MC3T3-E1 cells. The cultured cells were pretreated with various doses of tacrolimus for 60 min and then stimulated by 30 ng/ml FGF-2 or vehicle for 20 min. The extracts of cells were analyzed by Western blotting using antibodies against phospho-specific p38 MAP or p38 MAP kinase. The histogram shows quantitative representations of the phosphorylation level for p38 MAP kinase obtained from a laser densitometric analysis. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations.

sodium dodecyl sulfate (SDS), 50 mM dithiothreitol and 10% glycerol. The cytosolic fraction was collected as a supernatant after centrifugation at 125,000 x g for 10 min at 4˚C. SDS-polyacrylamide gel electrophoresis (PAGE) was performed according to Laemmli (28) in 10% polyacrylamide gels. The Western blot analysis was performed as described previously (29) by using phospho-specific p70 S6 kinase antibodies, p70 S6 kinase antibodies, phospho-specific p44/p42 MAP kinase antibodies, p44/p42 MAP kinase antibodies, phospho-specific p38 MAP kinase antibodies, p38 MAP kinase antibodies, phospho-specific SAPK/JNK antibodies or SAPK/JNK antibodies, with peroxidase-labeled antibodies raised in goat anti-rabbit IgG being used as second antibodies. The peroxidase activity on the polyvinylidine difluoride membrane was visualized on X-ray film by means of an enhanced chemiluminescence Western blotting detection system.

Determinations. The absorbance of enzyme immunoassay samples was measured at 450 nm with EL 340 Bio Kinetic Reader (Bio-Tek Instruments, Inc., Winooski, VT). A densitometric analysis was performed using the Molecular Analyst/Macintosh software program (Bio-Rad Laboratories, Hercules, CA).

Statistical analysis. The data were analyzed using ANOVA followed by the Bonferroni method for multiple comparisons between pairs, and a $p<0.05$ was considered to be significant. All data are presented as the mean ± SEM of triplicate determinations. Each experiment was repeated three times with similar results.

Results

Effects of tacrolimus or cyclosporine A on the FGF-2-stimulated VEGF release in MC3T3-E1 cells. To investigate the effects of immunosuppressants on the FGF-2-induced release of VEGF in MC3T3-E1 cells, we examined the effect of tacrolimus and cyclosporine A on VEGF release. Tacrolimus, which alone did not affect basal levels of VEGF, significantly amplified the FGF-2-induced release of VEGF in a time-dependent manner (Fig. 1). The amplifying effect of tacrolimus was dose-dependent in the range between 1 and 50 ng/ml (Fig. 2). Tacrolimus at 1 μg/ml caused ~100% enhancement in the FGF-2 effect. However, cyclosporine A failed to strengthen the FGF-2-induced release of VEGF in the range between 1 ng/ml and 1 μg/ml (<7.8 pg/ml for control; <7.8 pg/ml for 1 μg/ml cyclosporine A; 2686±145 pg/ml for 70 ng/ml FGF-2 alone; 2343±185 pg/ml for 70 ng/ml FGF-2 with 1 μg/ml cyclosporine A, as measured during the stimulation for 24 h).

Effects of tacrolimus on the FGF-2-induced phosphorylation of p44/p42 MAP kinase, p38 MAP kinase or SAPK/JNK in MC3T3-E1 cells. In our previous studies (16,17), we showed that p44/p42 MAP kinase and SAPK/JNK function as positive regulators in the FGF-2-stimulated VEGF release in osteoblast-like MC3T3-E1 cells and that FGF-2-activated p38 MAP kinase negatively regulates VEGF release. Therefore, in order to clarify whether the amplifying effect of tacrolimus on the FGF-2-stimulated VEGF release is through the activation of p44/p42 MAP kinase, p38 MAP kinase or

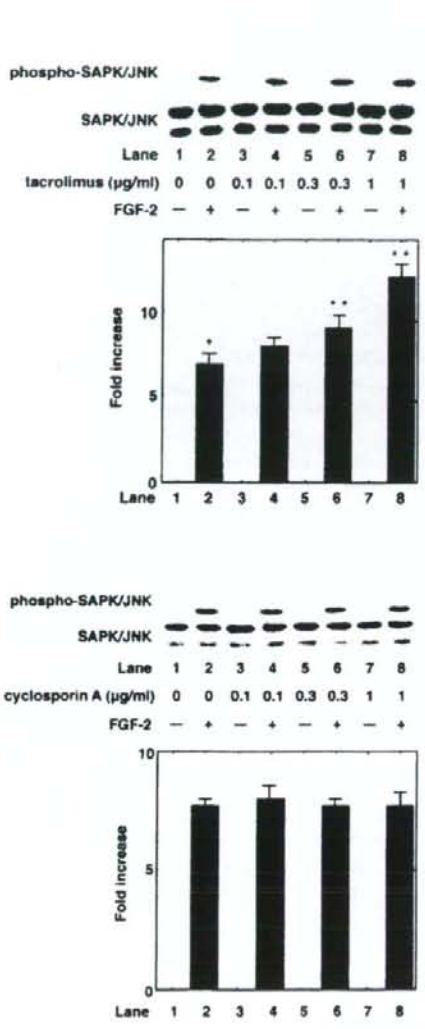

Figure 5. Effects of tacrolimus or cyclosporine A on the FGF-2-induced phosphorylation of SAPK/JNK in MC3T3-E1 cells. The cultured cells were pretreated with various doses of (A) tacrolimus or (B) cyclosporine A for 60 min and then stimulated by 30 ng/ml FGF-2 or vehicle for 20 min. The extracts of cells were analyzed by Western blotting using antibodies against phospho-specific SAPK/JNK or SAPK/JNK. The histogram shows quantitative representations of the phosphorylation level for SAPK/JNK obtained from a laser densitometric analysis. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}p<0.05$, in comparison to the value of FGF-2 alone.

SAPK/JNK in MC3T3-E1 cells, we next examined the effect of tacrolimus on the FGF-2-induced phosphorylation of p44/p42 MAP kinase, p38 MAP kinase and SAPK/JNK. The FGF-2-induced phosphorylation of p44/p42 MAP kinase (Fig. 3) and p38 MAP kinase (Fig. 4) was not affected by tacrolimus. On the contrary, tacrolimus, which by itself had no effect on the SAPK/JNK phosphorylation, significantly

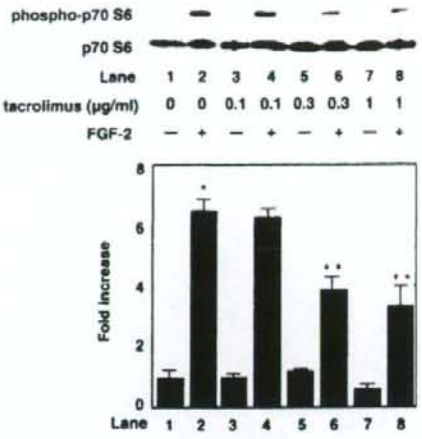

Figure 6. Effect of tacrolimus on the FGF-2-induced phosphorylation of p70 S6 kinase in MC3T3-E1 cells. The cultured cells were pretreated with various doses of tacrolimus for 60 min, and then stimulated by 30 ng/ml FGF-2 or vehicle for 20 min. The extracts of cells were analyzed by Western blotting using antibodies against phospho-specific p70 S6 kinase or p70 S6 kinase. The histogram shows quantitative representations of the phosphorylation level for p70 S6 kinase obtained from a laser densitometric analysis. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. $^{*}p<0.05$, in comparison to the value of FGF-2 alone.

enhanced the FGF-2-induced phosphorylation of SAPK/JNK (Fig. 5A). However, cyclosporine A had little effect on the phosphorylation of SAPK/JNK (Fig. 5B).

Effects of tacrolimus on the FGF-2-induced phosphorylation of p70 S6 kinase in MC3T3-E1 cells. We previously reported that p70 S6 kinase limits the FGF-2-stimulated release of VEGF via down-regulation of SAPK/JNK, composing a negative feedback system, in MC3T3-E1 cells (19). Thus, we examined the effect of tacrolimus on the FGF-2-induced phosphorylation of p70 S6 kinase. Tacrolimus markedly suppressed the FGF-2-induced phosphorylation of p70 S6 kinase (Fig. 6).

