propylfluorophosphate (DFP), ethylene diamine tetraacetate (EDTA), diethyl pyrocarbonate (DEPC) and 1,2-epoxy-3-(*p*-nitrophenoxy) propane (EPNP) were from Sigma-Aldrich (Tokyo, Japan). EnzCheck was purchased from Molecular Probes (Eugene, USA).

2.3. Antibodies

Monoclonal 4G8 antibody was from Signet Pathology Systems (Dedham, MA). 6F/3D (monoclonal) was from DAKO (Glostrup, Denmark) and anti 5–10 (monoclonal) was from QCB (Camarillo, CA). The epitopes recognised by the monoclonal antibodies were previously identified [12]: amino acid residues 17–21 (4G8), amino acid residues 9–14 (6F/3D) and amino acid residues 5–10 (anti 5–10 antibody). Alkaline phosphatase-conjugated, goat anti-mouse IgG from Promega (Madison, WI) was used as secondary antibody.

3. Methods

3.1. Denaturation of pH-modified Aβ42 with GdnHCl

Aβ42 (4 μg/ml) was incubated in either pH 4.6 or 7.4 buffer in Eppendorf tubes at 4 °C for 24 h and then plates were coated with 50 μl/well of solution for a further 24 h at 4 °C. After removing excess sample, 50 μl of GdnHCl was added to each well at the indicated concentrations and incubated for 30 min at 37 °C. After discarding the GdnHCl, the wells were washed three times with pH 7.4 buffer and the samples tested for reactivity with 6F/3D (1 μg/ml) using a standard enzyme-linked immunosorbent assay (ELISA) as described below.

3.2. Synthesis of the eight-residue peptides derived from Aβ42

A series of 8-mer peptides representing residues 1–32 of Aβ42 (overlapping by seven residues at the N terminus) were synthesized manually using solid phase peptide chemistry with Fmoc-protected amino acids on Rink Amide MBHA resin as previously described [12]. They were dissolved in water as indicated and used to inhibit Aβ42 aggregation and the proteolytic activity of Aβ42 for casein. Some of the peptides, Aβ16–23, Aβ17–24, Aβ18–25 and Aβ25–32, were dissolved in a minimal amount of DMSO before dilution with water.

3.3. Incubation of Aβ42 with the eight-residue peptides

Samples of Aβ42 (4 μg/ml) were mixed with each of the peptides (4 μg/ml) and incubated for 24 h at 4 °C in Eppendorf tubes in pH 4.6 or 7.4 buffer. The buffer used was 20 mM citric acid adjusted with disodium hydrogen phosphate.

3.4. Digestion of immobilized protein by proteinase K

Microtiter plate wells were coated with 50 μl of the mixtures described above for 24 h at 4 °C, and after removal of excess sample, the sample in each well was incubated with 50 μl of PK at 10 μg/ml in TBS, pH7.4 for 90 min at 37 °C. After removal of the proteinase K solution, the reaction was stopped by washing the well with TBST (TBS with 0.1% Tween-20, pH 7.4) followed by incubation with 3 mM PMSF at room temperature for 30 min, and rinsing twice with TBST. The samples were subsequently processed by standard ELISA assay as described below, to determine the amount of protein remaining.

3.5. ELISA assay

Samples after PK digestion were first incubated with TBS containing 3% BSA (pH 7.4) for 2 h at 37 °C, washed with TBST buffer and incubated for a further 2 h at 37 °C with 50 μl of anti 5–10 antibody (1 μg/ml) in TBS containing 1% BSA, pH 7.4. The wells were then washed with TBST, and incubated for an hour at 37 °C with 50 μl of a 1:5000 dilution of alkaline phosphatase-conjugated secondary antibody. After washing with TBST, bound antibody was detected by addition of *p*-nitrophenyl phosphate, and measured at 405 nm using a spectrophotometric plate reader (Molecular Devices) after 30 min. All washing steps were performed six times using a microplate autowasher, Model EL404 (BIO-TEK Instruments).

3.6. Cell line

The human glial cell line, KG-1-C, was obtained from RIKEN cell bank (Tsukuba, Japan) and cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal bovine serum and antibiotics at 37 °C in a humidified atmosphere containing 5% CO_2.

3.7. Cell culture with Aβ42 and peptides

Twenty-four-well flat-bottomed multiplates (Sumitomo Bakelite, Tokyo, Japan) were pre-coated with 100 μl per well of a mixture containing Aβ42 (4 μg/ml) and a short peptide (4 μg/ml), in either pH 4.6 or 7.4 buffer for 24 h at 4 °C. After removal of excess sample, wells were washed twice with phosphate-buffered saline (PBS), and 1.5 ml cells (2×10^4 cells/ml) was added per well, and incubated for 7 days. The cells were collected by scraper in 1 ml of PBS, adjusted to a density of 2×10^4 cells/ml in PBS and sampled for cell western dot blots.

3.8. Cell dot blots

One hundred microliters of each cell suspension was applied to a Dot Plate, DP-48 (Advantec, Tokyo, Japan) and blotted onto a methanol-immersed polyvinylidene difluoride

(PVDF) membrane (0.45 μm; Millipore, Bedford, MA) with absorption by vacuum pump.

The PVDF membrane was then removed, thoroughly air dried, and rinsed in TBS.

3.9. Digestion of samples with proteinase K

For digestion of the cells with proteinase K, the PVDF membrane were incubated with 5 ml of proteinase K at 10 μg/ml in TBS, pH 7.4 for 90 min at 37 °C with constant shaking. After removal of the proteinase K solution, the reaction was stopped by washing the membrane with TBST followed by incubation with 3 mM PMSF at room temperature for 30 min and rinsing twice with TBST. The membranes were subsequently processed for standard western blotting using 4G8 antibody to detect the protein remaining in the cell after PK digestion.

3.10. Cell western blot

PVDF membranes were blocked with 3% nonfat dry milk in TBST for 4 h, washed three times with TBST, and incubated with primary antibody 4G8 (1:10,000 dilution in TBS) for 2 h at 37 °C. This was followed by reaction with the secondary antibody, peroxidase linked anti-mouse IgG (1:5000 dilution in TBS) for 1 h at 37 °C. After three washes with TBST, the spots were detected by enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech., Uppsala, Sweden) according to the manufacturer's instruction.

3.11. Assay of hydrolytic activity of Aβ peptides

The hydrolytic activity of Aβ peptides for casein was assayed using EnzCheck. Enhancement of fluorescence was observed as a result of casein cleavage products. Activity was detected with a pH-insensitive green fluorescent BODIOY FL casein substrate according to the manufacturer's instructions with excitation/emission maxima of 485/538 nm (Perkin-Elmer LS-5B spectrofluorometer). The fluorescence intensity of samples after incubations was corrected with solvent and expressed as relative fluorescence intensity. The dose-dependence of the enzymatic activity of Aβ42 at pH 7.4 was examined after 60-min incubation with the indicated concentrations of Aβ42. We also examined the activity of Aβ42 (5 μM) and Aβ17–42 (5 μM) as a function of incubation time at pH 7.4 and 37 °C. In another experiment, we compared the activity of Aβ1–16, Aβ1–28, Aβ12–28, Aβ17–42, Aβ40 and Aβ42 each at 5 μM and pH 7.4. The effect of pH 7.4 versus pH 4.6 was also tested for Aβ42, Aβ17–42 and Aβ1–16.

3.12. Incubation of Aβ42 with metals

The Aβ42 (2.5 μM) was mixed with $CuCl_2$, $ZnCl_2$ and $FeCl_2$ at 25 μM, respectively and incubated at 37 °C for 1 h, and then tested for enzymatic activity using EnzCheck to study the effects of metal ions on the proteolytic activity of Aβ42 for casein. EDTA (50 μM) was also tested to reverse the effect of Cu^{2+} on the enzymatic activity.

3.13. Chemical modification of amino acid residues

Some of the amino acid residues of Aβ40 were modified by exposure to chemical compounds; di-isopropylfluorophosphate (DFP) for serine residues, diethyl pyrocarbonate (DEPC) for histidine residues and 1,2-epoxy-3-(*p*-nitrophenoxy)propane (EPNP) for aspartic acid residues. Aβ40 (1mg/ml) was incubated with 5 mM DEPC in 25 mM phosphate buffer (pH 7.4) for 2 h at 37 °C [24], and the formation of *N*-carbethoxy-histidine residues was followed by the characteristic increase in absorbance at 240 nm [25]. The sample was dialyzed to remove excess DEPC, and referred to as histidine-modified Aβ40: Aβ40 (H*). Aβ40 (2 μM) was incubated with 40 μM DFP in 1 ml water for 20 h at 37 °C, and after reaction it was adjusted to pH 7.4 with NaOH and the sample denoted serine-modified Aβ40 (S*). Similarly Aβ40 (2 μM) was incubated with 40 μM EPNP in 1 ml water for 20 h at 37 °C, yielding aspartic acid-modified Aβ40 (D*).

3.14. Inhibition of enzymatic activity by eight-residue peptides

Mixtures of 5 μM of each eight-residue peptide with Aβ42 (5 μM) were incubated at pH 7.4 and 37 °C for 60 min before reaction with casein substrate in EnzCheck. The enzymatic activity of the mixture was determined after 60 min incubation at 37 °C. As a control, the activity of Aβ42 without addition of any peptides was determined. The percent inhibition of Aβ42 activity by the short peptides was calculated as follows: percent inhibition=(1 – relative fluorescence intensity in the mixture of Aβ42 with short peptide/relative fluorescence intensity in Aβ42 control) × 100 (%).

4. Results

4.1. Cryptic epitopes of Aβ42

The reactivity of 6F/3D towards pH 4.6 treated-Aβ42 was greatly affected by increasing concentrations of the potent denaturant GdnHCl, whereas the sample incubated at pH 7.4 was unaffected. This finding suggests that GdnHCl induces unfolding of the sample when incubated at pH 4.6 (Fig. 2).

