ORIGINAL PAPER

Occlusal Disharmony Increases Amyloid-β in the Rat Hippocampus

**D. Ekuni · T. Tomofuji · K. Irie · T. Azuma ·
Y. Endo · K. Kasuyama · M. Morita**

Abstract Amyloid-β plays a causative role in Alzheimer's disease. Occlusal disharmony causes chronic psychological stress, and psychological stress increases amyloid-β accumulation. The purpose of the present study was to investigate whether occlusal disharmony-induced psychological stress affects the accumulation of amyloid-β and its related gene expressions in the rat hippocampus. Eight-week-old male Wistar rats ($n = 18$) were divided into three groups of six rats each: (1) a control group that received no treatment for 8 weeks; (2) an occlusal disharmony group that underwent cutoff maxillary molar cusps for 8 weeks; and (3) a recovered group that underwent cutoff maxillary molar cusps for 4 weeks followed by recovery for 4 weeks. Occlusal disharmony increased plasma corticosterone levels in a time-dependent manner. Levels of amyloid-β 40 and 42, glucocorticoid receptor (Gr) protein, and cleaved caspase 3 (Casp3) as well as gene expressions of amyloid precursor protein, beta-secretase, Casp3, and Gr in the hippocampus in the occlusal disharmony group were significantly higher than those in the control group ($P < 0.016$). These findings were significantly improved by recovery of occlusion ($P < 0.016$). These results indicate that psychological stress induced by occlusal disharmony reversibly induces amyloid-β 40 and 42 in the rat hippocampus through the glucocorticoid signal.

Introduction

Amyloid-β (Aβ) deposition and its neurotoxicity in areas of the brain, such as the hippocampus, play a causative role in Alzheimer's disease (AD) (Hardy and Higgins 1992). Aβ is generated by abnormal processing of amyloid-β precursor protein (APP) in the AD brain (Haass and Selkoe 2007; Chow et al. 2010). APP-cleaving enzyme 1 (BACE1), the neuronal β-secretase, is the first enzyme involved in the sequential cleavage of APP (Vassar et al. 2009). It has also been reported that Aβ40, Aβ42, and phosphorylated tau reflect the core elements of the disease process in AD (Strozyk et al. 2003; Buerger et al. 2006). Although the mechanisms through which Aβ exerts its toxicity are numerous, the detailed pathogenesis of AD is still not fully understood.

Several epidemiological studies have reported that tooth loss is associated with AD and dementia (Stein et al. 2007; Okamoto et al. 2010). Patients with AD have fewer teeth than patients with vascular dementia, and there is a significant dose–response relationship between the risk of dementia and the number of teeth in AD (Shigetomi et al. 1998). A direct relationship between the progress of dementia and reduced masticatory ability or occlusal force, which may be linked to occlusal disharmony, has been reported in elderly hospitalized patients (Okimoto et al. 1991). These findings suggest that occlusal disharmony caused by dysfunctional teeth may represent a potential risk factor for AD.

One risk factor for AD is psychological stress. Negative impacts on daily performance attributed to malocclusion

D. Ekuni (✉) · T. Tomofuji · K. Irie · T. Azuma · Y. Endo ·
K. Kasuyama · M. Morita
Department of Preventive Dentistry, Okayama University
Graduate School of Medicine, Dentistry and Pharmaceutical
Sciences, 2-5-1 Shikata-cho, Kita-ku, Okayama 700-8558, Japan
e-mail: dekuni7@md.okayama-u.ac.jp

may contribute to psychological stress in young adults (Ekuni et al. 2011). The placement of acrylic caps on both lower incisors in rats increases plasma corticosterone levels, an indicator of psychological stress (Yoshihara et al. 2001). Molarless aged SAMP8 mice have plasma corticosterone levels that are significantly greater than those in molar-intact control mice (Onozuka et al. 2002). These findings show that occlusal disharmony induces psychological stress. In addition, a recent report suggests that corticosterone itself increases Aβ accumulation and plays a central role in the development and progression of AD (Green et al. 2006). It is also known that psychological stress can increase Aβ accumulation and glucocorticoid receptor (Gr) expression in the hippocampus through the glucocorticoid signal (Dong et al. 2008). Therefore, chronic psychological stress by occlusal disharmony may increase Aβ levels in the hippocampus through the glucocorticoid signal. However, there has been no report regarding a causal relationship between occlusal disharmony and Aβ accumulation in the hippocampus.

The aim of this study was to investigate the effects of occlusal disharmony and its improvement on Aβ accumulation and its related genes, App and Bace1, in the rat hippocampus. Because Aβ causes apoptosis and triggers caspase activation (Allen et al. 2001), gene expressions of caspase 3 (Casp3) as a pro-apoptotic enzyme and Bcl2 as an anti-apoptotic enzyme in the hippocampus were also evaluated. In addition, plasma levels of corticosterone and Gr expression in the hippocampus were measured to evaluate the degree of psychological stress and the glucocorticoid signal (Yoshihara et al. 2001; Green et al. 2006).