Effects of SP600125 on the amplification by tacrolimus of the FGF-2-induced VEGF release in MC3T3-E1 cells. SP600125, a specific SAPK/JNK inhibitor (30), which by itself did not affect basal levels of VEGF, significantly reduced the enhancement by tacrolimus of FGF-2-induced VEGF release (Table I). The enhanced levels by tacrolimus of FGF-2-induced VEGF release were reduced by SP600125 similar to the levels by FGF-2 with SP600125 treatment.

Discussion

We previously reported that FGF-2 stimulates the release of VEGF, which is an endothelial cell-specific mitogen and an angiogenic inducer (1) in osteoblast-like MC3T3-E1 cells (16). Accumulating evidence suggests that immunosuppressants modulate bone metabolism (22-25). Thus, we investigated the effects of tacrolimus and cyclosporine A,

Table I. Effect of tacrolimus on the enhancement by tacrolimus of the FGF-2-induced VEGF release in MC3T3-E1 cells.

SP600125	Tacrolimus	FGF-2	VEGF (pg/ml)
-	-	-	<7.8
-	-	+	2438±132[a]
-	+	-	<7.8
-	+	+	5733±221[b]
+	-	-	<7.8
+	-	+	1125±85[b]
+	+	-	<7.8
+	+	+	1208±103[c]

The cultured cells were pretreated with 10 μM SP600125 or vehicle for 60 min and then incubated with 0.3 μg/ml tacrolimus or vehicle for 60 min. The cells were stimulated by 70 ng/ml FGF-2 or vehicle for 24 h. Each value represents the mean ± SEM of triplicate determinations. Similar results were obtained with two additional and different cell preparations. [a]$p<0.05$, in comparison to the control. [b]$p<0.05$, in comparison to the value of FGF-2 alone. [c]$p<0.05$, in comparison to the value of FGF-2 with tacrolimus pretreatment.

well known and clinically used immunosuppressive agents (20,21), on the FGF-2-stimulated VEGF release in MC3T3-E1 cells. Tacrolimus markedly enhanced the FGF-2-stimulated release of VEGF in these cells. On the other hand, we found that cyclosporine A did not amplify VEGF release. Therefore, we investigated the mechanism of tacrolimus underlying the amplifying effect on the FGF-2-induced VEGF release in osteoblast-like MC3T3-E1 cells.

Previously, we demonstrated that FGF-2 induces the activation of p44/p42 MAP kinase, p38 MAP kinase and SAPK/JNK in osteoblast-like MC3T3-E1 cells (16,17). The MAP kinase superfamily mediates intracellular signal transduction of a variety of extracellular factors and plays a central role in cellular functions including cell proliferation, differentiation, and apoptosis (18). It is well recognized that three major MAP kinases: p44/p42 MAP kinase, p38 MAP kinase, and SAPK/JNK are central elements used by mammalian cells to transduce the diverse messages (18). In our previous studies (16,17), we demonstrated that p44/p42 MAP kinase and SAPK/JNK acted as positive regulators in the FGF-2-induced VEGF release in MC3T3-E1 cells. On the other hand, FGF-2-activated p38 MAP kinase functions as a negative regulator in VEGF release. In the present study, tacrolimus failed to affect the phosphorylation of p44/p42 MAP and p38 MAP kinases. Based on these results, it seems unlikely that tacrolimus affects the FGF-2-stimulated release of VEGF through up-regulating the activity of p44/p42 MAP kinase or down-regulating the activity of p38 MAP kinase in osteoblast-like MC3T3-E1 cells. On the contrary, we showed here that the phosphorylation levels of FGF-2-induced SAPK/JNK were markedly enhanced by tacrolimus. In addition, the amplification by tacrolimus of the FGF-2-stimulated VEGF release was suppressed by SP600125, a specific inhibitor of SAPK/JNK (30), similar to levels of FGF-2 with SP600125. In addition, we demonstrated that cyclosporine A had no effect on the phosphorylation levels of SAPK/JNK induced by FGF-2. In light of our findings, it is probable that tacrolimus enhances the FGF-2-stimulated VEGF release via strengthening the activity of SAPK/JNK in osteoblast-like MC3T3-E1 cells.

In our recent study (19), we showed that FGF-2-stimulated p70 S6 kinase functions at a point upstream of SAPK/JNK in osteoblast-like MC3T3-E1 cells and negatively regulated VEGF release by FGF-2. These findings led us to speculate that tacrolimus might modulate the FGF-2-induced p70 S6 kinase activation in MC3T3-E1 cells. Thus, we investigated the effect of tacrolimus on the FGF-2-stimulated activation of p70 S6 kinase. The FGF-2-induced phosphorylation of p70 S6 kinase was significantly attenuated by tacrolimus. Based on our collective results, it is most likely that tacrolimus modulates p70 S6 kinase-regulated SAPK/JNK in osteoblast-like MC3T3-E1 cells, resulting in enhanced FGF-2-stimulated VEGF release.

Tacrolimus (FK506) and cyclosporine A are well known immunosuppressants that are used mainly in clinical organ transplantation (20,21). It has recently been shown that these agents have significant effects on bone metabolism (22-25). The immunosuppressants that were employed at high doses after organ transplantation, reportedly caused the reduction in bone mineral density (22,23). On the contrary, evidence is accumulating that tacrolimus induces the promotion of osteoblastic differentiation *in vitro* (24,25,31). Our present results indicate that not cyclosporine A but tacrolimus plays a role in the control of the production of VEGF, one of the key regulators of bone metabolism. It is generally recognized that new capillary extending is essential for bone remodeling (5). Since VEGF is a specific mitogen of vascular endothelial cells (1), our present findings suggest that tacrolimus-enhanced VEGF release from osteoblasts play an important role in the pathophysiological process of bone remodeling under the medication of this agent. It is probable that tacrolimus positively regulates microvasculature development in bones. Therefore, tacrolimus might be considered to be a potent therapeutic agent useful for the disorder of bone metabolism. However, the details regarding immunosuppressants in bone metabolism still remain unclear. Further investigation is necessary to elucidate the exact mechanism of tacrolimus in osteoblasts and in bone metabolism.

In conclusion, our present results strongly suggest that tacrolimus enhances FGF-2-stimulated VEGF release via up-regulation of SAPK/JNK through modulating p70 S6 kinase in osteoblasts.

Acknowledgements

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Relationship between oral function and general condition among Japanese nursing home residents

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ABSTRACT

The purpose of this study was to clarify the relationship between oral function and general condition among Japanese nursing home residents. The hypothesis was that oral function is one of the most important factors for the maintenance of general condition in dependent elderly. Seventy-nine residents of a nursing home in Japan participated in this study (54 women and 25 men, age range: 65–95 years, mean age: 82.2 ± 8.5). A water drinking test and gargling function were used as indicators of oral function. Indicators of general condition included cognitive function (mini-mental state examination; MMSE), ADL (Barthel index), and nutritional status (body mass index = BMI, and serum albumin level). To clarify the relationship between oral function and general condition among dependent elderly, statistical evaluations of correlations (Spearman rank correlation coefficient) and differences (Mann–Whitney *U* test, Student's *t* test) between groups were conducted. SPSS was used for the statistical analysis. The water drinking and gargling function tests showed a strong correlation ($p < 0.001$) with cognitive function and ADL. The water drinking and gargling function tests showed a correlation with BMI ($p < 0.005$, $p < 0.01$, respectively), and the water drinking test showed a correlation with serum albumin level ($p < 0.05$). However, no correlation was observed between the gargling function tests and serum albumin level. It is concluded that oral function is closely related to cognitive function, ADL, and nutritional status. Oral function may play an important role in maintaining general condition in dependent elderly. To prevent decreases in cognitive function, ADL and nutritional status in dependent elderly, the importance of improvements in oral function cannot be over-emphasized.