4.2. Screening eight-residue peptides that inhibit Aβ42 aggregation

We previously suggested that Aβ42 aggregates at pH 4.6 and that the aggregates exhibit PK resistance, whereas the Aβ conformer at pH 7.4 does not aggregate and remains sensitive

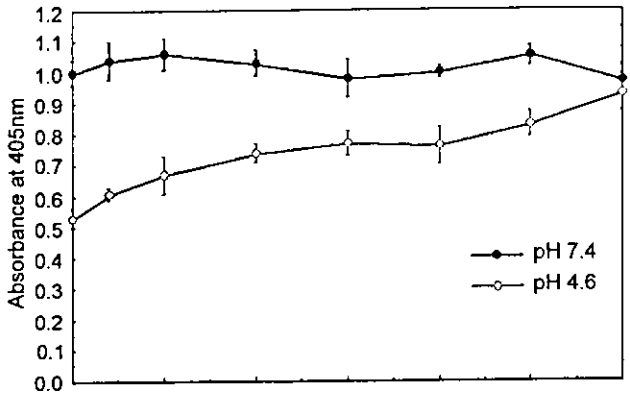

Fig. 2. GdnHCl-induced unfolding of pH-modified Aβ42. Samples of Aβ42 (4 μg/ml) incubated in pH 7.4 (●) and pH 4.6 (○) buffer were coated onto ELISA plates and treated with increasing amounts of GdnHCl prior to analysis by ELISA with 6F/3D antibody (1 μg/ml). Data are mean ± S.D. ($N=6$).

to PK [12]. We evaluated the ability of short peptides to interfere with aggregation of Aβ42 at pH 4.6 by estimating the percentage of the protein remaining after PK digestion. After incubation at pH 4.6, in the absence of any short peptide, 33% of the Aβ42 protein remained, while only 16% of the protein remained after incubation at pH 7.4. Only three of the short peptides, Aβ15–22, 16–23 and 17–24 reduced the amount of protein remaining from that of the control at pH 4.6, to that of the control at pH 7.4; their values were 16%, 14% and 17%, respectively ($p<0.001$; Fig. 3).

4.3. The active peptides also inhibit Aβ42 accumulation in glial cells

The inhibitory activity of the three short peptides on Aβ42 aggregation was confirmed by cell western dot blot assay. The signal from the spot of Aβ42 at pH 4.6 was reduced by co-incubation with Aβ15–22 and Aβ16–23 (Fig. 4a). The average pixel density of each spot was analysed with NIH imaging software and the percentages of protein remaining after PK digestion were calculated from the spot density. Protein remaining was 10% in the Aβ42 control at pH 7.4 and 62% in the control at pH 4.6. The percentage protein remaining at pH 4.6 was reduced by addition of Aβ15–22 and Aβ16–23 (42% and 37%, respectively; Fig. 4b). Aβ16–23 was more active in this assay than peptide LPFFD (45%), a derivative of Aβ17–20, LVFF (data not shown).

4.4. Dose-dependence of Aβ42 activity on casein

Fig 5a presents the hydrolytic activity of Aβ42 at pH 7.4 over the range of 1–20 μM. The activity of Aβ42 (2.5 μM) showed 1.7 fluorescence intensity and similar reactivity was observed for 200 pM trypsin (data not shown).

4.5. Time-dependence of hydrolysis

The hydrolysis of casein at pH 7.4 by Aβ 42 and Aβ17–42 was examined as a function of time at 37 °C. The relative fluorescence intensity immediately increase to 0.88 upon addition of Aβ42 peptide, and eventually reached a plateau at 2.2 after 60 min. The maximum activity of Aβ17–42 was about 30% of Aβ42, over a similar time course (Fig. 5b).

4.6. The proteolytic activity of Aβ peptides depends on pH

The plateau fluorescence intensity reached in the casein hydrolysis assay was reduced to 0.53 at pH 4.6, a decrease

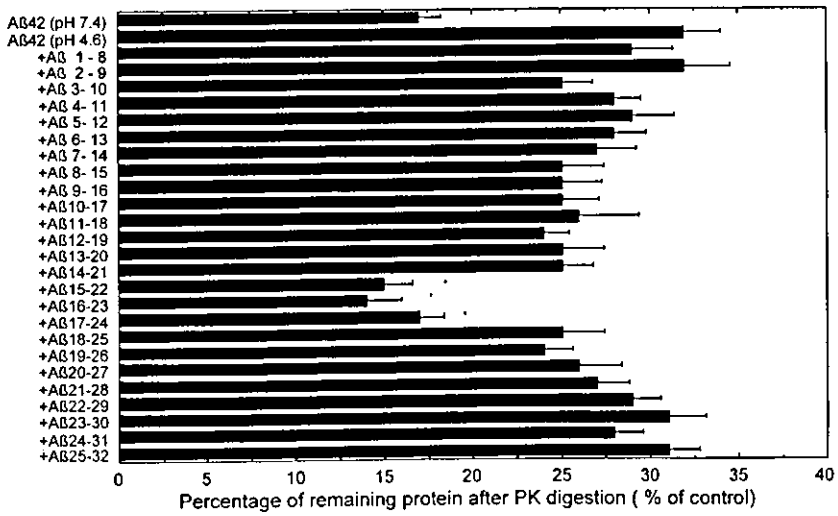

Fig. 3. Inhibition of acid-induced Aβ42 aggregation by Aβ fragments. Samples of Aβ42 (4 μg/ml) together with short peptides (4 μg/ml) at pH 4.6 were immobilized on the ELISA plate and, after digestion with PK at 10 μg/ml, the amount of protein remaining was determined with anti 5–10 antibody (1 μg/ml) by ELISA assay. The percentage of PK resistant protein in each sample was estimated as follows: (absorbance of remaining protein/initial protein) × 100 (%). Aβ42 (4 μg/ml) at pH 7.4 in the absence of any other peptide acted as control for PK sensitive Aβ42. Data are mean ± S.D. ($N=6$).

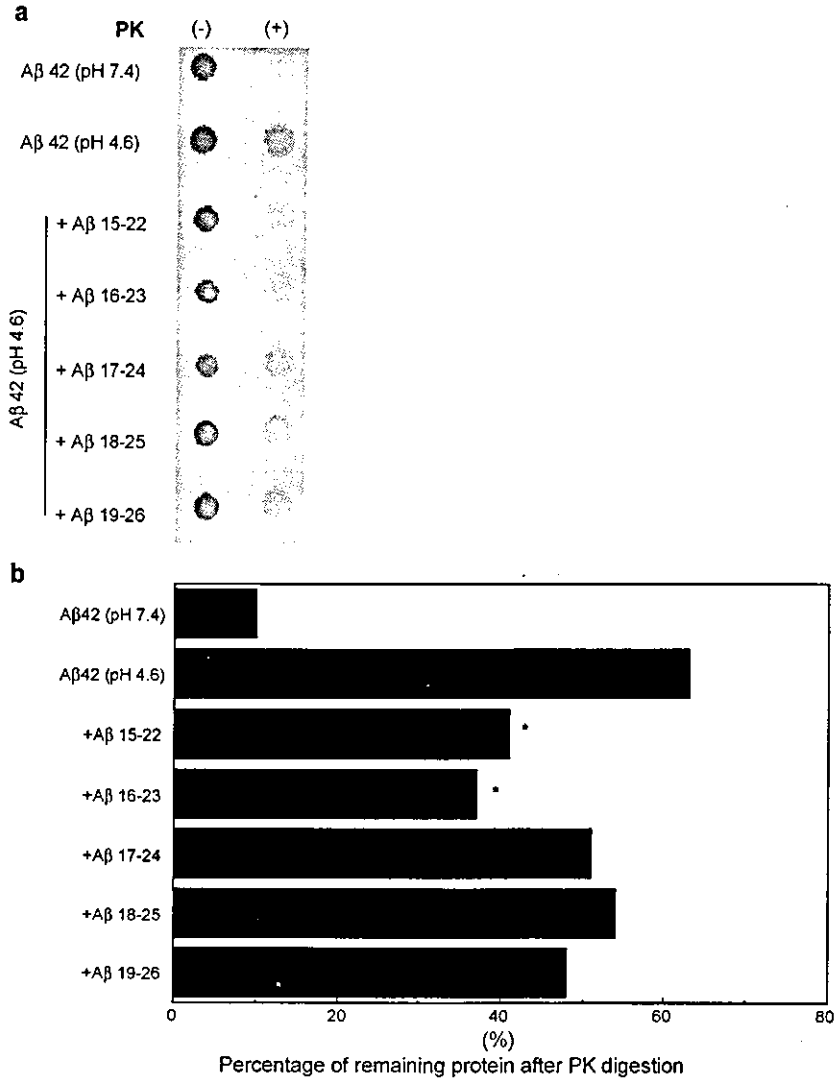

Fig. 4. Inhibition of Aβ42 accumulation in glial cells by short peptides. (a) Human glial cells (2×10^4 cells/ml) were cultured for 7 days with pH 4.6-modified Aβ42 (4 μg/ml) in the presence or absence of the eight-residue peptides (4 μg/ml), Aβ15–22 to Aβ19–26 and the fraction of protein remaining after PK digestion (10 μg/ml) determined using the cell western dot blot system as described in Methods. Aβ42 at pH7.4 served as a control. (b) Quantification of PK resistant Aβ by computerized densitometry. The percentage of PK resistant protein was determined as follows: (pixel density of spot after PK digestion/density before digestion) × 100 (%).

of about 80%. At neutral pH, Aβ17–42 had approximately 40% of the activity of Aβ42, and this was almost unchanged by acid exposure. Aβ1–16 had no activity at either pH (Fig. 6).

4.7. Dependence of proteolytic activity on peptide length and effects of metal ions for the activity

It would appear that at neutral pH the hydrolytic activity of Aβ, and small peptides derived from it, depends on peptide length. The activities of Aβ40 and Aβ42 were 1.9 and 2.2 fluorescence intensity, respectively: Aβ17–42 had 45% of this activity, while Aβ1–16 and Aβ12–28 displayed no activity at all. The activity of Aβ1–28 was 0.4 fluorescence intensity corresponding to about 20% of Aβ40 activity (Fig. 7a). The copper ion could inhibit the proteolytic activity of Aβ42 for casein by approximately 85% decrease and it was completely recovered with EDTA. Zinc and iron ions, however, had little affect on the activity (Fig. 7b).

4.8. Crucial amino acid residues for proteolytic activity

The activity of the Aβ40 derivatives in which glutamic acid is replaced by glutamine at position 11 or 22 was approximately 50% of Aβ40 itself. Chemical modification of the histidine residues at positions 6, 13 or 14, as well as modification of the serine residues at positions 8 and 26, almost completely abolished activity. Conversely, modification of the aspartic acid residues at positions 1, 7 or 23 had no effect (Fig. 8).