Materials and Methods

Animals

Eighteen male Wistar rats (8 weeks old) were used for this 8-week study. Animals were housed in an air-conditioned room (23–25°C) with a 12-h light–dark cycle. Animals had free access to a powdered diet (Oriental Yeast Co., Tokyo, Japan) and tap water. The experimental protocol was approved by the Animal Research Control Committee of Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences.

Experimental Design

Rats were randomly divided into three groups of six rats each: a control (C) group, which received no treatment for 8 weeks; an occlusal disharmony (D) group, in which all maxillary molar crowns were cut off for 8 weeks; and an occlusion recovery (R) group, in which all maxillary molar

crowns were cut off for 4 weeks and were then recovered with resin materials (Superbond C&B; Sun Medical, Shiga, Japan) for the remaining 4 weeks (Irie et al. 2011). In the D group, the bilateral maxillary molar crowns were cut off at the gingival margin using a dental turbine (Irie et al. 2011). In the R group, preliminary resin trays and the silicon-base impression material (Speedex; Coltene, Altstatten, Switzerland) were prepared and were used to make an impression of the maxillary teeth of each rat, following which the maxillary molar crowns were cut off using the same method as for the D group. After 4 weeks, the resin was carefully put on the crown-less teeth using the trays attached with the impression in order to cover occlusion (Irie et al. 2011). All procedures were done under general anesthesia by inhalation of 2–4% isoflurane delivered in an O$_2$ gas. The animals were killed under general anesthesia after 8 weeks.

Measurement of Plasma Corticosterone Levels

Plasma samples were collected from the tail vein at 4 weeks and from the heart at 8 weeks, between 7:00 and 9:00, in tubes containing EDTA (TERUMO, Tokyo, Japan). Tubes were immediately placed on ice and then centrifuged at 2,000×g for 10 min at 4°C. The supernatants were collected and stored at −80°C before use. Plasma corticosterone levels were determined using an EIA kit (Yanaihara Institute Inc., Shizuoka, Japan) according to the manufacturer's instructions. Measurement was taken in duplicate, and both intra- and inter-assay coefficients of variation were <5%.

Determination of Aβ40 and Aβ42 Levels in the Hippocampus

The left hippocampus was harvested, immediately frozen, and kept at −80°C. Approximately 150 mg of tissue was homogenized in 1 ml of 70% formic acid (Borchelt et al. 1996). Homogenates were centrifuged at 100,000 × g for 1 hr to remove particulate material, and the supernatant was recovered and neutralized with a 20-fold dilution in 1 M Tris base. Following neutralization, Aβ40 and Aβ42 level per total protein was determined using ELISA kits (Human/Rat β Amyloid (40) and Human/Rat β Amyloid (42) ELISA Kits, Wako Pure Chemical Industries, Ltd., Osaka, Japan) according to the manufacturers' instructions. Measurement was taken in duplicate, and both intra- and inter-assay coefficients of variation were <5%.

Determination of Gene Expressions by Real-Time Reverse Transcription-Polymerase Chain Reaction

The right hippocampus was harvested, immediately frozen, and kept in RNAlater, an RNA stabilization solution

(Ambion, Austin, TX, USA), at −80°C until it was processed using real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis. Total RNA was isolated from the hippocampus biopsy samples using Trizol reagent (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions. The isolated RNA was quantified by measurement of the absorbance at 260 nm, and its purity was determined by the 260/280 nm absorbance ratio. Samples with a ratio >1.8 were used in the procedure described below (Irie et al. 2011; Ekuni et al. 2010). Total RNA (2 µg) was reverse transcribed by AMV reverse transcriptase (TAKARA, Shiga, Japan) at 42°C for 30 min. The prepared cDNA was diluted 10-fold with yeast RNA (10 µg/mL). Real-time PCR was performed using TOYOBO SYBR Green PCR Master Mix (TOYOBO, Osaka, Japan) and the LightCycler™ (Roche Applied Science, Mannheim, Germany) system, for 45 cycles at 95°C for 20 s, at the appropriate annealing temperature for 20 s, and at 72°C for 20 s. The primer sequences were Gr, 5′-AGGCAGTGTGAAAT TGTATCCCAC-3′ and 5′-GAGGCTTACAATCCTCATT CGTGT-3′ (Xiao and Chen 2008); App, 5′-GGATGCGG AGTTCGGACATG-3′ and 5′-GTTCTGCATCTGCTCAA AG-3′ (Lin et al. 2009); Bace1, 5′-GCATGATCATTGGT GGTATC-3′ and 5′-CCATCTTGAGATCTTGACCA-3′ (Nie et al. 2010); Casp3, 5′-ATACCAGTGGAGGCCGAC TT-3′ and 5′-CAGCTTGTGCGCGTACAGTT-3′ (Gross et al. 2007); and Bcl2, 5′-ACCTGAATGACCACCTAGA G-3′ and 5′-ACTTCCGACTGAAGAGTGAG-3′ (Gross et al. 2007). The primers used to detect the internal control marker, β-actin, were 5′-TGTTGCCCTAGACTTCGAG CA-3′ and 5′-GGACCCAGGAAGGAAGGCT-3′ (Liu et al. 2008). Each gene expression was expressed in terms of the relative copy number ratio of target gene to β-actin for each sample.