1. Introduction

In a number of societies, the proportion of elderly in the population is rapidly increasing. In Japan, the proportion of elderly citizens has been increasing, and the government predicts that by the year 2015 more than 25% of all citizens will be over 65 years of age. With the rise in the elderly population, the issue of their health is becoming a social problem. It is estimated that approximately 3 million elderly Japanese people have physical or mental disabilities, and this number is predicted to rise to 5.3 million by 2025 (Ministry of Health, Labour and Welfare of Japan, 2003). Frail elderly people often have serious dental problems, including impaired oral functions. The main factors in death of elderly people are considered to be nutritional disorders and breathing problems, which are thought to be related to their impaired oral function.

Aspiration pneumonia is a prevalent and costly infection that is a significant cause of morbidity and mortality, especially in the elderly (Bentley, 1984). In addition to the risk factors of impaired cognitive function and ADL, aspiration due to impaired oral function is also recognized as one of the main causes of aspiration pneumonia. It has also been suggested that oral bacterial flora may function as a reservoir of potential respiratory pathogens that facilitate colonization on the oropharynx (Sumi et al., 2002, 2003, 2006, 2007).

In Japan more than 8000 elderly people die annually from asphyxia due to airway obstruction by food. A contributing factor in this is thought to be decreased oral function, including in elderly people who have no problems eating in their daily lives. There are also reports of a relation between the nutritional status of elderly people and their oral function, and the importance of improving oral function in the elderly has been indicated (Ikebe et al., 2006).

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The need to improve oral function was recognized in general society with the introduction in 2006 of measures to improve oral function in the new care insurance prevention benefits in Japan. Thus, it is important to examine the relationship between oral function and general condition among dependent elderly. However, the relationship between general health and oral function among frail elderly people is unclear. The purpose of this study was to clarify the relationship between oral function (water drinking and gargling function tests) and general condition (cognitive function, ADL, and nutritional status) in Japanese nursing home residents.

2. Subjects and methods

2.1. Subjects

Seventy-nine residents of a nursing home in Japan participated in this study (54 women and 25 men, age range: 65–95 years, mean age: 82.2 ± 8.5), after they or a family member fully understood the purpose of the study and gave consent for participation. Participants whose general condition was bad on the day of the examination or who could not be evaluated because of hospitalization or other reasons were excluded from the analysis.

2.2. Methods

Indicators for oral function evaluation were: (1) gargling function, a modified version of the gargling test (Ushiyama, 2003) was used (Table 1). (2) Water drinking test, a modified version of the water drinking test of Tohara et al. (2003) was used for evaluation (Table 2).

Indicators for general function evaluation were: (1) assessment of cognitive function, the mini-mental state examination (MMSE) (Folstein et al., 1975) was used by a psychiatrist. (2) Assessment of ADL, the Barthel index (Collin et al., 1988) was used for assessment. (3) Assessment of nutritional status, nutritional status was assessed with the indicators of serum albumin level and BMI, which were thought to reflect the nutritional status of the participants.

In the statistical analysis, Spearman's rank correlation coefficient was used for correlations. For tests between two groups, the Mann–Whitney U test, Student's t test, and Welch's t test were used with 4 as the threshold in the water drinking test and 2 as the threshold for gargling function. The SPSS statistical software package was used.

2.3. Ethical considerations

The ethical regulations of the National Center for Geriatrics and Gerontology were strictly followed. The study was approved by the National Center for Geriatrics and Gerontology.

3. Results

3.1. Relationship between oral function and cognitive function

The participants were divided into two groups according to gargling function, with 2 as the threshold, and cognitive function was investigated. It was found that the group with low gargling function (gargling function score of 3 or more) had significantly lower ($p < 0.001$) cognitive function (MMSE). In a test of correlation using Spearman's rank correlation coefficient, a negative correlation of $\gamma = 0.766$ ($p < 0.001$) was found between gargling function and cognitive function (Fig. 1).

Participants were divided into two groups of those with a score of 4 or less in the water drinking test and those with a score of 5, and cognitive function was investigated. The group with the lower score (4 or less) in the water drinking test had a significantly lower ($p < 0.001$) cognitive function (MMSE). Correlation was tested using Spearman's rank correlation coefficient, and a positive correlation of $\gamma = 0.634$ ($p < 0.001$) was found between gargling function and cognitive function (Fig. 2).

Table 1
Gargling test

1	Can be executed without problem
2	Can be executed with some difficulty
3	Water can be held in the mouth only
4	Water can be put in the mouth, but is swallowed
5	Water cannot be put in the mouth

Table 2
Water drinking test

1	No swallowing; gulping and/or changes in respiration
2	Swallowing; no gulping; changes in respiration or moist hoarseness
3	Swallowing, gulping
4	Swallowing, no gulping, no moist hoarseness
5	(In addition to 4) Swallowing can be repeated once in 30 s

3.2. Relationship between oral function and ADL

Participants were divided into two groups according to gargling function, with 2 as the threshold, and ADL was investigated. It was found that the group with low gargling function (gargling function score of 3 or more) had significantly lower ($p < 0.001$) ADL (Barthel index). In a test of correlation using Spearman's rank correlation coefficient, a negative correlation of $\gamma = 0.8000$ ($p < 0.001$) was found between oral function and ADL (Fig. 3).

Participants were divided into two groups of those with a score of 4 or less in the water drinking test and those with a score of 5, and ADL was investigated. The group with the lower score (4 or less) in the water drinking test had a significantly lower ($p < 0.001$) ADL (Barthel index). Correlation was tested using Spearman's rank correlation coefficient, and a positive correlation of $\gamma = 0.754$ ($p < 0.001$) was found between oral function and ADL (Fig. 4).

3.3. Relationship between oral function and nutritional status

Participants were divided into two groups according to gargling function, with 2 as the threshold, and BMI was investigated. It was found that the group with low gargling function (gargling function score of 3 or more) had significantly lower ($p < 0.005$) nutritional status (BMI). In a test of correlation using Spearman's rank correlation coefficient, a negative correlation of $\gamma = 0.344$ ($p < 0.005$) was found between oral function and BMI (Fig. 5).

Participants were divided into two groups of those with a score of 4 or less in the water drinking test and those with a score of 5, and BMI was investigated. The group with the lower score (4 or less) in the water drinking test had a significantly lower ($p < 0.05$) nutritional status (BMI). Correlation was tested using Spearman's rank correlation coefficient, and a positive correlation of $\gamma = 0.306$ ($p < 0.01$) was found between oral function and BMI (Fig. 6).

Serum albumin level was investigated in two groups divided according to gargling function, with 2 as the threshold. The group in which gargling function was lower (gargling function score of 3 or more) had a lower serum albumin level, but the difference between the two groups was not significant. In a test of correlation with Spearman's rank correlation coefficient, no correlation was found between gargling function and nutritional status (serum albumin level) (Fig. 7).

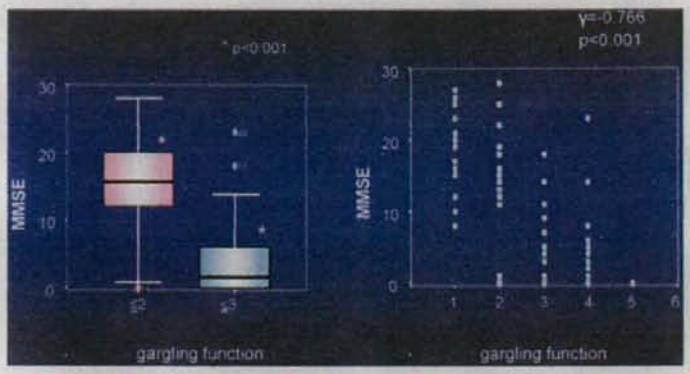

Fig. 1. Relationship between gargling function and cognitive function.