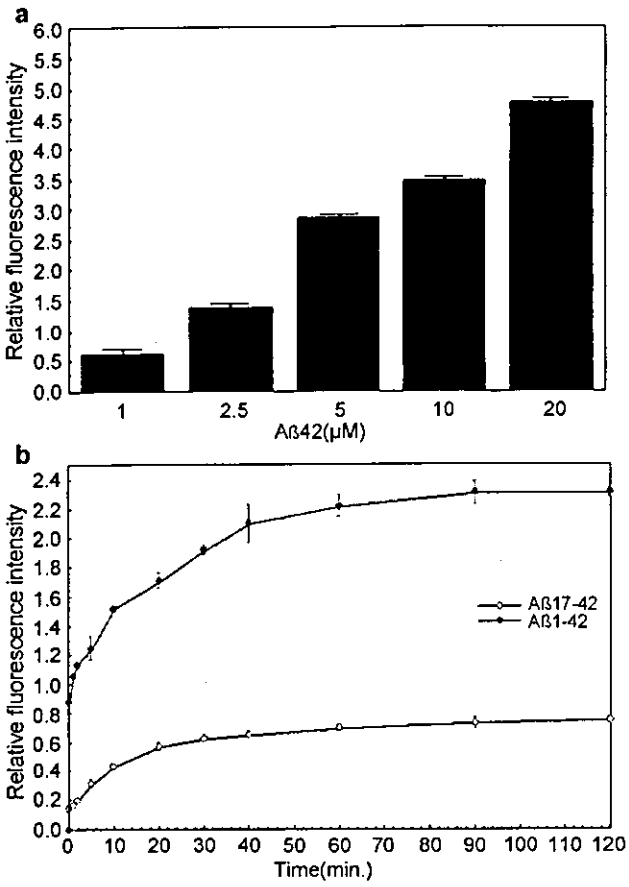

Fig. 5. (a) Dose-dependence of the proteolytic activity of Aβ42. The proteolytic activity of Aβ42 for casein at pH 7.4 was assayed using EnzCheck as described in Methods. The activity of the indicated concentrations of Aβ42 was determined after 1-h incubation at 37 °C. The relative fluorescence intensity was determined after subtraction of solvent fluorescence intensity. Data are mean ± S.D. (N=3). (b) Time dependence of the proteolytic activities of Aβ42 and Aβ17–42. The activity of Aβ42 (●) and Aβ17–42 (○) at pH 7.4 for casein was assayed by EnzCheck as a function of incubation time. Data are mean ± S.D. (N=6).

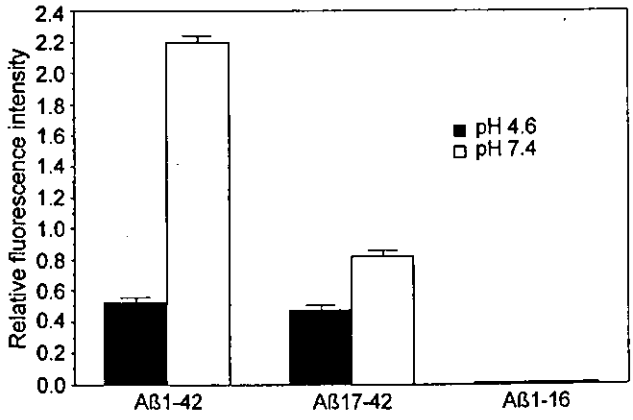

Fig. 6. pH-dependence of Aβ activity. Each of the Aβ-derived peptides of different length including Aβ42, Aβ17–42 and Aβ1–16 (5 μM) were assayed for proteolytic activity against casein using EnzCheck at pH 7.4 (□) and pH 4.6 (■). The activity was determined after 1-h incubation at 37 °C. Data are mean ± S.D. (N=6).

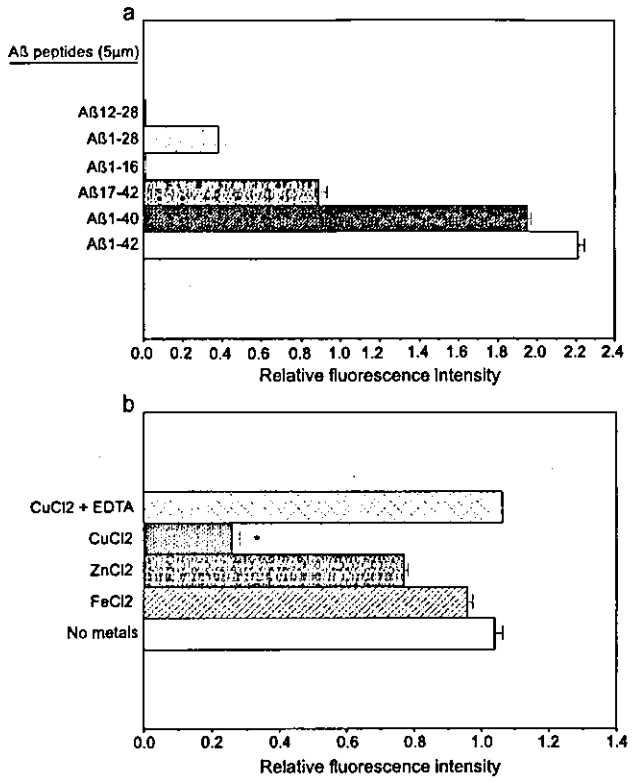

Fig. 7. (a) Dependence of the activity of Aβ peptides on peptide length. Aβ42, Aβ40, Aβ17–42, Aβ1–28, Aβ12–28 and Aβ1–16 at 5 μM were assayed for cleavage of casein at pH 7.4 using EnzCheck. Activity was determined after 1-h incubation at 37 °C. Data are mean ± S.D. (N=6). (b) Effects of metal ions for the Aβ42 activity. $CuCl_2$, $ZnCl_2$ and $FeCl_2$ at 25 μM was incubated with Aβ42 (2.5 μM), respectively, at 37 °C for 1 h, and they were tested for enzymatic activity using EnzCheck. EDTA (50 μM) was also tested to affect the Cu^{2+} in the enzymatic activity. Data are mean ± S.D. (N=6).

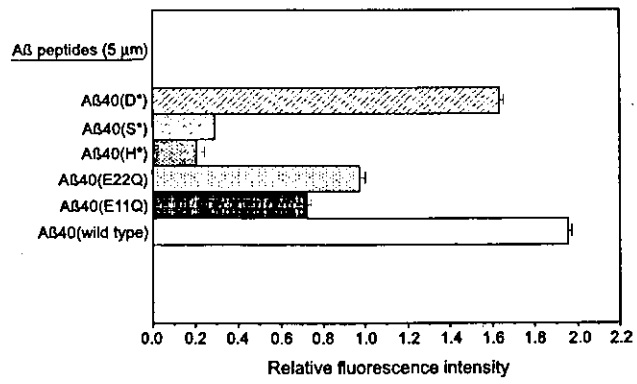

Fig. 8. Essential sequences and residues required for Aβ activity. Comparison of the proteolytic activities of Aβ40 (wild type), Aβ40 (E11Q), (E22Q), and chemically modified Aβ40; Aβ40 (*H), with histidine residues modified by DEPC; Aβ40 (*D), with aspartic acid modified by EPNP; Aβ40 (*S), with serine residues modified by DFP. Data are mean ± S.D. (N=6).

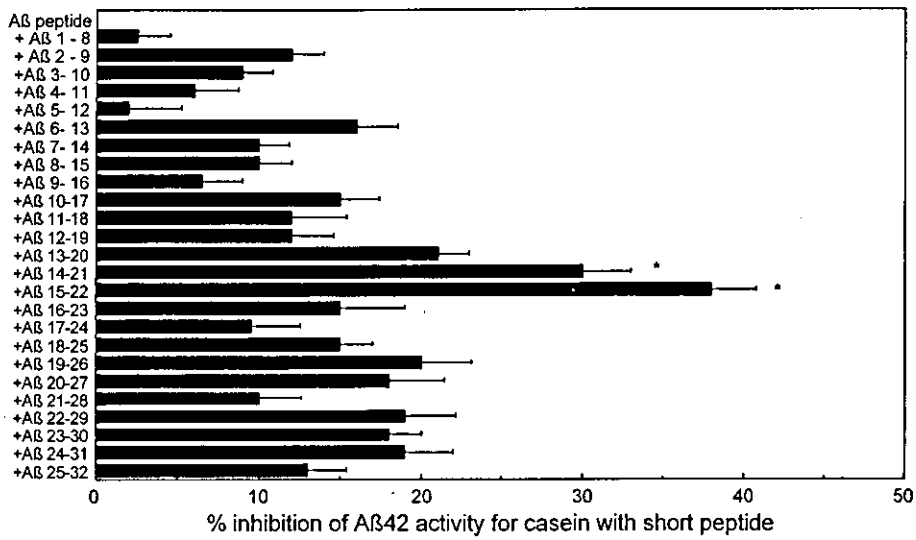

Fig. 9. Inhibition of Aβ42 activity by short peptides. Mixtures of each short peptide (5 μM) with Aβ42 (5 μM) were incubated at 37 °C and pH 7.4 for 60 min before assay with casein substrate. Proteolytic activity was determined after a further 60 min at 37 °C. As a control, the activity of Aβ42 without any short peptide was determined. Data are expressed as the percent inhibition as follows: percent inhibition=(1 – relative fluorescence intensity of mixture solution of Aβ42 and short peptide/that of Aβ42 without peptide) × 100 (%). Data are mean ± S.D. (N=6).

4.9. Inhibition of Aβ activity against casein by short peptides

The eight-residue peptides were tested for their ability to inhibit the hydrolytic activity of Aβ42 against casein. The percent inhibition was calculated as described in Methods. Only Aβ14–21 and Aβ15–22 were inhibitory, by about 30% and 38%, respectively (Fig. 9). Aβ15–22 also inhibited the hydrolytic activity of Aβ17–42 by about 45% (data not shown).