Determination of Protein Level of Gr and Casp3 in the Hippocampus

Samples of total protein were extracted from hippocampus samples using a detergent-based extraction buffer (T-PER Tissue Protein Extraction Reagent, Thermo Fisher Scientific Inc., Rockford, IL, USA) containing a protease inhibitor cocktail (Protein Stabilizing Cocktail, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The tissue samples were macerated in buffer (20 µl/mg of tissue) and centrifuged for 10 min at 11,700×g at 4°C. Aliquots (20 µg protein) were mixed with sample buffer (125 mM Tris–HCL (pH 6.8) with 20% glycerol, 4% sulfate polyacrylamide gel, 0.04% bromophenol blue, and 10% 2-mercaptoethanol) and heated at 95°C for 3 min. The samples and controls along with marker were loaded onto 10% polyacrylamide gel for SDS–PAGE, and proteins were separated over 30 min at 200 V. After gel electrophoresis,

the separated proteins were electroblotted onto a 100% Triton-free nitrocellulose membrane (pore size = 0.2 µm) (Bio-Rad Laboratories, Hercules, CA, USA) in a transfer buffer (0.3% Tris–HCl, 1.44% glycine) using 24 V for 60 min. The membrane was blocked with 5% (W/V) nonfat milk in 0.1% Tween-20 in Tris-buffered saline for 1 h at room temperature. The membranes were probed with mouse monoclonal antibody to BuGR2 (Abcam, London, UK, 1:20000) (Sarabdjitsingh et al. 2010), rabbit polyclonal antibody to cleaved Casp3 (Cell Signaling Technology, Beverly, MA, USA, 1:20000) (Siu et al. 2009), and mouse monoclonal antibody to α-tubulin (1:30000) (Sigma-Aldrich, St. Louis, MO, USA, 1:20000). Membranes were washed in a washing buffer three times for 5 min each and incubated with secondary antibodies conjugated to horseradish peroxidase (1:50000) for 1 h at room temperature and given a final wash in buffer. Subsequently, signals were visualized with an Amersham ECL Plus Western Blotting Analysis System (GE Healthcare, Little Chalfont, UK). The Western blot bands were quantified by densitometry using an Epson perfection scanner (Epson Europe, Amsterdam, The Netherlands). Each data point was then normalized relative to the α-tubulin present in that sample (Spiga and Lightman 2009).

Statistical Analysis

All data analyses were done using a statistical software package (SPSS 15.0 J for Windows; SPSS Japan, Tokyo, Japan). Comparisons between groups were made using the Mann–Whitney U-test with Bonferroni correction. The level of significance was set at $P < 0.016$. The Wilcoxon signed-rank test was used to investigate intragroup differences. A P value <0.05 was considered significant.

Results

There were no significant differences in food consumption among the three rat groups over the experimental period. Body weights (median [25%, 75%]) for the C, D, and R groups were 413.1 (405.0, 430.7), 395.9 (373.6, 407.7), and 387.1 (380.3, 398.3) g at 4 weeks, and 505.2 (497.4, 523.4), 493.2 (460.6, 498.8), and 481.0 (475.4, 490.0) g at 8 weeks, respectively. There was no significant difference in body weight among the groups.

Plasma corticosterone levels in the D group increased in a time-dependent manner, and the value was significantly higher than values in the C and R groups at 8 weeks ($P < 0.016$) (Fig. 1). No significant differences in plasma corticosterone levels were detected between the C and R groups at 8 weeks.
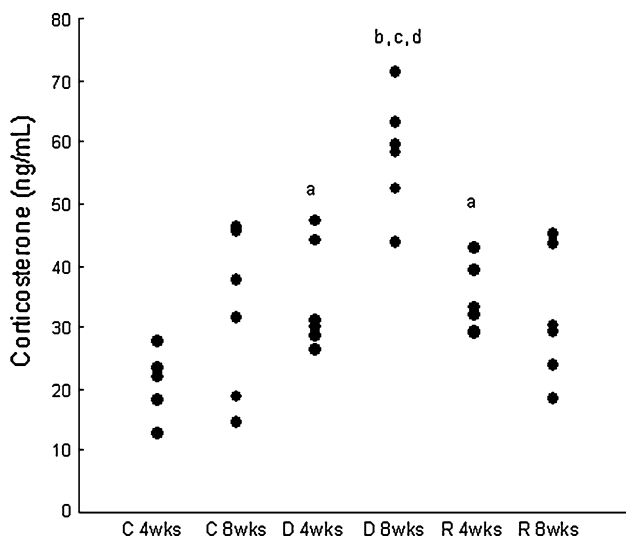
Fig. 1 Serum levels of corticosterone in the control (*C*), occlusal disharmony (*D*), and recovered (*R*) groups. Each *black circle* represents data from each rat. [a]$P < 0.016$ compared with the *C* group at 4 weeks (Mann–Whitney *U*-test with Bonferroni correction). [b]$P < 0.016$ compared with the *C* group at 8 weeks (Mann–Whitney *U*-test with Bonferroni correction). [c]$P < 0.016$ compared with the *R* group at 8 weeks (Mann–Whitney *U*-test with Bonferroni correction). [d]$P < 0.05$ compared with the *R* group at 4 weeks (Wilcoxon signed-rank test)