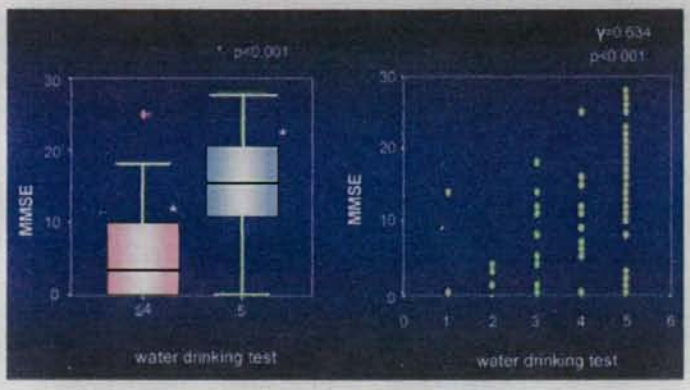

Fig. 2. Relationship between water drinking test and cognitive function.

Serum albumin level was investigated in participants divided into two groups of those with a score of 4 or less in the water drinking test, and those with a score of 5. The group with the lower score on the water drinking test (4 or less) had a significantly ($p < 0.05$) lower nutritional status (serum albumin level). In a test of correlation with Spearman's rank correlation coefficient, a correlation of $\gamma = 0.280$ ($p < 0.05$) was found between oral function and serum albumin level (Fig. 8).

4. Discussion

Relatively little attention has been paid to the impact of oral health, and a similar diet is generally provided for all residents regardless of oral function in institutions. Many elderly people wish to take meals without feeding assistants, but they often have physical functional disorders or mental illness that may influence their eating ability. Self-feeding ability is an important part of ADL

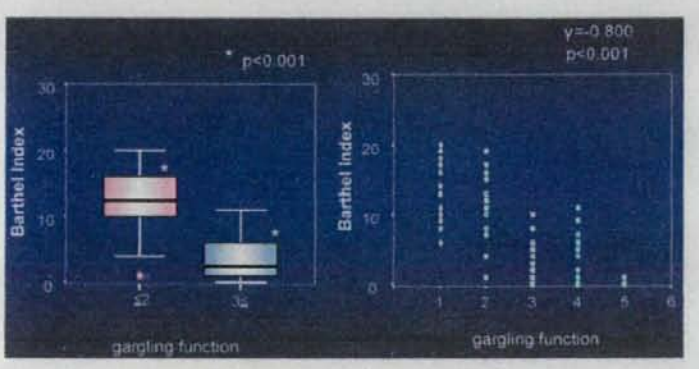

Fig. 3. Relationship between gargling function and ADL.

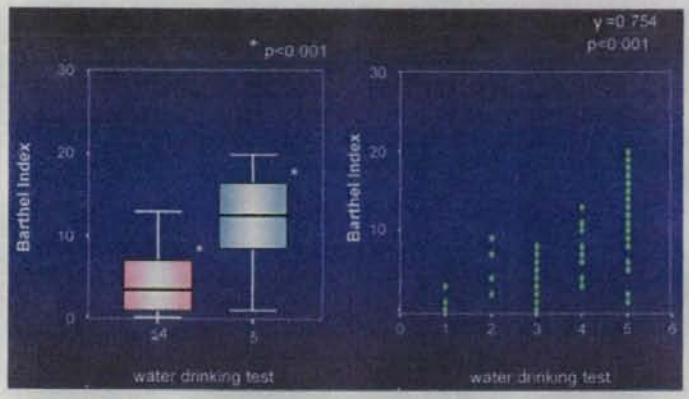

Fig. 4. Relationship between water drinking test and ADL.

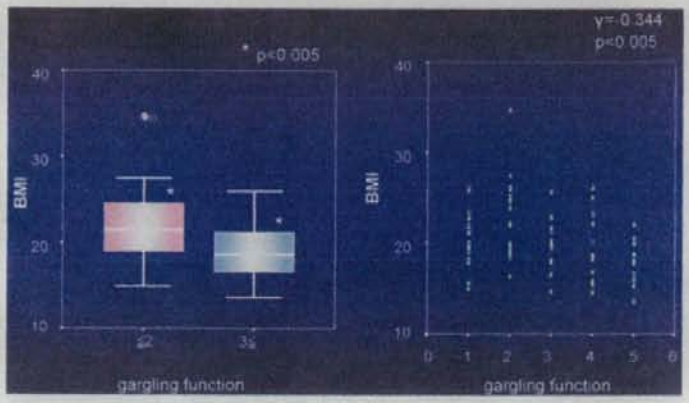

Fig. 5. Relationship between gargling function and BMI.

throughout human life. Our previous report indicated that the highest risk for losing self-feeding ability was the inability to wash one's own mouth (gargling function) (Arai et al., 2003). Gargling function is also very important for the maintenance of oral health, as oral pathogenic microbes are spit out with washing agents from the oral cavity. If elderly people have frail airways and defective oral functions, oral pathogenic microbes might intermittently invade their airways (Preston et al., 1999). In fact, it was reported that a higher rate of morbidity, especially aspiration pneumonia, and mortality were found among persons who required assistance

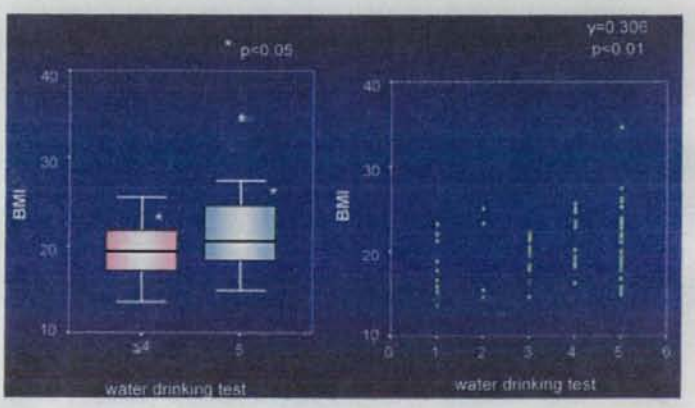

Fig. 6. Relationship between water drinking test and BMI.

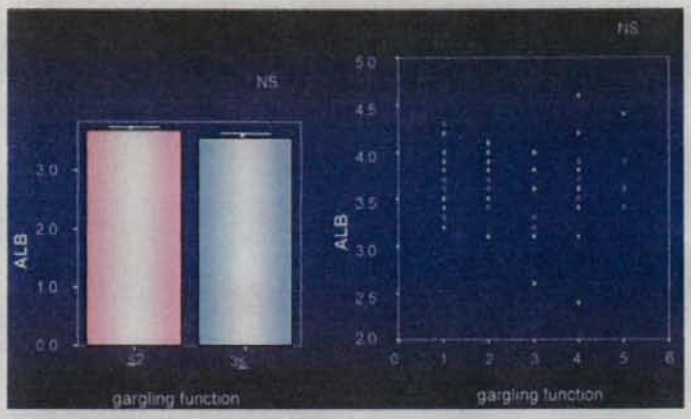

Fig. 7. Relationship between gargling function and serum albumin level.

with eating when compared with those who did not (Blaum et al., 1995; Langmore et al., 1998).

One fundamental way for the elderly to maintain the quality of life (QoL) is to obtain nutrition by safely enjoying delicious food. To maintain QoL of the elderly, prevention of dental disease and maintenance of oral function is essential. Maintenance or improvement of oral function is related to prevention of the life-threatening disease of aspiration pneumonia, as well as to prevention of asphyxia, dehydration, and poor nutrition, and is therefore extremely important from the perspective of extending healthy life and improving QoL.