5. Discussion

Guanidine hydrochloride (GdnHCl) is a commonly used protein denaturant and at high concentrations unfolds the molten globule state. However, at low concentrations it can refold acid-unfolded proteins such as apomyoglobin and cytochrome *c*, stabilizing their molten globule state [26]. We have shown in the present study that it exposes amino acid residues 9–14 in Aβ42 (Figs. 1 and 2), supporting our previous observation that this region is hidden at pH 4.6. In addition GdnHCl caused unfolding of partially aggregated Aβ42, which may correspond to the intermediates that acquire proteinase resistance [12].

Studies of Aβ42 aggregation kinetics have suggested that aggregation of the hydrophobic form of Aβ42 can be selectively inhibited by the more soluble form, and that aggregation is driven by a hydrophobic effect of Aβ42 [27]. The central region of Aβ42 has been implicated in various biological functions including interaction with other proteins such as apolipoprotein E [3] and this region, comprising amino acid residues 19–25 has a very important influence on the aggregation and secondary structure of Aβ peptide [28]. It has been suggested that residues10–23 may provide the structural basis of the hydrophobic behaviour under physiological conditions [29]. Amino acid substitution studies indicate that the hydrophobic residues at position 17–20 are crucial for the amyloidogenic properties, with the very hydrophobic carboxy-terminal residues 29–42 corresponding to the transmembrane domain of Aβ42 [30,31] (Fig. 1). The five-residue peptide LPFFD, which is homologous to the central hydrophobic region 17–21 (LVFFA) of Aβ42, has been reported to inhibit Aβ fibrinogenesis in vitro, prevent neuronal cell death in culture and reduce Aβ deposition in the rat brain [32]. It has also been claimed that this peptide can reverse pre-existing Aβ fibrils [33]. Our pervious study of the pH-induced conformational transitions of Aβ42 also suggested that amino acids residues at positions 9–14 and 17–21 were responsible for the changes [12].

The ability of partial Aβ fragments around Aβ16–23 to inhibit Aβ42 aggregation was proved by both ELISA and cell western dot blot analysis (Figs. 3 and 4a,b). This may be due an ability to bind to the central hydrophobic region of Aβ42, including the pH-sensitive region, thereby destabilizing the interaction between Aβ monomers and/or oligomers necessary for fibril stability. As a result, the site of proteinase cleavage would be exposed, and it would become sensitive to proteinase K. Short fragments around Aβ16–23 may be produced in vivo in the normal processing steps, since α-secretase is reported to act between residues 16 and 17 [34], and cathepsin D in the lysosome acted in the region of residue 21 [35,36]. Moreover, exposure to the proteinase responsible for insulin degradation generates the Aβ17–24 fragment [37]. Hence, peptides around Aβ16–23 could be physiological products and may play an important role in inhibiting self-aggregation

of Aβ42 in vivo. In our present assay system Aβ16–23 inhibited Aβ42 aggregation better than the five-residue peptide LPFFD (data not shown). We have suggested that short peptides could facilitate the formation of mixed aggregates with Aβ42 that are sensitive to proteinase K and these findings raise the possibility that peptides around Aβ16–23 could be useful for treating amyloid plaque in the AD brain.

We have confirmed the proteolytic activity of Aβ42 for casein at neutral pH and have shown that Aβ17–42 also has some activity (Figs. 6 and 7a). pH shift from 7.4 to 4.6 decreased the activity of Aβ42 by about 76% and resulted in the same level of activity as Aβ17–42, which was almost unaffected by acid pH (Fig. 6). These data indicate that residues important for Aβ42 activity are pH sensitive and that these must be present in the region between residues 1 and 16. We speculate that residues around 9–14 and 17–21, which are affected by pH and induce conformational changes [12], may participate in the activity. Though Aβ42 requires the first 16 residues for full activity, Aβ1–16 itself has no activity (Figs. 6 and 7a). As there are no amino acid residues in Aβ29-42 capable of forming the active site, the region 1–28 must contain all of the amino acid residues essential for activity. Aβ1–28 corresponds to the extracellular domain and contains the first α-helix (Fig. 1), and had a low level of activity. It should be noted that although NMR studies of micelle-bound Aβ42 revealed the existence of an α-helix in this region [38], examination in water did not support that finding [39]. Despite the fact that the lengths of Aβ1–28 and Aβ17–42 are almost the same, Aβ17–42 showed higher activity than Aβ1–28 (Fig. 7a). We suggest that the region 29–42, which contains a second a-helix and corresponds to the transmembrane domain, is essential for full activity of Aβ42 and that serine 26 may contribute to the partial activity of Aβ17–42. Furthermore, residues 29–42 may be essential for stabilizing the first α-helix (residues 9–23), because these residues are reported to be essential for stabilizing the fibrils [40,41]. Although Aβ1–16 and Aβ12–28 may contribute residues crucial for Aβ42 activity, both are shorter than Aβ1–28 and lack the region required for stabilization. Metal ions including copper and zinc could induce conformational transition of Aβ42 at neutral pH and amino acids residues 9–14 (6F/3D epitope) participate to the changes [12] and in the present study, copper could also inhibit the enzymatic activity of Aβ42 (Fig. 7b). These results suggest that the 6F/3D region, which is associated with copper, might participate to the formation of the catalytic site of Aβ42.

Chemical modification of the serine, as well as the histidine residues of Aβ40, dramatically reduced proteolytic activity. However, modification of the aspartic residue has no affect (Fig. 8). Substitution of glutamic acid by glutamine at positions 11 and 22, in the putative α-helical region, also decreased activity by 50–60% (Fig. 8). The mutation at position 22 (E22Q) is known as the "Dutch type" and gives rise to familial early onset AD [42–44]. Such protein has potent aggregative ability and neurotoxicity in PC12 cells (rat pheochromocytoma) [45]. Our previous data suggested that the glutamic acid at position 11 is very sensitive to acidic pH and is a key residue for preserving the conformation around region 9–21 [12]. The present findings suggest that serine and histidine residues participate directly in the proteolytic activity of Aβ42, indicating that this may be a serine proteinase-like activity. The glutamic acids at positions 11 and 22 may be required to preserve the conformation around the catalytic site and the 29–42 region may be essential to stabilize the conformer. Further experiments are required to identify the serine and histidine residues responsible for activity.

Interestingly, the proteolytic activity of Aβ42 was only inhibited by Aβ14–21 and Aβ15–22 (Fig. 9). Thus, Aβ15–22 can inhibit both Aβ42 aggregation at acidic pH and its serine proteinase-like activity at neutral pH, and we speculate that oligomerisation of Aβ42 may be required for full proteolytic activity. Aβ15–22: QKLVFFAE may be useful in the treatment of AD.

Acknowledgements

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神経心理学

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abstract

アルツハイマー病では主に脳の後方領域が障害され，記憶障害の他，失語や失行がみられる．近年，画像診断技術が進歩し，また，MMSEをはじめ痴呆の神経心理検査バッテリーの開発，普及もめざましい．しかし，神経心理学的所見とは，単に認知機能検査の結果をさすものではなく，神経心理症状ないし精神神経症候の的確な把握，記載が望まれる．

I はじめに

近年の画像診断技術の進歩により，痴呆を含む脳損傷者ならびに健常人を対象とした脳の研究が発展し，また数々の痴呆の神経心理検査バッテリーの開発，普及もめざましいが，神経心理学的症状の把握はテストを施行すれば可能であるというものではないことに留意する必要がある．アルツハイマー病（AD）では変性過程が進行すると，さまざまな道具障害，すなわち一般知性の底にあって道具となりうる言語，認知，記憶といった作能の障害である，失語，失行，失認，健忘などの巣症状が出現し，同時に一般知性障害や全般性注意障害が加わる．このため個々の道具障害を純粋に抽出することが困難となる．したがって，長谷川式簡易知能評価スケール（HDS-R）[1]やMini-Mental State Examination（MMSE）[2,3]の下位項目の粗点の低下は，単にある一つの道具障害によるものではなく，さまざまな要因が関与してくるようになる．したがって，そこでは神経心理症状ないし精神神経症候の的確な把握が求められる[4,5]．ここではADにみられる代表的な神経心理学的所見と，実際の神経心理学的検査について解説する．

II 記憶障害

ADの初期症状として注目される健忘あるいは物忘れは記銘力障害であり，エピソード記憶の障害である．臨床的には即時記憶は比較的保たれており，その場その場での会話は成立するものの，著明な近時記憶の障害を呈し，数分前のことも忘れてしまう[6,7]．例えば，診察時に3つの物品を引き出しに隠し，なにがあったか答えさせると，直後には正答するが，数分後にもう一度尋ねると正しく答えられなかったり，隠したこと自体も忘れていたりする．

III 失語[8]

通常は語が思い出せないという語想起障害（喚語困難）に始まり，さらに了解障害が加わり，まれには非流暢性失語像を呈することもあるが，通常は流暢性の失語像を呈する．進行した例ではウェルニッ

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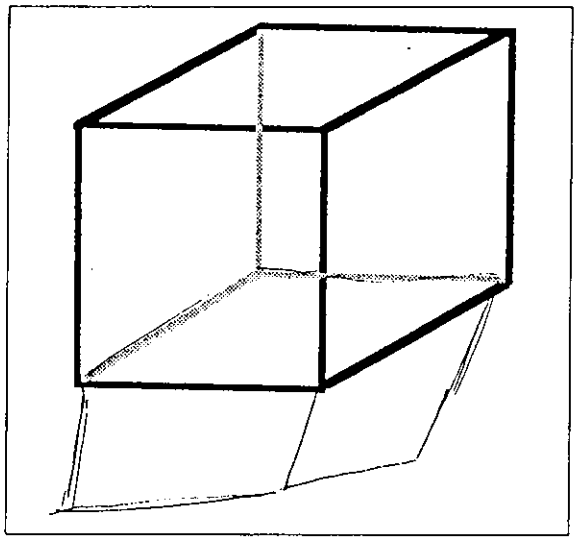

図1　アルツハイマー病患者にみられたなぞり描き(closing in)

ケ失語のように錯語やジャルゴンを呈する場合もあるが，本質的には復唱は保たれ呼称や理解に障害を認め，超皮質性失語の範疇でとらえられる場合が多い．復唱障害とされている場合でも実際には復唱の指示が十分入っていなかったり，取り繕い反応のため，なにを検査したのか判然としない場合もあり，注意を要する．

