Study on dentinhypersensitivity-like toothache induced by steroid therapy -Clinical survey and basic study on the mechanisms-

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博士論文

Study on dentinhypersensitivity-like toothache induced by steroid therapy
-Clinical survey and basic study on the mechanisms-
(副腎皮質ステロイド剤投与による象牙質知覚過敏様歯痛に関する研究
-その実態調査とメカニズムの検討について-)

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Chapter 1

Clinical survey of patients with dentinhypersensitivity-like toothache evoked by steroid therapy in Tohoku University Hospital

Introduction

Dentine hypersensitivity (DH) is a common dental pain condition characterized as an intense, transient pain resulting from stimulation of exposed dentine, typically in response to chemical, thermal, tactile or osmotic stimuli (West NX, 2013). In Tohoku University Hospital, some patients with steroid therapy complained of DH-like toothache. However, there is no academic report indicating that steroid therapy induces DH-like toothache as a side effect, although many other side effects of steroid have been known such as lipodystrophy, neuropsychiatric disorders, skin disorders, muscle cramp, proximal muscle weakness and so on (Fardet L et al., 2007). In fact, many patients who take steroid therapy complained of severe DH-like toothache on personal weblog, twitter or facebook. Some of them tweeted that this toothache was one of the most annoying side effects as well as moon face. This information prompted us to determine the

relationship between steroid therapy and DH-like toothache.

In this study, we focused on the DH-like toothache that appeared after taking steroid and defined it as steroid derived (SD)-toothache. We first retrospectively surveyed using a questionnaire the patients in the department of Hematology and Rheumatology of Tohoku University Hospital in order to determine both the prevalence and features of SD-toothache. Second, the patients with SD-toothache were examined whether they suffered from typical DH toothache or not, and the details of its features through the clinical examination including the pain score by visual analog scale (VAS) in the department of Oral Diagnosis.

Subjects and Methods

Ethics statement

The present study was approved by the Ethics Committee of Tohoku University Graduate School of Dentistry, and carried out at Tohoku University Hospital between June 2009 and July 2012. Written informed consent was obtained from all participants. This clinical survey was conducted following the ethical principles of medical investigation involving

human subjects under the Helsinki Declaration of the World Medical Association (http://www.wma.net).

Inclusion and exclusion criteria

The subjects were comprised of 220 patients (male: n = 40; female: n =180), 17 to 87 years of age (mean = 49.9 years) who attended the department of Hematology and Rheumatology of Tohoku University Hospital. Their primary diseases for steroid therapy consisted of systemic lupus erythematosus (SLE: n=93), rheumatoid arthritis (RA: n=70), Sjögren syndrome (SS: n=17), Takayasu's arteritis (TA: n=16), mixed connective tissue disease (MCTD: n=9), dematomyositis (DM: n=5) and so on. After a questionnaire survey, participants who experienced DH-like toothache after taking steroid were referred to the department of Oral Diagnosis of Tohoku University Hospital. Patients with dental caries including wedge-shaped defect and with 5 or less remained vital teeth were excluded from the subjects of this project, after clinical examination (dental checkup) including the confirmation on toothache written in questionnaire. Finally, we defined the DH-like toothache that appeared after taking steroid as steroid derived

(SD)-toothache.

Methods

A cross-sectional study were performed using a questionnaire included the items on gender, age, the primary disease to attend the hospital, the treatment for the diseases [e.g., steroid therapy (1~200 mg/day) and/or steroid pulse therapy (500 or 1000 mg/day)], experience of SD-toothache, and its details (the region, the onset point, the trigger, and the degree of pain by using VAS). The relationship between the above factors and SD-toothache were statistically analyzed between patients groups with and without SD-toothache.

Statistical Analysis

Fisher's exact test and chi-squared test for comparison of qualitative data were used. The relationship between steroid dose and the pain sensation evaluated by pain score was analyzed by correlation coefficient (ρ). PASW statistics, version 18.0 (SPSS INC. Chicago, IL 60611, USA) was applied for these statistical analyses. Differences were considered significant at $p <$

0.05.

Results

SD-toothache prevalence of patients taking steroid was 17.7% (39 subjects out of 220). There were no significant differences in prevalence of SD-toothache between genders (p=0.7135) and among primary diseases of SLE (93 subjects), RA (70 subjects), or other diseases (97 subjects) (p=0.4374). The prevalence of SD-toothache was significantly more frequent in the patients treated with steroid pulse therapy (41.2%: 7 subjects out of 17) than in those with non-pulse therapy (15.8%: 32 subjects out of 203; $p<0.05$, Fig. 1). The correlation coefficient (ρ) between steroid dose and pain score was 0.642, indicating a positive correlation (Fig.2). Interestingly, dose reduction of steroid relieved SD-toothache in all cases (data not shown).

With regard to the onset of SD-toothache, the appearance within a month and within 1~3 months were the most frequent except the subjects who answered "unknown" (Fig. 3A). The mean days of the onset of SD-toothache were 94.8 days. Fig. 3B shows the types of triggers for SD-toothache. The most of the subjects (approximately 84%) felt toothache

by cold water and approximately 24% of the patients felt pain even by hot water. The pain occurred not in one tooth but in a number of the vital teeth at the same time in all cases (data not shown). Most of the pain was characteristically continuous (37/38, 97.4%), different from the typical DH toothache. Fig. 3C shows the degree of SD-toothache, “tolerable” was the most frequent (approximately 64%), but 31% of the patients complained of “intolerable” symptoms. The patients who answered “hardly anxious” were few (Fig. 3C).