Levels of Aβ40 in the hippocampus in the C, D, and R groups at 8 weeks were 46.8 (32.1, 63.8), 128.5 (123.4, 134.3), and 45.0 (40.1, 49.8) pmol/mg, respectively (Fig. 2). The value was significantly higher in the D group than those in the C and R groups ($P < 0.016$) (Fig. 2).
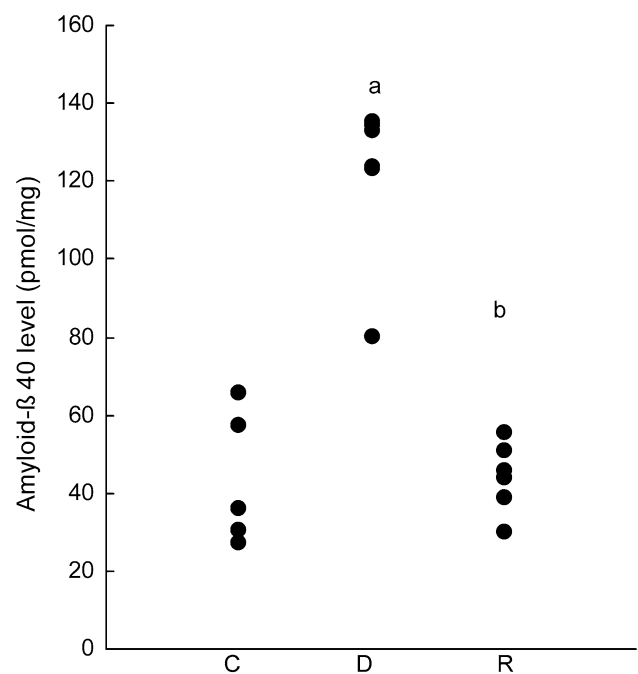
Levels of Aβ42 in the hippocampus in the C, D, and R groups at 8 weeks were 7.2 (6.0, 8.5), 19.4 (16.6, 21.6), and 10.2 (6.9, 11.2) pmol/mg, respectively (Fig. 3). The value was significantly higher in the D group than those in the C and R groups ($P < 0.016$) (Fig. 3).

Gene expressions of Gr, App, Bace1, and Casp3 were significantly higher in the D group than in the C and R groups at 8 weeks ($P < 0.016$) (Table 1). On the other hand, there was no significant difference in the Bcl2 expression between groups.

The protein expressions of Gr and cleaved Casp3 were significantly higher in the D group than those in the C and R groups at 8 weeks ($P < 0.016$) (Table 2; Fig. 4).

Discussion

Epidemiological studies have shown that tooth loss, which may be linked to occlusal disharmony, is associated with AD (Shigetomi et al. 1998; Stein et al. 2007; Okamoto et al. 2010). Occlusal disharmony induces psychological stress (Yoshihara et al. 2001; Onozuka et al. 2002; Irie et al. 2011; Ekuni et al. 2011). Psychological stress

Fig. 2 Amyloid-β 40 levels in the hippocampus in the control (*C*), occlusal disharmony (*D*), and recovered (*R*) groups. Each *black circle* represents data from each rat. [a]$P < 0.016$ compared with the *C* group (Mann–Whitney *U*-test with Bonferroni correction). [b]$P < 0.016$ compared with the *D* group (Mann–Whitney *U*-test with Bonferroni correction)
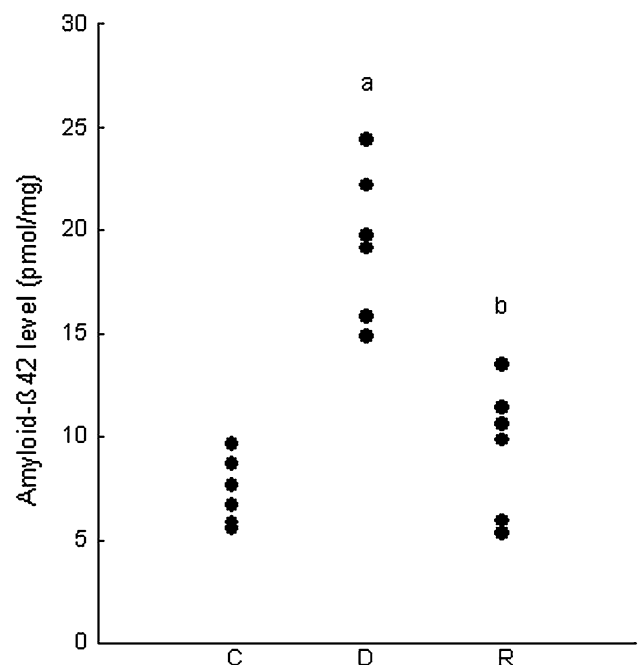
Fig. 3 Amyloid-β 42 levels in the hippocampus in the control (*C*), occlusal disharmony (*D*), and recovered (*R*) groups. *Each black circle* represents data from each rat. [a]$P < 0.016$ compared with the *C* group (Mann–Whitney *U*-test with Bonferroni correction). [b]$P < 0.016$ compared with the *D* group (Mann–Whitney *U*-test with Bonferroni correction)