Ⅳ 構成障害

問題行動として徘徊はよく挙げられるが，空間的見当能力の障害が強くなると，よく知っている場所でも迷うようになり，家の中でも迷ってしまうことがある[5),6)]．ゴルフのパターがうまく打てない，自動車を車庫にうまく入れられない，バスなどのバスカードをうまく差し込めない[9)]といった客体を空間的に正しく定位できない症状から，さらにはベッドに斜めに寝る，電車の中で他人の膝の上に座るといった自己身体さえも空間的に正しく定位できない症状もみられる[10)]．検査では立方体の模写ができなかったり，clock drawing test（CDT）で正しく時計の絵を描くことができなかったりする．模写の課題では重症の場合にはなぞり描きがみられることもある（図1）．CDTはごく短時間で実施でき，視空間構成能力をはじめ，抽象概念や数の概念などの言語理解能力，言語的記憶などさまざまな認知機能を評価でき，ADのスクリーニングや重症度評価の補助手段としても有用である[11),12)]．

Ⅴ 神経心理学的検査施行にあたっての一般的な注意事項

難聴や視力障害，言語障害などが存在する場合，検査の成績が低下するのは当然であり，これらの障害の有無や程度を考慮する必要がある．また，身体症状（体調）や精神状態は特に注意集中に影響する．施行当日の体調不良や拒否が強いなどの不安定な精神状態では，実際の能力よりも低く評価されるか，施行できない場合もある．また高齢の患者の場合は注意集中を維持することが困難な場合もあり，患者の様子を見ながら1回の検査時間を短くしたり，途中に休憩を挟んだりする必要がある．また検査の項目で施行の順番が厳密に決まっていないものであれば患者の様子を見ながら順番を入れ替えるか，中断可能なものであれば何度かに分けて施行するといった工夫も必要である[13),14)]．

評価にあたっては対象者の教育歴（教育年数）も確認することが必要である[15)]．対象者が高い教育歴を有する場合，簡易知能検査であればたやすく回答してしまったり，WAIS-R成人知能検査法（WAIS-R）においても正常範囲内，ないし軽度の低下にとどまったりする場合がある．高い教育歴の患者では，より多様性のある認知機能障害を示すという報告もある[16)]．また，検査の得点や時間だけでなく，施行時の患者の様子などを文章で記載しておくことも重要である．非協力的な患者の0点も協力的な患者の0点も，数字上は同一であるが，症候学的には意味が異なるためである．

Ⅵ 実際の検査

1）一般的認知機能検査[15)]

認知機能検査には対象者本人に対して行う質問式（直接式）と対象者の状態をよく知る家族などの主たる介護者から情報を得る観察式（間接式）がある．

SMQ　評価　年　月　日　氏名　（回答者　）	できない	時にはできる	大体はできる	いつもできる
1. 昨日着ていた服装を覚えていますか？	1	2	3	4
2. いつも利用するバス/電車の停留所を覚えていますか？	1	2	3	4
3. 自分の家の電話番号を言えますか？	1	2	3	4
4. 雑貨店で，メモを持たずに5つの品物を忘れずに買うことができますか？	1	2	3	4
5. いつでも自分の眼鏡をどこに置いたか覚えていますか？	1	2	3	4
6. いつでも自分の鍵をどこに置いたか覚えていますか？	1	2	3	4
7. 家族の誕生日を覚えていますか？	4	3	2	1
8. 誰かに訊ねられると，自分の家への道筋を教えることができますか？	1	2	3	4
9. 外出したときに，家の戸締まりをしたか覚えていますか？	1	2	3	4
10. スーパーを出るときにお釣りをいくらもらったかを覚えていますか？	1	2	3	4
11. 先週の日曜日の午後に，なにをしたかを話すことができますか？	1	2	3	4
12. 家の人や他の人が頼んだ用事を，覚えておくことができますか？	1	2	3	4
13. 言おうとしている言葉がすぐに出てきますか？	4	3	2	1
14. 自分でお金の管理ができますか？（支払い，銀行口座，預貯金など）	1	2	3	4
注意：7番と13番の得点は合計から減じる。	得点　/46点			

表1
日本語版short memory questionnaire（SMQ）
〔文献20）より引用〕

それぞれに長所短所があり，両者を組み合わせることにより，より正確な認知機能を測定することが可能である．また，ピック病にみられるような考え無精[5),6)]や，せん妄のような注意障害がある場合には教示そのものが正しく保持されないため，認知機能を正しく評価することはできない．したがって検査施行にあたっては，最初からWAIS-Rやウエスクラー記憶検査法（WMS-R），前頭葉機能検査などを施行するのではなく，まずMMSEのような簡易な検査を施行したうえで，より複雑な課題ないし特殊な課題を施行することが望ましい．

①MMSE

Folsteinら[2)]によって考案され，わが国では森ら[3)]によって邦訳，標準化された．見当識，記銘力，注意と計算，言語，構成の課題からなり，30点満点である．通常15分程度で施行可能であり，痴呆による認知機能の低下を検出するための，簡便で信頼度の高い検査である．邦訳版では23/24点をカットオフ値とすると，痴呆の検出率は83%，健常者を痴呆なしと判別できる特異性は93%を示す．老年期の典型的なADでは，記銘力（3単語の遅延再生），時の見当識，計算，構成，場所の見当識，言語の順に障害される．若年性のADなどでは構成の著しい障害がみられることもある．また，会話の印象や通常の行動からかけ離れて成績が悪く，3段階命令や，書字命名，計算課題などの障害が目立つ場合には，失語があるか，せん妄などなんらかの原因で注意力低下をきたしている可能性がある．本検査は簡便で，かつ認知機能を多角的にとらえられるためスクリーニングに適していると思われる．わが国でよく使用されているHDS-RはMMSEと比較して記憶に重点が置かれ，近時記憶障害の検出に優れている．また，語産生課題により前頭葉機能低下や喚語困難を検出できる項目が含まれていることも特徴である．しかし，記憶障害の著しい症例では意欲低下や拒否をきたしやすく，施行時に配慮が必要であると思われる[14)]．

②リバーミード行動記憶検査

イギリスのリバーミードリハビリテーションセンターで日常記憶の障害を発見し，治療による変化を観察するために開発されたテストバッテリーである[17)]．日常記憶とは実際の日常生活場面で必要とされる記憶のことである．この検査では，名前の記憶や会話内容の記憶，展望記憶（約束），絵の記憶，行為（用件を含む）の記憶，見当識など日常生活に直接影響を与えるような記憶障害を検出できるのが特徴である．これまでの記憶の検査では評価されることのなかった展望記憶（近い将来の約束や実行の記憶）が評価できる唯一の記憶検査である．また，リハビリテーションの効果判定という繰り返しの評価を前提に作成されており，同等の難易度の4つの平行検

評価　年　月　日　氏名

A. 電話の使い方	
1. 自由に電話をかけることができる	1
2. いくつかのよく知っている番号であればかけることができる	1
3. 電話で対応できるが電話をかけることはできない	1
4. 全く電話を使うことができない	0
B. 買い物	
1. 1人で買い物ができる	1
2. 少額の買い物であれば1人でできる	0
3. だれかが付き添っていれば買い物ができる	0
4. 全く買い物ができない	0
C. 食事の支度	
1. 人数にあった支度をして必要十分な用意ができる	1
2. 材料が用意してあれば食事の支度ができる	0
3. 食事をつくることはできるが，人数にあった用意ができない	0
4. 他人に支度をしてもらう	0
D. 家　事	
1. 力仕事など以外は1人で家事をすることができる	1
2. 食事のあとの食器を洗ったり布団を敷くなどの簡単なことはできる	1
3. 簡単な家事はできるが，きちんとあるいは清潔に維持できない	1
4. 他人の助けがなければ家事をすることができない	1
5. 全く家事をすることができない	0
E. 洗　濯	
1. 1人で洗濯できる	1
2. 靴下などの小さなものは洗濯できる	1
3. 他人に洗濯してもらう	0
F. 移動・外出	
1. 自動車を運転したり，電車やバスを利用して出かけることができる	1
2. タクシーを自分で頼んで出かけられるが，電車やバスは利用できない	1
3. 付き添いがあれば電車やバスを利用することができる	1
4. 付き添われてタクシーや自動車で出かけることができる	1
5. 全く出かけることができない	0
G. 服薬の管理	
1. きちんとできる	1
2. 前もって飲む薬が用意されていれば自分で服薬できる	0
3. 自分では全く服薬できない	0
H. 金銭の管理	
1. 自分でできる（家計費，家賃，請求書の支払い，銀行での用事など）	1
2. 日常の買い物はできるが，大きな買い物や銀行へは付き添いが必要	1
3. 金銭を扱うことができない	0

得点　男性　/5，女性　/8

表2
日本語版instrumental activities of daily living scale（IADL）
得点は，男性では0～5点，女性では0～8点
〔文献25）より引用〕

査が用意され，繰り返しによる練習効果の影響を排除できる．わが国では2002年に邦訳版が標準化され[18]，さらに軽症AD患者における有用性が報告された．本検査は初期AD患者の検出や，治療効果の判定のみならず，AD患者の日常生活上の問題を予測し家族や介護者に対する介入法の指導に役立つものと期待される．

③short memory questionnaire（SMQ）

AD患者の記憶障害に対する自覚は，比較的早期に失われる．また医療機関を家族などに連れられて受診している状況では，診察，検査を含めて本人の協力が得られない場合もある．したがって記憶障害の程度については家族などの主たる介護者から聴取することが多い．SMQはKossら[19]によってADの早期発見を目的として開発された観察式の評価尺度で，筆者ら[20]により邦訳，標準化された．**表1**に日本語版SMQを示す．SMQはRiegeらが高齢者の記憶の自己評価用として作成した30項目の質問内容[21]から健常高齢者と軽度AD患者の鑑別に有用であった14の項目をKossらが抜粋したもので，それぞれ1から4までの4段階で評価する．7番と13番の2項目の点数を他の12項目の合計点から減じたものが得点と