Low body weight and rapid unintentional weight loss are highly predictive of mortality and morbidity in elderly populations (Fischer and Johnson, 1990). Malnutrition and dehydration are common problems in the institutionalized elderly (Simmons et al., 2001, 2003; Castellanos et al., 2004) The factors that contribute to poor nutritional status include alternations in the gastrointestinal tract, functional disabilities, lower socioeconomic status, social isolation, and chewing problems. It has been reported that dental status can affect food preference, dietary intake, and nutrition. The results of some epidemiological studies suggest that oral health is related to physical, mental and social health (Avlund et al., 2001; Shimazaki et al., 2001). In particular, it has been reported that chewing ability influences nutritional status, overall health, and ADL in the elderly (Agerberg and Carlsson, 1980; Horwath, 1989; Miura et al., 1997, 1998). In an assessment of the relationship between oral health status and nutritional condition, it was reported that older people in Britain with more than 20 teeth are more likely to have a normal BMI (Sheiham et al., 2002). Sahyoun et al. (2003) reported that dental health is closely associated with nutritional status and suggested that status of dentition should be considered in nutritional counseling and assessment of older adults. However, compared to number of remaining teeth or type of dentition, oral functions such as gargling function and water drinking have received less attention. There are many studies on dental health and general condition, but few reported studies on the relationship between oral function (gargling function and water drinking test) and cognitive function, ADL and nutritional condition.

The results of this study indicated that the water drinking and gargling function tests were strongly correlated ($p < 0.001$) with cognitive function and ADL. The water drinking and gargling function tests showed a correlation with BMI ($p < 0.005$, $p < 0.01$, respectively), and the water drinking test showed a correlation with serum albumin level ($p < 0.05$). However, no correlation was observed between the gargling function test and serum albumin level.

Based on the results of this study, it is concluded that oral function is closely related to cognitive function, ADL, and nutritional status. It is suggested that oral function is one of the

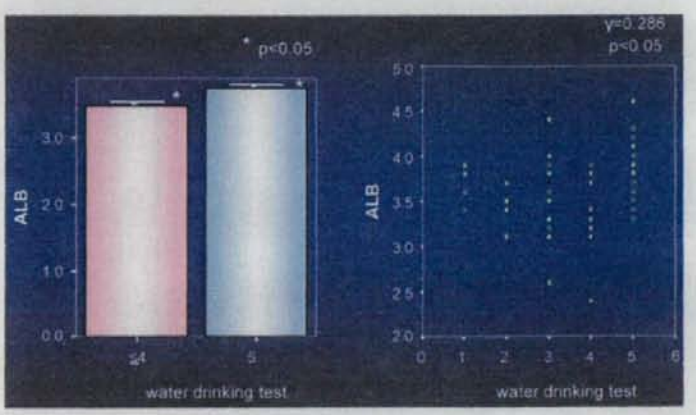

Fig. 8. Relationship between water drinking test and serum albumin level.

most important factors for the maintenance of general condition in dependent elderly. To prevent decreases in cognitive function, ADL and nutritional status in dependent elderly, the importance of improvements in oral function cannot be overemphasized. From the perspective of preventing the need for new or more intensive care in the elderly, the necessity of strategies to prevent decreased oral function has become clear.

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九州実験動物雑誌，24，3～8，2008

総　説

実験動物の大腿骨

Femur in Laboratory Animals

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はじめに

長寿科学は，高齢者，特にfrailとされる（後期）高齢者の生活の質(QOL: Quality of Life)と生活の尊厳(ROL: Respect of Life）の維持はもとより，その向上までを目指すものである。社会医学的な貢献が重要であるが，臨床医学的な寄与と貢献も必須である事は自明である。しかしながら，基礎医学，とりわけ実験動物学に期待され，実のある寄与と貢献は首記の二分野に比べると明確ではない。

厚生労働省は，「厚生労働省の所管する実施機関における動物実験等の実施に関する基本指針」，いわゆる厚生労働省版の動物実験ガイドラインの中で，文部科学省や環境省のものとは異なり，動物実験を医学の進展や国民の福利厚生のために必須のものと謳っている。実験動物からの貢献が，むしろ厚生科学研究やその一翼である長寿科学研究にも，明確に希求されているといえる。しかし，この希求と現実の動物実験からの貢献の間には大きな乖離があるのではないだろうか。というのは，長寿科学の基盤に，老年学・老年医学はあるが，実験動物学にあってはとてもではないが十分な認識に基づいていると言えない状況が垣間見られるからである。先ず，げっし目実験動物の加齢変化を知ろうとしても，正確な寿命の特定はおろか，何より研究に必須の健常・健康加齢個体を得ることが極めて困難である。この獲得が困難でありながら，加齢個体を育成しようとする気風が，例え小型げっし目にあっても，わが国では極めて希薄でインフラ整備がなされず，いたずらに短命動物や系統を是とし，求めるきらいがある。種差や系統差が大きいげっし目実験動物では，収斂はおろか，成果から距離を隔てるばかりにさえ見える。基礎医学的に，実験動物のレベルから長寿科学を，老化学を研究するという土壌に根本的に欠けているのかもしれない。端的なのは，本稿冒頭にも挙げたが，高齢者や加齢個体を弱ったものと決め付けていることではないだろうか。ラットやマウスといった小型げっし目では，系統に特異な疾病を避け得た，健常加齢個体は，外観，体毛の艶や長さから容易に区別でき，体重や臓器重量でのばらつきは大きくなり，失調に弱く，多少動きが少なくなるものの，決して脆弱（frail）ではない。近交系ラットF344/Nでは33月齢，近交系マウスC57BL/6では28月齢に至っても成熟卵胞を有している。体重がラット程度である，小型真猿のコモンマーモセットでは，生殖を終え（させ），加齢し，歯が抜け落ち，下顎骨や大腿骨に著しい骨粗鬆症が明確となり，アミロイドを検出するとされる単クローン抗体4G8陽性の小粒がおびただしい個数，脳内に認められても，飼育ケージ内を飛び回る様は若齢個体と区別できない。高齢妊娠を避け，生殖を終えさせるべく，雌雄を別居せねばならないほどの交尾行動をとり，妊娠が成立して雌が死に至ることさえある。これは妊娠しながら，正常に出産ができず，出産死に至るためである。言い方を替えると，出産できる体力がなくとも交尾ができ，妊娠には至るといえる。自然界ではあり得ず，実験室飼育で初めて認められ

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る事実だろうが，出産できない個体を身ごもるという事実は，個体保存・維持といった生物の根幹に触れるように見える。

あまり事実や見識，確認に基づいたとはいえない風聞や思い込みにだけ左右されていては，高齢者のQOL維持を目指す長寿科学と，実験動物や動物実験の貢献に深い隔たりができかねない。そこで，いわゆる「ねたきり」に着目し，動物実験や実験動物の貢献を考えてみたい。

長寿科学の目標

高齢者のQOLを著しく害するのは，首記の「ねたきり」であって，その基質的原因の大きな部分は骨粗鬆症と認知症で占められ，疾病に対する医学的ならびに社会的解明と打開は長寿科学が最も希求する大望である。ここでの目標は，社会の需要と制度に応じたもので，かなり具体的な貢献を要求しており，動物実験への要求も同様に貢献を視野に入れたものでなくてはならないのは当然である。考えたい動物実験の関わりとは関係なく，骨粗鬆症，特に閉経後のこの疾病については，一度の投薬で対応がかなう薬剤が開発されており，わが国でもその承認の待たれるところである。予防薬や治療薬の開発の常であるが，必ずしも疾病の機序の解明から始まって薬剤が開発される場合だけとは限らない。ために，今後も機序の解明については動物実験の貢献は必要とされるものと考えられる。

認知症についても，同じく多くの薬剤開発が進んでおり，この疾病の大きな部分を占めるアルツハイマー病についてはドネペジルなどがよく知られている。アルツハイマー型認知症の原因は，病理学的解析に基づく老人斑の検出とその主たる成分が不溶性アミロイドの沈着である事から，アミロイドの沈着とされ，いわゆるアミロイド学派が構築されている。この学派は，アミロイドが脳内に蓄積されなければ，例え蓄積されても消去できれば，認知症に対応できると考え，老人斑の形成を，例えば重合の抑止や融解など，種々のレベルで阻害する事や，アミロイドそのものを消去する事が根本治療につながると考えてきた。こうしてアミロイドワクチンが開発されるに至ったが，安全性を確かめた動物実験では全く認められなかった脳炎で死亡する患者が出現し，このプロジェクトは頓挫した。しかし，認知能力の回復までは確認できず，死亡患者の剖検脳からは炎症の跡は見られるものの，アミロイド斑が消失している事も確認された。このため，一部のアミロイド学派の研究者は，このアミロイドワクチンを改良する事で脳炎を回避できるのではないかと考えた。これは，アミロイドが根本原因で，その消去こそが根本治療であると考えたからのようであった。このアミロイドワクチンは，わが国で変更が加えられ，腸管免疫型としただけのものが動物実験において著効が示されたことをもとに，一日も早い臨床応用が提起された。