Table 1 Gene expression/β actin in the hippocampus in the control (C), occlusal disharmony (D), and recovered (R) groups

Gene	C	D	R
Gr	8.4 (2.8, 9.0)[a]	10.3 (10.0, 10.8)*	8.2 (7.7, 9.3)**
App	5.0 (4.5, 5.5)	8.2 (7.1, 8.4)*	5.2 (4.2, 5.9)**
Bace1	10.4 (5.4, 10.5)	16.6 (13.9, 21.0)*	6.5 (3.4, 9.5)**
Casp3	7.0 (6.5, 7.3)	8.8 (8.5, 9.5)*	6.4 (5.1, 7.5)**
Bcl2	43.5 (34.4, 51.8)	55.6 (47.0, 61.6)	29.7 (27.2, 34,6)

*Gr* glucocorticoid receptor, *App* amyloid precursor protein, *Bace1* beta-secretase, *Casp3* caspase 3

* $P < 0.016$ compared with the C group (Mann–Whitney U-test with Bonferroni correction)

** $P < 0.016$ compared with the D group (Mann–Whitney U-test with Bonferroni correction)

[a] Median (25%, 75%)

increases Aβ accumulation and Gr expression in the hippocampus through the glucocorticoid signal (Dong et al. 2008). However, the mechanism by which occlusal disharmony induces Aβ accumulation in the hippocampus was not clearly understood. To the best of our knowledge, this is the first study to assess the casual relationship between occlusal disharmony and Aβ accumulation in the hippocampus. The increased levels of Aβ40, Aβ42, and Gr in the rat hippocampus were induced by occlusal disharmony. Plasma levels of corticosterone in the occlusal disharmony group increased in a time-dependent manner. These levels were decreased by occlusal recovery. These results suggest that psychological stress induced by occlusal disharmony reversibly induces Aβ40 and Aβ42 accumulation in rat hippocampus through the glucocorticoid signal.

Recently, a study using a mouse AD model reported that high levels of glucocorticoids found in AD mice played a central role in the development and progression of AD (Green et al. 2006). Corticosterone elevated Aβ40 and Aβ42 levels through increases in APP and BACE1 in that model. The primary mode of action by which glucocorticoids modulate Aβ levels appears to be mediated by binding to stress-activated Grs (Green et al. 2006). On the other hand, there have been other studies investigating the effects of occlusal disharmony on plasma levels of
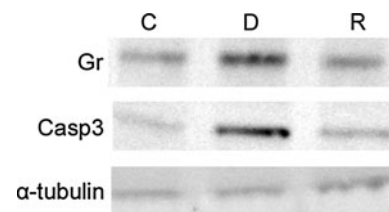
Fig. 4 Western blot analysis of glucocorticoid receptor (Gr) and cleaved caspase 3 (Casp3) in rat hippocampus. The protein expressions of Gr and cleaved Casp3 in the occlusal disharmony group (D) were higher than those in the control (C) and recovered (R) groups. α-Tubulin was used as an internal control

corticosterone. For instance, mean plasma levels of corticosterone in rats with incisal caps are significantly higher than those in rats without incisal caps (Yoshihara et al. 2001). Rats in the molarless group exhibited a significantly higher level of corticosterone than those in the control group at 24 weeks of age (Aoki et al. 2010). These observations are consistent with the present results that the increased mRNA levels of Gr, App, and Bace1 in the rat hippocampus were induced by occlusal disharmony and plasma levels of corticosterone increased in a time-dependent manner.

The median level of corticosterone in the D group was 59 ng/ml. This was 1.7 times compared with that in the C group at 8 weeks. In the other rat chronic stress models, the values were increased 3.5 times by isolation stress (Dong et al. 2008), three times by immobilization stress (Radecki et al. 2005), 1.9 times by cusp-less stress (Irie et al. 2011), and 1.3 times by molarless stress (Aoki et al. 2010) compared with each control group. These findings suggest that the stress level in our study was milder than that in other stress models.

In the D group in this study, the median Aβ40 and Aβ42 levels in the rat hippocampus were 128.5 pmol/mg, which was close to 19,200 pmol/mg of brain, and 19.4 pmol/mg of total protein, which was close to 2,800 pmol/mg of brain, respectively. In a transgenic mouse model of AD, the values of Aβ40 and Aβ42 were about 1,000 pmol/mg and 2,600 pmol/mg of brain (prefrontal cortex) using a similar method, respectively (Seo et al. 2010). Although the area (hippocampus vs. prefrontal cortex) or species (rats vs.