評価　年　月　日　氏名

A. 排　泄	
1. 排泄は全く介助を要しない	1
2. 誘導あるいは後始末に介助が必要，時に（多くても週に1度）失敗がある	0
3. 週に1度以上，寝ている間に失禁がある	0
4. 週に1度以上，日中に失禁がある	0
5. 常に失禁がある	0
B. 食　事	
1. 介助なしで食事ができる	1
2. 食事のときに多少の介助が必要，特別な調理法が必要あるいは食事のときに汚したものを片づけてもらう	0
3. 食事に介助が必要であり，食べるときにも散らかってしまう	0
4. 常に介助が必要	0
5. 自力では全く摂取できない	0
C. 着替え	
1. タンスから適切な服を選んで自分で着替えられる	1
2. 多少の介助で脱ぎ着できる	0
3. 服を選んだり，脱ぎ着に手助けが必要	0
4. 着替えに介助を要するが，本人も協力する	0
5. 常に介助が必要であり，着替えに拒否的	0
D. 身繕い（身だしなみ，髪や爪の手入れ，洗面など）	
1. いつも身だしなみがきちんとしている	1
2. 1人で身繕いができるが髭などは剃ってもらう	0
3. いつも多少は手伝ってもらう	0
4. 常に介助を要するが，そのあとはきちんとしていられる	0
5. 介助に抵抗する	0
E. 移動能力	
1. 1人で出かけることができる	1
2. 家の中か家の周囲まで出かけることができる	0
3. 杖（　），歩行器（　），車椅子（　）の助けが必要	0
4. 椅子や車椅子に座っていられるが，自分では動かせない	0
5. 終日の半分以上は寝たきり	0
F. 入　浴	
1. 介助なしで入浴できる	1
2. 浴槽の出入りには介助が必要	0
3. 手や顔は洗えるが他の部分を洗えない	0
4. 自分では洗えないが協力的	0
5. 介助に抵抗する	0
得点　/6	

表3
日本語版physical self-maintenance scale (PSMS)
得点は0～6点
〔文献26)より引用〕

なる．日本語版SMQは日本語版MMSEと高い相関を示し，39/40をカットオフ値とした場合，ADの検出率は100%，特異度は94%であり，スクリーニング検査として優れている．

2) ADLの評価方法

痴呆が，脳の器質性の要因により，いったん獲得，成立した知的機能に欠損が生じ，それまで可能であった日常生活や社会生活に支障をきたした状態をさす以上，痴呆患者の総合的機能評価に際しては認知能力の障害と並んで日常生活活動能力（ADL）の障害の評価が不可欠である．ADLには，更衣，排泄などの基本的な生活機能であるbasic ADLと買い

物，電話の使用などのより高次の生活機能であるinstrumental ADLがあり，この両者を評価する必要がある．LawtonとBrodyは高齢者の日常生活における自立度の簡便な評価尺度として，instrumental ADLに対してinstrumental activities of daily living scale（IADL）を，basic ADLに対してphysical self-maintenance scale（PSMS）を考案し，その有用性を示した[22)~24)]．

表2に日本語版IADL[25)]を示す．評価にあたっては，本人の日常生活をよく知る家族ないし主たる介護者から聴き取りを行う．IADLは8項目から構成されているが，対象が男性の場合には「食事の支度」「家事」「洗濯」については評価しない．したがって，得点範囲は男性では0～5点，女性では0～8点となる．PSMS[26)]（**表3**）は男女とも6つの基本的な生活機能を評価し，合計点を算出する．必ずしもIADLとPSMSの両者を評価する必要はなく，必要に応じていずれか一方のみの使用も可能である．

Ⅶ おわりに

神経心理学とは「高次精神活動を脳の構造との関連において研究する分野」であり，失語，失行，失認などの代表的巣症状のみならず器質性の幻覚，記憶障害，知能障害，情動障害をも対象としている[27)]．前述したように最も重要なことは検査を施行して点数を算出することではなく，診察や検査を通じてAD患者を含む対象者の症候を的確に把握し，記載することである．

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Sleep health, lifestyle and mental health in the Japanese elderly
Ensuring sleep to promote a healthy brain and mind

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Abstract

The Ministry of Health, Labor and Welfare in Japan proposed a plan called "Health Japan 21," which adopted sleep as one of the specific living habits needing improvement. This has led to increased interest in mental health needs at community public health sites. In addition, it was reported from a recent 2000 survey that one in five Japanese, and one in three elderly Japanese, suffer from insomnia. Insomnia is becoming a serious social problem; so much so that alarm bells are ringing with insomnia listed as one of the refractory diseases of the 21st century. Against this background, in January 2001, Japan began a national project called "Establishing a Science of Sleep."

This article is an overview of sleep and health in the elderly, sleep mechanisms and the characteristics of insomnia among the elderly. At the same time, it introduces the scientific basis for lifestyle guidance that is effective for ensuring comfortable sleep, an essential condition for a healthy, energetic old age, with actual examples from community public health sites. The present authors reported that a short nap (30 min between 1300 and 1500 h) and moderate exercise such as walking in the evening are important in the maintenance and improvement of sleep quality. The study was to examine the effects of short nap and exercise on the sleep quality and mental health of elderly people. "Interventions" by short nap after lunch and exercise with moderate intensity in the evening were carried out for 4 weeks. After the "intervention," wake time after sleep onset significantly decreased and sleep efficiency significantly increased, showing that sleep quality was improved. The frequency of nodding in the evening significantly decreased. As a result, the frequency of nodding before going to sleep decreased, and the quality of nocturnal sleep was improved. Present results demonstrated that the proper awakening maintenance during evening was effective in improving sleep quality. After the "intervention," mental health also improved with improving sleep quality. Furthermore, physical health also improved with improving sleep quality. These results suggest that this "intervention" technique is effective for the quality of life (QOL) and the activity of daily living (ADL) of elderly people.

Keywords: Elderly; Exercise; Intervention; Lifestyle; Nap

The aging of Japanese society and measures against insomnia

As of 2000, the proportion of elderly people over the age of 65 in Japan had reached 17% of the total population, a proportion that is expected to exceed 22% by 2010 (Ministry of Health, Labor, and Welfare white paper). In the 21st century, we will face a rapidly aging society, and, as seen from the calls for improved quality of life (QOL) rather than simple longevity, there is now a strong desire to achieve longevity with both health and true wellbeing. In June 2000, the World Health Organization (WHO) first published *Healthy Life Expectancy*, which estimates the age to which people can expect to live in health. Of the 191 countries surveyed, Japan was reported to have the longest healthy life expectancy of all, at 74.5 years (males 71.9 years, females 77.2 years; mean lifespan was also the longest in the world at 80.9 years), followed closely by Australia at 73.2 years. The number of elderly people with dementia, on the other hand, is expected to increase 1.8-fold from the current number of about 1.6 million to 2.9 million in 2020. This will also increase the burden and stress placed upon family and caregivers.

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In recent years, it has been reported that sleep is closely related to physical and mental health [1–13], and that one in three Japanese elderly suffer from insomnia [14]. In Japan's aging society, dealing with insomnia among the elderly has become a major social issue. Lack of sleep or sleep disorders in the elderly can lead to social maladjustment, including decreased motivation or depression, and there are physical affects as well with an increased risk for lifestyle-related diseases.

Approach to comfortable sleep from lifestyle improvements

Ensuring proper sleep is crucial for people to enjoy an energetic, vital old age without becoming senile or bed-ridden. However, if an elderly person does have difficulty sleeping, administration of sleep medication can in many cases be problematic due to low responsiveness to the drug, the risk from combined use with medications for other diseases and regular dose-dependence or side effects from long-term use. To assure proper sleep in the elderly, therefore, lifestyle improvements can play a key role. Sleep science in recent years has shown that regular short daytime naps can help relieve fatigue in the brain and improve nighttime sleep [10,15,16]. Naps are also reported to be effective in lowering the risk of dementia of the Alzheimer's type to one-fifth and in preventing lifestyle-related diseases [17]. These findings strongly suggest that re-examining lifestyles and ensuring high-quality sleep will be effective in greatly reducing the number of elderly with dementia or who are confined to bed. The numbers of such elderly are expected to dramatically increase in the future. Comfortable sleep in old age will not only result in a clear increase in the QOL of elderly people themselves, but will also be important in leading to increased well-being in the family and caregivers of the elderly, and society as a whole.

Changes with age in sleep architecture and biological rhythms

As a person ages, the time he or she goes to bed, gets up and sleeps tend to become earlier [8] (Fig. 1). In addition, it is reported that sleeping time (time in bed) increases with age for people beyond the age of 60, exceeding 8 h for those older than 80. Thus, there is a marked increase in sleeping time with age.

Fig. 2 shows a model of changes in sleep architecture and biological rhythms. Sleep in the elderly may be characterized in a word as shallow, inefficient sleep. With age there is a considerable decrease in deep sleep (slow-wave sleep, Stages 3–4) and an increase in night awakenings, and thus conspicuous interruptions in sleep. Moreover, there is also an increase in early morning awakening when a person cannot get back to sleep. Another phenomenon is completely sleepless nights, in which a person tries to sleep but cannot, and greets the morning still awake. A considerable number of elderly wake up in a bad mood or low spirits because of poor quality sleep, and so are lethargic throughout the day. The reduction in slow-wave sleep that accompanies age means a less efficient process during sleep of relieving stress or sleep pressure built up during the day. The decrease in slow-wave sleep and increase in night awakenings may be considered signs that the maintenance and control system that manages sleep is aging. As people age, it becomes more difficult to obtain sufficient sleep, as a result of which time in bed inevitably becomes longer. Sleep efficiency is poor (there is not enough sand in the hourglass at night), and so even if a person sleeps for a long time there is a lack of sharpness between sleeping and waking, and the person tends to feel intensely drowsy during the day. Furthermore, age differences in the REM latency (the duration of NREM sleep before the first REM period of the night), with older subjects showing shorter latencies than younger subjects, have been shown in some studies of normal and depressed patients [1,18–20]. The

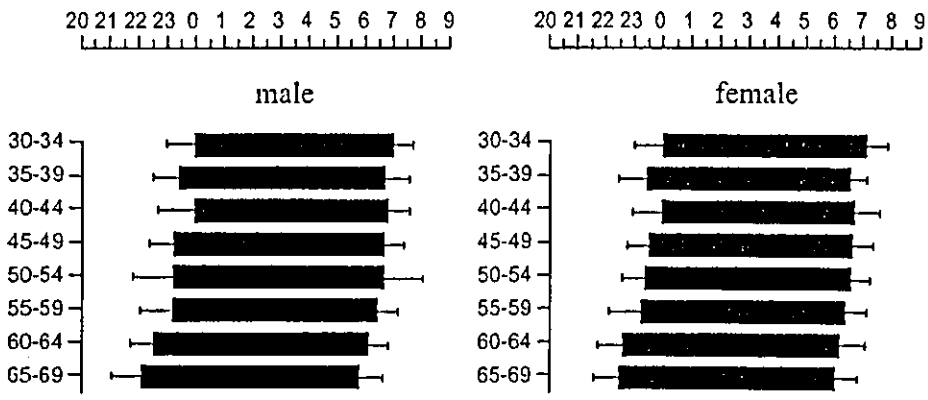


Fig. 1. Changes in sleeping habits from middle age to old age.