ところが，最近，Lancet誌にこのアミロイドワクチンが認知症罹患のヒトで，症状の軽快や軽減はもとより生存性の改善も一切もたらさず，明確な脳炎による死亡はない事を報ずる論文が，80名を対象とした2000年9月からの長期に渡るPhase I 研究の結果を基に掲載された[2]。もともと安全性は動物実験で確認され，ヒトへの展開が図られた際，一部に脳炎が発症し，死亡例が出たことから警鐘がならされ，アミロイド融解に炎症反応を利用していることに批判が出た。そこでマイルドな認識（ポリオワクチンを例に）を目指した腸管免疫のワクチンが開発され，その効用が喧伝された[1, 3]。ここには動物実験が大きく関わり，ヒト家族型アルツハイマー病遺伝子を導入したゲノム改変マウス，アフリカミドリザル，カニクイザルなどを用いた結果が基とされた。この中で，誤りなく断言できそうなのは，改良型のアミロイドワクチンは，従前のワクチンと同じく，蓄積されているはずのアミロイドを，有効に消去するだろうことのみであった。加えて，効能に認知能力が回復したことまで言及したのは，マウスの行動実験を利用したものだけで，アフリカミドリザルでは認知能力の検討はなされず，アミロイドの消失にだけ注目がなされた。カニクイザルでは，使用個体の若さに不足が明らかであるだけでなく，この種で有効とされる指迷路による知能検査での効果の判定が望めるレベルにすらたどり着けなかった。

それゆえ，アミロイドの蓄積と認知症の間には，十分な相関が証明されていないとする研究者たちの間ではこれら一連の動物実験に強い疑義を唱え，そこにヒトでのフォロウアップ研究の結果が報告され，これは動物実験，改変マウスの行動実験だけの結果を基に報告された提言とは相容れないものであった。アミロイド学派にも，老人斑は代謝老廃物を貯めているのから，必要悪だという解釈まである。種々の解釈は別としても，これらげっし目動物実験の結果に基づいた主張は，コモンマーモセットでの行動と脳内所見の実際や，ヒトでのフォロウアップに基づくものとは大きくかけ離れてしまった。ヒトで効果が認められないと報告されたのに，動物実験では，げっし目の行動実験だけがワクチンの効果を認めるように結果が得られ，結果の解釈や行動実験の意義に見直しが必要ではないかという指摘が生まれた。

サル類の行動実験や設定年齢から，全くワクチンの効

能が証明されず，コモンマーモセットの加齢変化におけるアミロイド検出が異常行動の原因とならないという事実とも一致せず，マウスでだけ効能がありと唱えられたことは，改変動物で，本来あるはずのない化合物（ここではアミロイド）が小さな脳に異常に蓄積され，不動化などの行動障害を惹起したが，ワクチンの炎症作用（マウスはこの炎症で死亡しない）でアミロイドが消されたので行動能力が回復し，行動量を認知能力に読み換える行動実験ではこれが認知能力の回復と評価・解釈された，とするとヒトへの連続性での矛盾や改変げっし目だけの乖離がある程度整理でき，げっし目の不足が明白となろう。マウスの行動実験の成否ついての指摘はこの場合だけの留まらず，これに基づく向神経薬の開発や評価が信頼を置けないことにも因っていた。加齢ラットの場合，加齢で行動量が減る系統ではこれらがうつ症状となったと判定され，切歯を抜去すると，このうつを亢進するという報告までも，成果として出ており，げっし目の不足が如何にわきまえられていないかという背景もあった。

げっし目実験動物の認知能力については，その存否から始まり，どの程度のものかを論ずることそのものがナンセンスとする立場から，意義があるとするまでの立場にまで亘ることはつとに知られたところである。生死を司り，運動を司り，知能を司る能力が脳にあるのであれば，辺縁系より下位しかないげっし目の脳の制約は自明にも見える。一部の昆虫などを例に取れば神経節細胞だけで小さな生命は維持されている。しかし，社会活動を行い，そこでの支障となる認知症を脳の相同性がないげっし目で，双方向の確認手段がない，一方的な解釈ベースの行動実験で薬剤の効能を判定することには危険が大きすぎると見える。体重がラットと同様で，高々脳の重さが5倍とはいえ，形態学的に辺縁系より上位の分化が顕著で，大脳皮質の存在では，ヒトに近い形態の脳を有するコモンマーモセットで，20年を超えて生存し，加齢で夥しいアミロイドが検出されることは大いに参照されねばなるまい。20歳のコモンマーモセットを実験に供せられる規模で育成する事は容易でないが，価値ある加齢モデル動物として，げっし目の不足を補い，より寿命の長いカニクイザルの加齢変化の解釈を助け，ヒトへの貢献を目指した翻訳動物としての期待がいやがうえにも増すものといえる。このような前提で，コモンマーモセット脳の海馬領域に注目し，構築細胞数を経時的に半定量的な特定にもあたっている。この際，動物種の特異性に紛れないよう十分に注意している事は言うまでもない。

問題は認知症だけ

長寿，科学にあって動物実験に指摘される不足や制約は，解釈や脳の比較解剖学的な自明の違いを考慮しない認知症だけではなさそうである。骨粗鬆症にあっては，すでに生涯に一度投与されれば後世この病気で苦しむことがない薬剤が開発されている。この過程で，安全性試験には動物実験の貢献があったようであるが，開発やその途上ではどうであったろう。

表1には，さまざまな動物種で，さらし骨標本とした大腿骨を，二重X線骨塩測定装置（DXA：Double X-ray absorptiometry，ALOCA，東京，DSC-600EX-IIIR，動物用）で測定した結果が示されている。このDXA法は，骨粗鬆症の診断はもとより治療薬の開発や基礎研究でヒトから動物まで広範に用いられている。この方法で測定される骨密度（BMD; bone mineral density）は，種差検出目的であれば，むしろ素晴らしいといえるほどの違いを検出している。ここでの大きな疑義は，これら総てが骨を測定したものであることである。化学的には全く同じもの，カルシウムアパタイト，から構築された組織を測定しているにもかかわらず，類似の値を検出する測定値がない。このような不足を補うものとして，pQCT（peripheral quantitative computed tomography）が開発され，骨の部位による強度や細胞の集積の違いなどから，骨質という概念も提唱されるようになった。ところが，この方法では，例えば大腿骨などを，特に生体で，骨全体を測定する機種は少なく，骨の一部を，断面的に測定するものが多く，臨床や研究レベルで蓄積されてきた膨大なDXAによる測定結果とは互換利用できるものではなかった。

生体では，関節や姿勢などの制約が大きいので，動物実験であり，単離骨の獲得が比較的容易であることから，これに基づいてDXAによる測定値を読み直す事を考えた。というのは，DXAは骨を構成する塩量，すなわちカルシウムアパタイトの量（骨塩量：BMC bone

表1．さらし骨標本としたさまざまな動物種の大腿骨の特性

	骨塩量：BMC mg	骨面積：AREA cm²	骨密度：BMD mg/cm²
Human	13620.4	164.5	829.6
Japanese Monkey	19573.3	37.2	525.7
Cynomolgus Monkey	5165.2	16.6	310.4
Common Marmoset	329.4	3.1	106.2
Rat	393.2	3.1	127.8
Mastomys	78.7	1.1	71.4
Mouse	12.3	0.4	29.1