Table 2 Western blot analysis of glucocorticoid receptor (Gr) and cleaved caspase 3 (Casp3) in the hippocampus in the control (C), occlusal disharmony (D), and recovered (R) groups

Protein	C	D	R
Gr	1.14 (1.13, 1.16)[a]	2.24 (2.18, 2.36)*	1.08 (1.03, 1.17)**
Cleaved Casp3	0.17 (0.11, 3.34)	5.60 (3.09, 7.94)*	1.41 (1.17, 1.62)*, **

* $P < 0.016$ compared with the C group (Mann–Whitney U-test with Bonferroni correction)

** $P < 0.016$ compared with the D group (Mann–Whitney U-test with Bonferroni correction)

[a] Median (25%, 75%) (expression/α-tubulin)

mice) is different between our study and the study by Seo et al. 2010, the Aβ42 levels were similar. The median Aβ40 and Aβ42 levels in the rat hippocampus in the D group were both 2.7 times higher compared with those in the C group. In other studies, when compared with levels of the control groups, Aβ40 levels in the hippocampus in the AD model groups were 1.9 times (Iwata et al. 2001), 2.5 times (Dong et al. 2008), and more than 3 times higher (Wahrle et al. 2005), and Aβ42 levels in the hippocampus in the AD model groups were 1.8 times (Iwata et al. 2001), 2.3 times (Dong et al. 2008), and more than three times higher (Wahrle et al. 2005). It is feasible that the degrees of increased Aβ40 and Aβ42 level in our study were within the range of previous reports using the AD model.

The gene expression of Casp3 (a pro-apoptotic marker) and protein expression of cleaved Casp3 in the D group were significantly higher than those in the C and R groups, whereas Bcl2 (anti-apoptotic) gene expression did not change. These findings suggest that neuronal apoptosis in the hippocampus is induced by occlusal disharmony. Apoptotic neurons have been detected in the AD brain, and programmed cell death is thought to be one of the key factors contributing to the pathogenesis of AD (Carter 2008). In a cell model of AD in vitro, Aβ42 increased Casp3 (Liang et al. 2009). In humans with pre-AD stage, referred to as amnestic mild cognitive impairment, hippo-campal levels of Bcl2 and Casp3 were elevated (Sultana et al. 2010). These findings support that up-regulation of Casp3 and neuronal apoptosis in the hippocampus are induced by occlusal disharmony. However, the level of Bcl2 in the D group was not significantly higher than that in the other groups with a tendency for higher expression of Bcl2. The reasons for the discrepancy between our results and the previous study (Sultana et al. 2010) might depend on the species used (rats vs. human, respectively).

Previous clinical studies suggest that tooth loss may affect dementia through its chronic effects rather than acute effects (Stein et al. 2007; Okamoto et al. 2010). Therefore, the current experimental period of 8 weeks may be too short to induce dementia. However, it is already reported that the rat molar loss model causes memory impairment at 1 week after tooth extraction (Andoh et al. 2009). The present study also showed the increased level of amyloid-β in the rat hippocampus. Although further studies are needed in humans, these suggest that the tooth loss and/or molar loss could relate to an initiation of dementia at even a short period.

In this study, we hypothesized that the increased level of psychological stress following occlusal disharmony could have an influence on the hippocampus. A clinical study shows that salivary cortisol is significantly associated with tooth loss (Rai et al. 2011). It is also known that plasma cortisol levels are increased in individuals with mild cognitive impairment and in AD patients (Sauro et al. 2003; Csernansky et al. 2006). Moreover, it has been demonstrated that persons who were exposed to psycho-logical stress were more likely to develop AD than persons who were not (Wilson et al. 2005). These findings support the concept of our hypothesis. However, it is also possible that chronic underlying infections affect the hippocampus. Therefore, measuring infectious markers may also be important to clarify the mechanisms by which tooth loss or molar loss induces pathological changes in the hippocam-pus. This is a limitation of this study.

There are the other limitations to this study. First, we did not perform memory tests, such as those done using the radial arm water maze. Therefore, it is still unclear how Aβ40 and Aβ42 accumulation following occlusal dishar-mony affects brain function. Further experiments are nee-ded to clarify this issue. Second, it was not clear whether the pathogenesis of AD was established in this model because amyloid plaque was not confirmed in the brain. Although we found Aβ40 and Aβ42 accumulation in our model, a diagnosis of AD will be required in future studies.

In conclusion, psychological stress induced by occlusal disharmony reversibly induces Aβ40 and Aβ42 in rat hippocampus through the glucocorticoid signal.

References

Allen, J. W., Eldadah, B. A., Huang, X., Knoblach, S. M., & Faden, A. I. (2001). Multiple caspases are involved in beta-amyloid-induced neuronal apoptosis. *Journal of Neuroscience Research, 65*, 45–53.