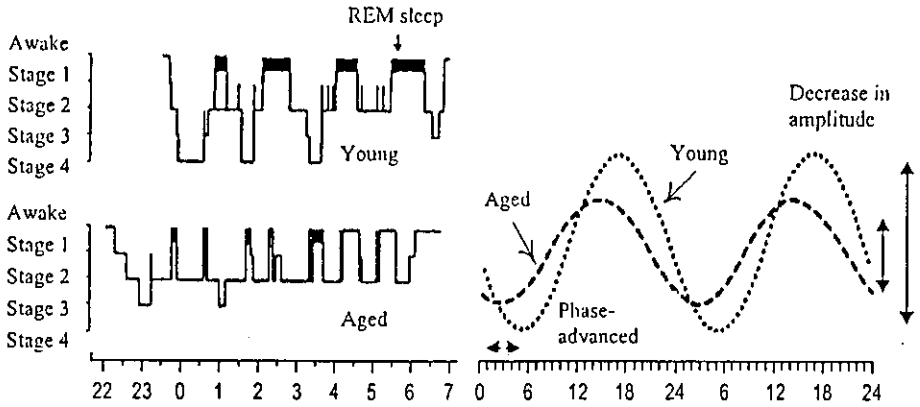


Fig. 2. A model of the deterioration in sleep functions and body rhythm (temperature rhythm) functions due to aging.

results may be a function of the decreased Stages 3 and 4 sleep within that cycle as a function of aging. One additional possibility in explaining short REM latencies in aged individuals is an age-dependent change in the circadian timing system [1].

Elsewhere, deterioration of biological rhythms due to age is seen in various circadian rhythms such as core body temperature, and the core body temperature curve is phase-advanced and a decrease in rhythm amplitude (Fig. 2, right). However, biological rhythms periods are not exactly 24 h but closer to 25 h. Therefore, to match our rhythms to the 24-h day–night rhythms of the outer world, we make a correction in our daily lives of about 1 h each day, through such means as sunlight. With the rise of the sun our eyes catch the sunlight, and the light signal is then carried to a location called the nucleus suprachiasmatic nuclei of the hypothalamus of the brain. The nucleus suprachiasmatic nuclei holds our body clock, and entrains this clock to the 24-h light–dark cycle of a single day. The factor that synchronizes circadian rhythms to the 24-h cycle of the environment is called the entraining agent, and in humans it is known to act in response to bright light, feeding, social contact and exercise. The reduction of occasion to be exposed to entraining agents caused by the transition of living surroundings and/or the deteriorated function of biological clock makes the circadian rhythm ability worse with aging.

Effects of insufficient or disordered sleep on brain function and physical and mental health: sleep health and mental and physical health

Figs. 3–6 show comparisons of day and night activity, condition of mental health and daytime drowsiness over 1 week between elderly who get poor sleep and those who get good sleep. The gray band is the time in bed at night. The height of the vertical black bars indicates the level of activity, with the parts indicating extremely low activity showing sleeping or dozing. There are a greater number of black bars during the nighttime or time in bed for elderly with poor sleep, and the length of night awakening is nearly 2 h (first night, top row). This shows that activity edges down to an extremely low level during the day, with the person dozing off many times. Thus, elderly with poor

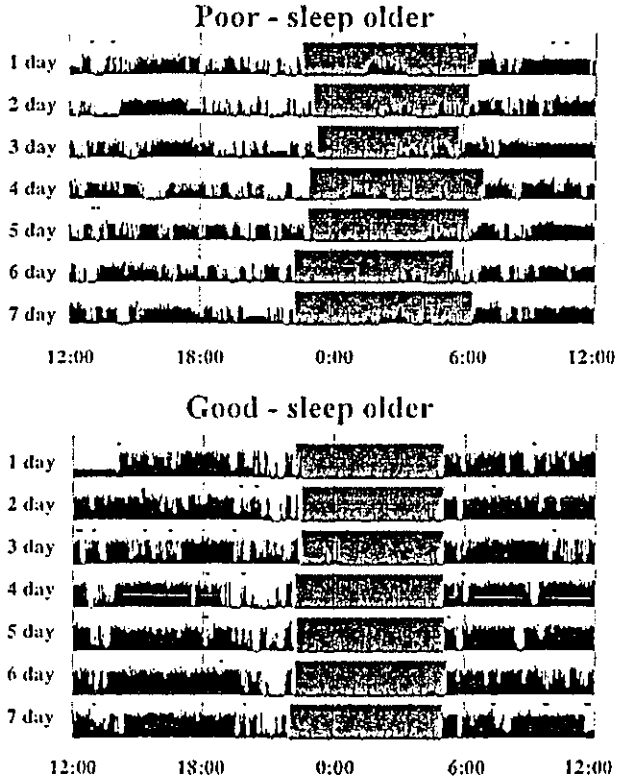


Fig. 3. Comparison of activity levels in elderly people who get good and poor sleep. This figure compares the activity level of elderly people who get poor and good sleep. Subjects wore wristwatch activity meters (actigrams) continuously for 1 week to investigate daytime and nighttime activity. The numeral 0:00 at the midpoint of the vertical axis is 12:00 midnight. The height of the black part shows the activity level (higher equals a greater activity level), and the parts with extremely low activity indicate sleeping or napping.

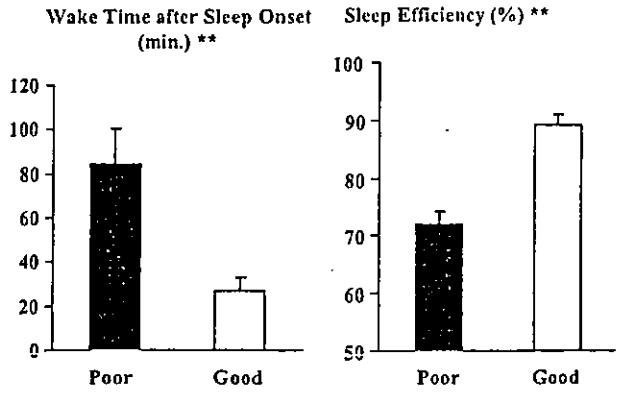


Fig. 4. Comparison of actigraph data between good sleep group and poor sleep group. ** $P<0.01$.

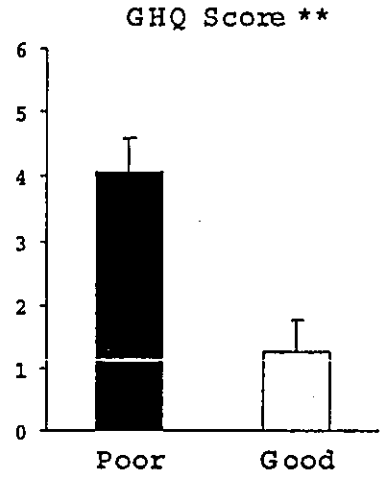


Fig. 6. Comparison of mental health between good sleep group and poor sleep group. ** $P<0.01$.

nighttime sleep doze off many times and have a low level of activity during the day.

Elderly people who get good sleep (Fig. 4), on the other hand, have a high level of activity during the day, are energetic and feel a sharp distinction between sleep and wakefulness. Their subjective feeling of drowsiness during the day is also low (Fig. 5), and they have good mental health (Fig. 6). Elderly people who get good sleep, moreover, are reported to be confident in their own life as well as being confident that they have the trust of others (a high level of social confidence), and to be healthy and volitional [21]. Recently, our studies [10] ($n=467$, 65–94years) have shown that people who get better sleep have higher levels of morale [22] (social adaptability) and satisfaction with regard to their own roles in society, higher levels of activities of daily living (ADLs) (Fig. 7) and fewer illnesses and strong subjective feelings of health. In the above study, a questionnaire based on life habits and sleep health was used. From the questionnaire involving life habits and sleep health [23,24] (Appendix), five sleep-health risk factors were determined by factor analysis, and these were scored as fellows: (1) sleep maintenance problems, (2) parasomnia-like problems, (3) sleep apnea, (4) difficulty waking up and (5) difficulty initiating sleep. Furthermore, the total

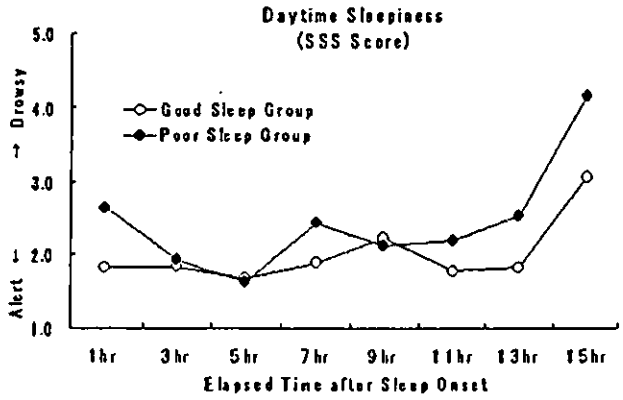
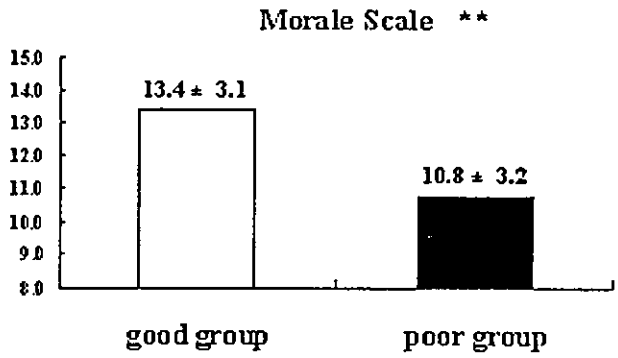


Fig. 5. Comparison of sleepiness between good sleep group and poor sleep group.