表2．さらし骨標本としたさまざまな動物種の大腿骨の特性－2

	骨塩量：BMC mg	骨面積：AREA cm^2	骨密度：BMD mg／cm^2	骨塩量：BMR mg／骨重量
Human	136420.4	164.5	829.6	0.557
Japanese Monkey	19573.3	37.2	525.7	0.541
Cynomolgus Monkey	5165.2	16.6	310.4	0.501
Common Marmoset	329.4	3.1	106.2	0.509
Rat	393.2	3.1	127.8	0.542
Mastomys	78.7	1.1	71.4	0.529
Mouse	12.3	0.4	29.1	0.377

mineral contents）を測定するものであって，結果として得られる骨密度（BMD）は，このBMCをX線の影となる部分の面積（AREA: bone area）で割ったものだったからである[5]こういった算出方法に基づく骨密度は，骨体が大きいものほど高い骨密度に結果する事となる。表1からも明確なように骨体が大きいものほど骨密度が高い。これは，本来密度が，骨塩量を骨体の体積で割って算出されねばならないものが面積で割られたためであった。この弊害は，機器の開発業者や一部研究者の間では十分認識されていて，種間や大きさが異なる骨の比較には適さない，とされてきた。ところが単離骨では重量が得られることから，これに基づく是正を考え，骨塩率（BMR: bone mineral ratio）という骨塩量を骨重量で割る指標を考えた（表2，5）。果たして，骨塩率では0.5に近い値が種を越えて得られ，骨を比較する上での共通指標として用いうる可能性が支持された。この指標では，下顎骨形態[4]に加え，F344系統群では亜系統による違い，ミュータント系との違い（表3）も検出できた。

このBMRでは同じげっし目でありながら，ラットはヒトやサル類に近いのに，マウスがとりわけ低値となった。体重の支持を考えると，直立歩行を行うヒトで下肢が高い能力を有することの理解は容易である。しかし，サル類ではその行動様態を見れば明白なように前肢の体重分担があるが，どの程度であるか予想すらできない。四肢で体重を支えるという行動形態での同一さが何ゆえマウスとラットで大きく異なるBMRとなったのかには慎重な検討が必要である。命・運動・知能を支える器官とその生物学的な違いを十分に考えないと，確認手段で一方通行の動物実験の結果を有効の利用できないことに直結することが懸念される。

大腿骨の形状

図1には，15歳前後の雄コモンマーモセットの大腿骨が示されている。大腿骨は，やや湾曲した表面が滑らかなほぼ円筒型を，特に骨幹部では，呈する。図2のラットでも同様である。ところがこれら2例では，先ず大きさでかなりの違いがある。長さでの違いはまま見られ，個体差と解釈されている。これらの2例も繁殖つがいの雄親として貢献してきた。ところが部位に若干違いはあるものの本来滑らかな大腿骨前面に褶曲とも見える高まりが，I 478（上）では膝関節の上部に，N 029（下）では骨幹の大腿関節に寄った部位で見られる（図1，矢印）。前者では，この高まりの両側で骨稜のような広がりを認め，普通円筒形の骨を三角形に見せている。このような骨表面の異型性は，加齢変化というより，付着する筋でかかったひずみと考えられている。大腿骨には，内転筋や大腿四頭筋などが付着しており，これらが付着面積の増大を要求し，骨表面が褶曲や稜形成でこれにこたえたと考えられる。ここで大切なことは，骨形状が大きく変化しているのに，行動の違いや異常がなかったかという点である。ここまで骨の異常が顕著で，ヒトであっても触診で検出可能で，動きに違いがあろうと推定されるのに，まったく異常は見られなかった。行動による異常をまったく露呈することなく，コモンマーモセットでは高齢とされる15年を生存した。すなわち，生理異常に伴う違いさえ行動から推し量ることは不可能であった。げっし目よりはるかに複雑な動きを見せるコモンマーモセットにおいてさえである。行動実験の解釈の難しさはこういった事実からもうかがわれ，げっし目での結果の解釈

表3．4つのげっし目系統間での大腿骨特性の比較

Species	Strains	Days of age	Femur weight	BMC	AREA	BMD	BMR
Rattus norvegicus	F344/N male	212	742.6	394.9	2.966	133.1	0.532
	F344/N female	212	410.5	212.8	2.097	117.3	0.518
Rattus norvegicus	ODS-od/od male	210	678.2	359.7	2.846	126.3	0.530
	ODS-od/od female	210	353.9	173.6	1.918	90.5	0.491
Praomys coucha	MKS	208	149.7	79.9	1.11	72	0.534
	MKS	208	103.4	52.3	0.906	57.7	0.506
Mus musculus	C57BL/6	233	45.3	20.9	0.576	36.2	0.461

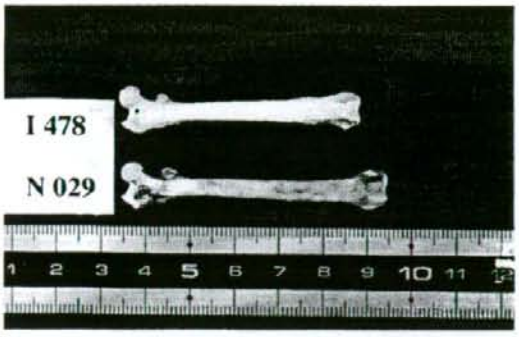

図1．コモンマーモセット大腿骨前面
1．15歳前後の雄コモンマーモセットの大腿骨。本来平滑である骨前面に撓曲（矢印）あり，側面に稜が形成されている（上）ことに注目。

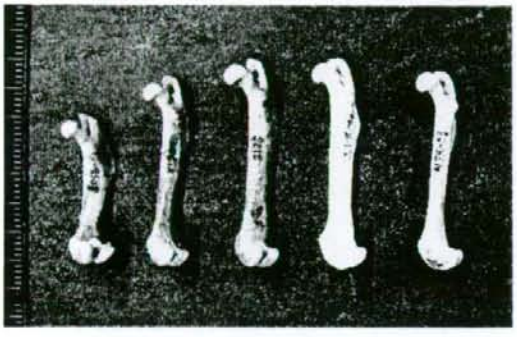

図2．ラット大腿骨の系統差
2．ODS-od/od，ODS-+/+，Jcl: Wistar，F344/N 雌の大腿骨。

には慎重な姿勢が望まれる。加齢すると，ラットの下顎骨の下顎頭に鞍状のへこみが形成されることがある。マウスでは切歯を実験的に切ると同様のへこみが形成される。しかしながら見かけの咀嚼行動には全く異常が見られない。増幅をかけた動態解析では違いが検出されるようであるが，目に見える行動での判定など，容易でないというより，不可能である。ところがげっし目の行動実験では，はるかに高次の機能である精神活動が推定できることになっている。動物種の制約をわきまえた，慎重な検討が望まれる所以である。一方，ラットの大腿骨では骨特性での系統の弁別（表3）が果たせるだけでなく，図2のように，その形状からミュータント系が識別できる。骨特性で低値を示したODSラットの雌の大腿骨は短くなっている。短いだけでなく，骨塩率が小さい事は，形態だけでなく性質も他の系統とは異なる事が示されたといえる。全体の形状からは，大腿骨頭部と体部が短縮し，太く，短く見えるが，コモンマーモセットのような骨表面の異型性（図1）には至らず，平滑さを保っている。これら異常の違いも種差を考えれば相似性や相同性の検討に興味を馳せさせるものがある。