Andoh, T., Sakuma, Y., Yamamoto, S., Matsuno, A., Maeda, T., & Kotani, J. (2009). Influences of molar loss of rat on learning and memory. *Journal of Prosthodontic Research, 53*, 155–160.

Aoki, H., Kimoto, K., Hori, N., Hoshi, N., Yamamoto, T., & Onozuka, M. (2010). Molarless condition suppresses prolifera-tion but not differentiation rates into neurons in the rat dentate gyrus. *Neuroscience Letters, 469*, 44–48.

Borchelt, D. R., Thinakaran, G., Eckman, C. B., Lee, M. K., Davenport, F., Ratovitsky, T., et al. (1996). Familial Alzheimer's disease-linked presenilin 1 variants elevate Abeta1–42/1–40 ratio in vitro and in vivo. *Neuron, 17*, 1005–1013.

Buerger, K., Ewers, M., Pirttilä, T., Zinkowski, R., Alafuzoff, I., Teipel, S. J., et al. (2006). CSF phosphorylated tau protein correlates with neocortical neurofibrillary pathology in Alzhei-mer's disease. *Brain, 129*, 3035–3041.

Carter, C. J. (2008). Interactions between the products of the Herpes simplex genome and Alzheimer's disease susceptibility genes: relevance to pathological-signalling cascades. *Neurochemistry International, 52*, 920–934.

Chow, V. W., Mattson, M. P., Wong, P. C., & Gleichmann, M. (2010). An overview of APP processing enzymes and products. *Neuromolecular Medicine, 12*, 1–12.

Csernansky, J. G., Dong, H., Fagan, A. M., Wang, L., Xiong, C., Holtzman, D. M., et al. (2006). Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia. *American Journal of Psychiatry, 163*, 2164–2169.

Dong, H., Yuede, C. M., Yoo, H. S., Martin, M. V., Deal, C., Mace, A. G., et al. (2008). Corticosterone and related receptor expression are associated with increased beta-amyloid plaques in isolated Tg2576 mice. *Neuroscience, 155*, 154–163.

Ekuni, D., Furuta, M., Irie, K., Azuma, T., Tomofuji, T., Murakami, T., et al. (2011) Relationship between impacts attributed to malocclusion and psychological stress in young Japanese adults. *European Journal of Orthodontics* (Epub ahead of print).

Ekuni, D., Tomofuji, T., Irie, K., Kasuyama, K., Umakoshi, M., Azuma, T., et al. (2010). Effects of periodontitis on aortic insulin resistance in an obese rat model. *Laboratory Investigation, 90*, 348–359.

Green, K. N., Billings, L. M., Roozendaal, B., McGaugh, J. L., & LaFerla, F. M. (2006). Glucocorticoids increase amyloid-beta and tau pathology in a mouse model of Alzheimer's disease. *Journal of Neuroscience, 26*, 9047–9056.

Gross, J., Machulik, A., Amarjargal, N., Moller, R., Ungethüm, U., Kuban, R. J., et al. (2007). Expression of apoptosis-related genes in the organ of Corti, modiolus and stria vascularis of newborn rats. *Brain Research, 1162*, 56–68.

Haass, C., & Selkoe, D. J. (2007). Soluble protein oligomers in neurodegeneration: Lessons from the Alzheimer's amyloid beta-peptide. *Nature Reviews Molecular Cell Biology, 8*, 101–112.

Hardy, J. A., & Higgins, G. A. (1992). Alzheimer's disease: The amyloid cascade hypothesis. *Science, 256*, 184–185.

Irie, K., Ekuni, D., Tomofuji, T., Azuma, T., Endo, Y., Kasuyama, K., et al. (2011). Occlusal disharmony induces BDNF level in rat submandibular gland. *Archives of Oral Biology, 56*, 35–40.

Iwata, N., Tsubuki, S., Takaki, Y., Shirotani, K., Lu, B., Gerard, N. P., et al. (2001). Metabolic regulation of brain Abeta by neprilysin. *Science, 292*, 1550–1552.

Liang, J. H., Du, J., Xu, L. D., Jiang, T., Hao, S., Bi, J., et al. (2009). Catalpol protects primary cultured cortical neurons induced by Abeta(1–42) through a mitochondrial-dependent caspase pathway. *Neurochemistry International, 55*, 741–746.

Lin, H. B., Yang, X. M., Li, T. J., Cheng, Y. F., Zhang, H. T., & Xu, J. P. (2009). Memory deficits and neurochemical changes induced by C-reactive protein in rats: implication in Alzheimer's disease. *Psychopharmacology (Berl), 204*, 705–714.

Liu, G., Sun, Y., Li, Z., Song, T., Wang, H., Zhang, Y., et al. (2008). Apoptosis induced by endoplasmic reticulum stress involved in diabetic kidney disease. *Biochemical and Biophysical Research Communications, 370*, 651–656.