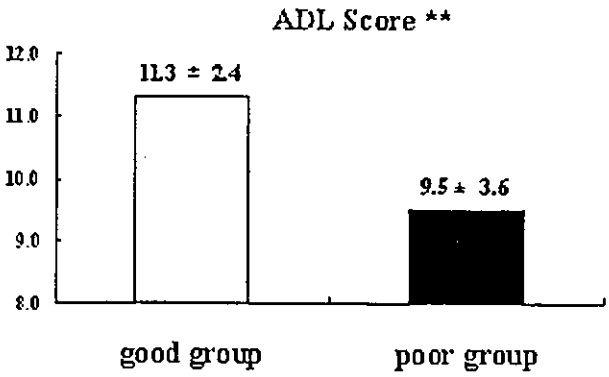


Fig. 7. Comparison of morale and ADL between good sleep group and poor sleep group.

score of each factor score was calculated as the Sleep-Health Risk Index [23,24] (Fig. 8, Appendix A). According to the rank of the Sleep-Health Risk Index, 117 (1/4 higher ranking) subjects were classified in the poor sleep-health group, and 117 (1/4 lower ranking) subjects were classified in the good sleep-health group. The two groups were then compared.

Ensuring good sleep, then, would clearly seem to occupy an important position in maintaining and promoting the mental and physical health of the elderly. The elderly have an increased incidence of various physical diseases, and suffer a corresponding increase in sleep disorders. Sleep has an active role in maintaining the body's functions, and sleep disorders have various affects on the maintenance of life. These can be life-threatening risks for elderly people, whose health is already more fragile than before.

Effects on the brain, mind and body from sleep lack or disorder

The effects of a lack of sleep or sleep disorder on brain function include decreased memory and learning functions, and lower powers of attention and concentration. Maintaining the powers of attention and concentration is sometimes difficult in the elderly, so that there is an increased risk of accidents such as falls and broken bones. Physically, lack of sleep or sleep disorders can cause decreases in the restorative functions of the body and protective (immune) maintenance functions. Decreased immune function means decreased resistance to infectious diseases, and the elderly in particular have an increased risk of infection. In addition, respiratory disorders during sleep have a great impact on the cardiovascular system, and are known to raise the risk of ischemic heart diseases, hypertension, dementia and other diseases. Known effects on mental health include lower levels of emotional control, motivation and creativity.

Survey of insomnia among the elderly: characteristics of insomnia in the elderly

Insomnia is an experience of inadequate or poor quality sleep characterized by one or more of the following: (1) difficulty falling asleep (sleep-onset insomnia), (2) difficulty maintaining sleep, (3) waking up too early in the morning (early morning awaking) and (4) nonrefreshing sleep (non-restorative sleep). Insomnia also involves daytime consequences, such as "tiredness, lack of energy, difficulty

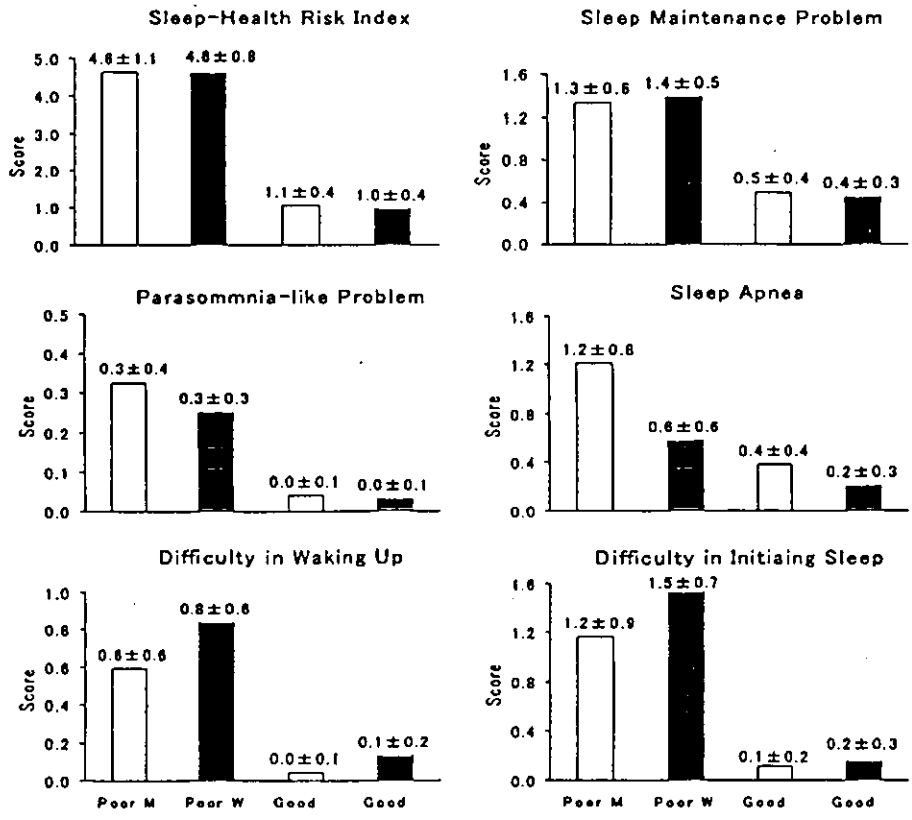


Fig. 8. Comparison of Sleep-Health Risk Index between group and sex difference. ** $P<0.01$.

concentrating, irritability" [4]. A recent national survey [14] of 3030 people aged 20 years or over in Japan found that 21.4% had experienced insomnia within the previous month. Thus, about one in five people today live day-to-day with insufficient or poor quality sleep. By age, the percentages were 18.1% for those aged 20–39 years, 18.9% for those 40–59 years and 29.5% for those 60 years and above. Thus, insomnia increases with age, with about one in three elderly people suffering this condition. About 1 in 10 elderly people are reported to have difficulty falling asleep, 1 in 5 to have night awakenings and 1 in 8 to awake too early in the morning. The causes of insomnia, other than the effects of age, are considered to include lack of exercise and difficulty dealing with stress.

In a survey on sleep disorders in 6466 outpatients (aged 3–99 years) at general hospitals nationwide (1996), the percentages of people reporting the use of sleeping medication or tranquilizers to assist them in falling asleep were 8.2% overall; 11.2% of men and 17.8% of women in their 60s; and 16.3% of men and 20.9% of women in their 70s. Use of such medication was thus shown to be particularly high among elderly women. In addition, people suspected of having restless legs syndrome or periodic limb movement disorder, which tend to be misdiagnosed as sleep-onset disorders or deep sleep disorders, accounted for 1.8% of males and 1.4% of females. Among those 80 years of age or above, they were 4.8% of males and 5.9% of females.

Reconsidering lifestyles is key to improving sleep: learning from the elderly of the "Longevity Prefecture," Okinawa

To clarify the type of lifestyle and specific measures that strongly impact sleep health, we compared the sleep health and lifestyles in Okinawa, where pre-urbanization lifestyle of Japan is considered to remain and people live unhurried lives, and Japan's largest city of Tokyo. We found an overwhelmingly smaller number of elderly people in Okinawa who were troubled because of sleep, and that elderly people in Okinawa have good sleep health. In terms of lifestyle, many Okinawans took short daytime naps, went out for evening strolls and exercised regularly. It was found in particular that elderly people who took a short nap of less than 30 min between 1300 and 1500 h had good nighttime sleep.

A considerable number of elderly people in Tokyo took naps in the evening or before going to bed, and their poor nighttime sleep was a major cause of deterioration in the proper arousal maintenance function during the day. It was thus found that elderly Okinawans slept well, and that a nonurban lifestyle with daytime naps, evening walks and appropriate exercise played a key role in the maintenance and promotion of sleep health. The elderly of Okinawa, known as the prefecture of long life, is reported to sleep better compared with those in the Tokyo metropolitan area [1]. Factors contributing to this include regular sleeping hours, as well as daily habits such as short naps and light exercise in the evenings [9,23]. In addition, it has been reported [10] that even inside the prefecture as well, there were regional differences in sleep health and lifestyle, furthermore, in ADLs. It thus seems necessary to re-examine the essential human lifestyles of earlier ages that were better for us physically. As may be understood from the foregoing, ensuring comfortable sleep is essential to a healthy, energetic old age.

Reconsidering naps: short daytime naps are effective in preventing senility and lifestyle-related diseases

Until recently, daytime naps were considered to interfere in falling asleep and the maintenance of sleep at night, and to be a cause of insomnia. In lifestyle guidance for the elderly with sleeping problems, naps were forbidden and enhanced daily activities were emphasized. Recently, however, it has come to be understood that healthy elderly tend to regularly take short daytime naps, and that naps of less than 30 min prevent nighttime insomnia among the elderly. We should therefore re-examine our thinking with regard to napping by the elderly. Moreover, although naps were thought to interfere with nighttime sleep, it is now known that this is only true of long naps of more than 1 h (which have a negative effect because the person goes into deep sleep) that produce sleep inertia (the bad mood and disorientation that a person feels upon waking), and naps close to bedtime.

It has recently been reported [15,16] that short daytime naps inhibit drowsiness and feelings of fatigue, and are effective in improving task results and EEG activity and lowering blood pressure. Short naps, therefore, are beneficial for brain function and relieving fatigue, and effectively prevent lifestyle-related diseases. Moreover, there are further benefits of regular short daytime naps. A habit of taking short daytime naps is known to be a preventive factor for dementia of the Alzheimer type [17]. Short naps of less than 30 min have been shown to reduce the risk of developing dementia to less than one fifth, and daytime naps of between 30 min and 1 h to reduce the risk to less than one half. Naps longer than 1 h, on the other hand, are associated with a twofold increase the risk of dementia of the Alzheimer type. Thus, regular short naps are effective, whereas naps that are too long have the opposite effect. An extremely interesting finding has also been reported that, by taking regular naps of less than 30 min, people with apolipoprotein E4, which is a risk factor for the development of dementia of the Alzheimer type, can greatly reduce the risk of developing the disease. Naps of less than 30 min improve nighttime sleep and alleviate brain fatigue, which is thought to elevate immune function and thereby reduce the risk of developing disease. Comparing the above with