さいごに

動物実験の結果や成果を，ヒトにおけると同様の用語で語り，伝えられれば，語りやすく伝えやすい。同じ生命の営みを名状しているのであって，一見不足はないようにもみえる。しかし，ヒトと動物の隔たりは自明であって，十分な注意と留意の基になされないと大きな過誤を生じかねない。同じ霊長目のサル類であっても，彼らは買い物もしなければ調理もしない。MCI（mild cognitive impairment）で同じ食材を買い続けたり，同じ料理を大量に作り続けるといったヒトでの臨床症状をミミックする事もない。ましてや，大脳皮質がほとんどないげっし目でこのようなことが想定できよう筈もない。ところが行動実験を経ると，感知する部位や起動する部位，大脳皮質，がないのにいきなりヒトと遜色のない知能と感情に長けたものに化けたことにできるといった事態が招来する。このような拡大解釈の危険性に対する指摘は，既に本誌でも行ってきた[6,7]。動物実験が，科学でなくなってしまうと懸念されたからだ。骨を構成する成分が同じだからと，類似の数値が検出できたとしても，ヒト・サル類・げっし目では骨の性状を左右する体重の支持や運動様態を同一視できないので慎重な検討が必要となる。動物実験は，コミュニケーションできない動物の力を借りて行うものあることを忘れてはならない。加齢の研究で，唯一寿命を延伸する手段として食餌制限がある。この方法は，飽食型の下等動物でより成果が得られるものの，ヒトやサル類での効果はさほどとされている。ところが，理想的な食餌制限，具体例は不明ながら，げっし目の寿命を今より更に延ばすと盲信する研究者が基礎老化学にあることと類似し，自省に値すると考えられる。動物種には，その種に特異な制約が寿命にもあり，これがさまざまな環境要因で修飾されている[6,7]。マウスやマストミスといった小型のげっし目では，手で捕定しただけで，反射的に排尿する。テステーブで調べるときなどは便利な反応だが，高齢者に多く，対策の待たれる排尿に関わるトラブルのモデルをたずねるときなど，留意が必要な生理といえる。老化は，ゲノムでは到底解決できない最後の生理なのだろう。如何なるハザードがあろうと，われわれは，加齢個体の価値（20歳のコモンマーモセットの育成には20年，3歳のラットを育成するには3年を要する事も含めて）を承知し，これらを用いる動物実験の中からヒトや科学に貢献できる，外挿できる事象を探索せねばならない。これこそが真の動物実験の使命である。

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疾患別 VF・VEのみかた

連載目次

パーキンソン症候群

長屋政博[1)]　中澤 信[1)]

key words　パーキンソン病　VF　進行性核上性麻痺　レビー小体型認知症　多系統萎縮症

1 はじめに

パーキンソン病(以下PD)は，筋固縮，安静時振戦，運動減少症，および姿勢反射異常などを主要症候とする錐体外路系疾患で，黒質緻密層ドーパミン性神経細胞の変性と線条体ドーパミン量の低下を主病変とする原因不明の変性疾患の一種であり，脳のドーパミン枯渇状態を示す疾患である．わが国では，PDの有病率は，人口10万当たり100人前後と推定されている．発症年齢は50代が最も多く，40歳代，60歳代がこれに続き，神経疾患のなかでは，頭痛，脳卒中，てんかんについで多く，特に最近，わが国では高齢社会を迎えるようになって，年齢の増加とともにPDの患者数はしだいに増加していると考えられる．そのため日常の嚥下障害外来およびリハビリテーション(以下リハ)診療を行ううえで，PDの摂食・嚥下障害の評価およびリハに携わる機会が多く，本稿では，PDおよびパーキンソン症候群における摂食・嚥下の特徴や対策について概説する．

2 PDの症状

PDの臨床症状は多彩であるが，①安静時振戦，②筋固縮，③動作緩慢または無動，および④姿勢保持障害を4大症状として，その他に前屈姿勢で

表1 パーキンソン病にみられる主要な障害症状[1)]

中核症状
振戦：安静時振戦(4～6Hz)，丸薬丸め運動
筋強剛(固縮)：鉛管現象，歯車現象
運動緩慢・無動
姿勢反射障害，立ち直り反射障害
その他の障害・症状
表情減少(仮面様顔貌)
まばたき頻度の減少，滑動性眼球運動障害
発話障害：小声症，単調，早口，吃(どもり)，構音障害
協調性運動障害：反復拮抗運動ができない，巧緻動作障害
歩行障害：すくみ足，引きずり足，小刻み歩行，加速歩行，突進現象
姿勢異常：前傾姿勢，脊椎後弯，脊椎側弯
小字症
寝返り困難
精神症状：抑うつ，心気的，知的機能障害，思考の転換が遅い
自立神経症状：便秘，流涎，脂顔，起立性低血圧，多汗，消化管の蠕動運動障害，排尿障害，四肢循環障害
摂食・嚥下障害：低栄養，低体重，肺炎

1) 国立長寿医療センターリハビリテーション科

図1 パーキンソン病における重症度と嚥下障害の自覚症状

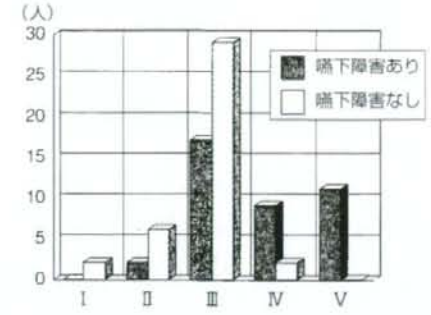
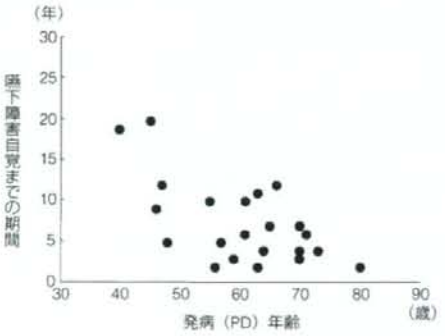


図2 発病時の年齢と嚥下障害自覚までの期間



の小刻み歩行，加速歩行などの歩行障害，あるいは早口で平坦で小声などの構音障害が認められる（**表1**）[1]．PDでの頻度が高い自律神経徴候は，便秘，眼脂，流涎である[2]．その他には，排尿障害，起立性低血圧，多汗，網状青斑，嚥下障害，体温調節障害，血管運動障害，除萎などである．PDでは，一般的に10～20%に認知症がみられる[3]．

わが国でのPD患者における死因は，肺炎・気管支炎約39%，悪性新生物13%，心疾患11%，窒息約7%，栄養障害約7%，脳血管障害6%，その他11%であると報告されている[4]．PDにおいては，肺炎・気管支炎，窒息，栄養障害による死亡が多く，摂食・嚥下障害との関連が大きいことが考えられる．

表2 パーキンソン病の重症度分類

Hoehn & Yahrの重症度分類		生活機能障害度（異常運動疾患調査研究班）	
Stage I	症状は一側性．機能障害はないかあっても軽微．	I度	日常生活・通院にほとんど介助を要さない．
Stage II	両側性の障害があるが，姿勢保持の障害はない．日常生活，職業は多少の障害はあるが行いうる．		
Stage III	立ち直り反射に障害がみられる．機能障害は，軽ないし中程度で職種によっては仕事は可能である．日常生活はある程度制限されるが一人での生活が可能である．	II度	日常生活・通院に介助を要する．
Stage IV	重篤な機能障害を呈し，自力のみによる生活は困難となるが，まだ支えられずに立つこと，歩くことがどうにかできる．		
Stage V	立つことが不可能となり，介助による車椅子移動または寝たきりとなる．	III度	日常生活に全面的な介助を要し，歩行・起立不能

3 PDの摂食・嚥下障害の特徴

PD患者では，摂食・嚥下障害の自覚に乏しく，むせを伴わない誤嚥，不顕性誤嚥が多い．しかしながら自ら摂食・嚥下障害を認めない患者もいる．症例によっては，嚥下障害がPD初期から存在することもある．嚥下障害はPDの経過中に高率に出現し，30～60%ともいわれ，90%という報告もある．筆者らがPD患者78名（男性37名，女性41名）に対してアンケート調査をした結果，50%になんらかの嚥下障害を自覚していた（**図1**），またこの自覚症状は，Hoehn-Yahrの重症度分類（**表2**）や日常生活動作の障害度の進行とともに，頻度が増す傾向がみられた．Stage IIのときから嚥下障害を自覚している患者もいて，stage Vでは全例で嚥下障害を自覚していた．また，発症から嚥下障害の自覚症状が出現する時期を**図2**に示すと，発病時の年齢が低ければ，嚥下障害の自覚までの期間が長く，高齢発症であれば嚥下障