Nie, J., Luo, Y., Huang, X. N., Gong, Q. H., Wu, Q., & Shi, J. S. (2010). Icariin inhibits beta-amyloid peptide segment 25–35 induced expression of beta-secretase in rat hippocampus. *European Journal of Pharmacology, 626*, 213–218.

Okamoto, N., Morikawa, M., Okamoto, K., Habu, N., Hazaki, K., Harano, A., et al. (2010). Tooth loss is associated with mild memory impairment in the elderly: the Fujiwara-kyo study. *Brain Research, 1349*, 68–75.

Okimoto, K., Ieiri, K., Matsuo, K., & Terada, Y. (1991). The relationship between oral status and the progress of dementia at senile hospital. *Journal of Japanese Prosthodont Society, 35*, 931–943.

Onozuka, M., Watanabe, K., Fujita, M., Tonosaki, K., & Saito, S. (2002). Evidence for involvement of glucocorticoid response in the hippocampal changes in aged molarless SAMP8 mice. *Behavioural Brain Research, 131*, 125–129.

Radecki, D. T., Brown, L. M., Martinez, J., & Teyler, T. J. (2005). BDNF protects against stress-induced impairments in spatial learning and memory and LTP. *Hippocampus, 15*, 246–253.

Rai, B., Kaur, J., Anand, S. C., & Jacobs, R. (2011) Salivary stress markers, stress and periodontitis: A pilot study. *Journal of Periodontology, 82*, 287–292.

Sarabdjitsingh, R. A., Meijer, O. C., & de Kloet, E. R. (2010). Specificity of glucocorticoid receptor primary antibodies for analysis of receptor localization patterns in cultured cells and rat hippocampus. *Brain Research, 1331*, 1–11.

Sauro, M. D., Jorgensen, R. S., & Pedlow, C. T. (2003). Stress, glucocorticoids, and memory: a meta-analytic review. *Stress, 6*, 235–245.

Seo, J. S., Jung, E. Y., Kim, J. H., Lyu, Y. S., Han, P. L., & Kang, H. W. (2010). A modified preparation (LMK03) of the oriental medicine Jangwonhwan reduces Abeta(1–42) level in the brain of Tg-APPswe/PS1dE9 mouse model of Alzheimer disease. *Journal of Ethnopharmacology, 130*, 578–585.

Shigetomi, T., Asano, T., Katou, T., Usami, T., Ueda, M., & Kawano, K. (1998). A study on oral function and aging—an epidemiological risk factor for dementia-. *Journal of Japan Stomatological Society, 47*, 403–407.

Siu PM, Tam EW, Teng BT, Pei XM, Ng JW, Benzie IF, et al. (2009) Muscle apoptosis is induced in pressure-induced deep tissue injury. *Journal of Applied Physiology, 107*, 1266–1275.

Spiga, F., & Lightman, S. L. (2009). Dose-dependent effects of corticosterone on nuclear glucocorticoid receptors and their binding to DNA in the brain and pituitary of the rat. *Brain Research, 1293*, 101–107.

Stein, P. S., Desrosiers, M., Donegan, S. J., Yepes, J. F., & Kryscio, R. J. (2007). Tooth loss, dementia and neuropathology in the Nun study. *Journal of the American Dental Association, 138*, 1314–1322.

Strozyk, D., Blennow, K., White, L. R., & Launer, L. J. (2003). CSF Abeta 42 levels correlate with amyloid-neuropathology in a population-based autopsy study. *Neurology, 60*, 652–656.

Sultana, R., Banks, W. A., & Butterfield, D. A. (2010). Decreased levels of PSD95 and two associated proteins and increased levels of BCl2 and caspase 3 in hippocampus from subjects with amnestic mild cognitive impairment: Insights into their potential roles for loss of synapses and memory, accumulation of Abeta, and neurodegeneration in a prodromal stage of Alzheimer's disease. *Journal of Neuroscience Research, 88*, 469–477.

Vassar, R., Kovacs, D. M., Yan, R., & Wong, P. C. (2009). The beta-secretase enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential. *Journal of Neuroscience, 29*, 12787–12794.

Wahrle, S. E., Jiang, H., Parsadanian, M., Hartman, R. E., Bales, K. R., Paul, S. M., et al. (2005). Deletion of Abca1 increases Abeta deposition in the PDAPP transgenic mouse model of Alzheimer disease. *Journal of Biological Chemistry, 280*, 43236–43242.

Wilson, R. S., Barnes, L. L., Bennett, D. A., Li, Y., Bienias, J. L., Mendes de Leon, C. F., et al. (2005). Proneness to psychological distress and risk of Alzheimer disease in a biracial community. *Neurology, 64*, 380–382.

Xiao, L., & Chen, Y. (2008). Culture condition and embryonic stage dependent silence of glucocorticoid receptor expression in hippocampal neurons. *Journal of Steroid Biochemistry and Molecular Biology, 111*, 147–155.

Yoshihara, T., Matsumoto, Y., & Ogura, T. (2001). Occlusal disharmony affects plasma corticosterone and hypothalamic noradrenaline release in rats. *Journal of Dental Research, 80*, 2089–2092.