Discussion

We demonstrated in this experiment that the patients taking steroid therapy frequently felt SD-toothache. We found that (1) the most of the patients with steroid therapy suffered from SD-toothache, (2) the pain was not transient but continuous, and severe, (3) the pain was often triggered by cool water, sometimes hot one (Fig.3B), (4) the pain occurred not in one tooth but in a number of the vital teeth at the same time in all cases. Thus, there were some differences in quality and triggers between SD-toothache and well-known DH toothache.

We further found that the steroid pulse therapy evoked SD-toothache more frequently than non-pulse therapy ($p<0.05$, Fig. 1). In addition, the correlation coefficient between the dose of prednisolone and pain sensation showed a positive correlation (Fig. 2). These results could show that SD-toothache appear in a dose-dependent manner in the patients treated by steroid therapy. Interestingly, dose reduction of steroid relieved SD-toothache in our all cases, possibly indicating an apparent relationship between steroid therapy and induced pain (SD-toothache). This is the first report to demonstrate the close relationship between steroid therapy and DH-like toothache. However, the mechanisms of the DH-like toothache evoked by steroid are still unknown.

It is well-known that adrenocorticosteroid is an agent composed of glucocorticoid, one of adrenal cortical hormones, and is widely applied to the treatment for various diseases because it strongly suppresses inflammation and controls immunity (Clark JH et al., 1992; Herold et al., 2006; Meldy JC., 1974). On the mechanisms underlying the steroid-induced DH-like toothache, both of the peripheral (tooth pulp) and the central nerves factors should be considered. In the tooth, it is reported that steroid dose-dependently inhibits

dentin formation in prenatal rat (Wang YJ et al., 2000). But no morphological changes by steroid have ever been reported in the dental pulp. On the other hand, some studies suggested that the relationship between steroid and histological reactions in the brain system. Steroids can cross the blood brain barrier (Bannwarth et al., 1997) and the brain has the steroid receptors (Fuxe et al., 1985; Cintra et al., 1994). Furthermore, steroid administration induces degeneration and cell death of neurons as well as proliferation of glial cells (Haynes et al., 2001; Ramos-Remus et al., 2002; Tan et al., 2002). It is consequently suggested that there is a possible relationship between steroid and neuronal or glial reactions relating to pain regulation and/or modification. Further study should be needed to find out the mechanisms of this toothache.

Conclusion

The patients taking steroid frequently complained of SD-toothache (17.7%). The degree of pain was in accordance with the dose of steroid therapy. The features of this SD-toothache were continuous and severe pain. All the patients with SD-toothache complained this toothache in many vital

teeth at the same time by cold water (sometimes even by hot water). This is the first report to demonstrate the close relationship between steroid therapy and steroid-evoked toothache.

Chapter 2

Hystopathological study on the microglial activation induced by prednisolone in the subnucleus caudalis of the rat trigeminal sensory complex

Introduction

Our clinical survey demonstrated that the patients taking steroid were more subject to the DH-like toothache than the patients who do not. However, its mechanism is still unknown. We suggested possible mechanisms that the neuronal or glial reactions by steroid could exert an influence on pain regulation and/or modification in the Chapter 1. In the Chapter 2, we further investigated the mechanisms in the brain with steroid administrated rat.

The brainstem has several sensory and motor nuclei of the cranial nerves. These include trigeminal sensory and motor, facial and hypoglossal nuclei. Injury of their peripheral nerves causes degeneration or cell death of sensory and motor neurons in the trigeminal ganglion (TG) and brainstem. Axotomy results in increase of TG neurons and cranial motoneurons which express c-Jun activating transcription factor 3 (ATF3), a marker for neuronal degeneration (Tsuzuki et al., 2001; Park et al., 2011). Expression of calcitonin gene-related peptide (CGRP) is also elevated in injured

motoneurons within the facial and hypoglossal nuclei (Streit et al., 1989; Grothe, 1993; Fukuoka et al., 1999). In neonatal animals, axotomy activates caspase-3 and apoptosis in the TG, brainstem trigeminal sensory complex and facial nucleus (Vanderluit et al., 2000; Sugimoto et al., 2004, 2005). Transection of peripheral nerves also causes glial activation in cranial sensory and motor nuclei (Laskawi and Wolff, 1996; Lee et al., 2010; Ichikawa et al., 2011). In the sensory trigeminal complex, expression of p38 mitogen-activated protein kinase (MAPK) in glial cells is increased by lingual nerve injury (Terayama et al., 2011).

Glucocorticoids (GCs) are a class of steroid hormones that bind to the glucocorticoid receptor (GR). GR is located in lymphocytes and GCs have inhibitory effects on lymphocyte proliferation (Herold et al., 2006). GCs are widely used to treat chronic inflammatory diseases including allergies, asthma and autoimmune diseases. Previous immunohistochemical studies have demonstrated the presence of GR in the brain (Fuxe et al., 1985; Cintra et al., 1994). Neuronal and glial cells express GR-immunoreactivity (-IR). In the prefrontal cortex and hippocampus, GCs are known to induce degeneration and cell death of neurons as well as proliferation of glial cells

(Haynes et al., 2001; Ramos-Remus et al., 2002; Tan et al., 2002). These findings are probably associated with learning and memory impairment after chronic exposure to GCs. Because GR is distributed in sensory and motor-related area of the brain (Cintra et al., 1994), GC is also considered to influence sensory and motor systems. A previous study has demonstrated that GC can affect microglial response to peripheral nerve transection in the facial nucleus (Kiefer and Kreutzberg et al., 1991). However, little is known about GC-induced degeneration, cell death or glial reaction in the TG, and cranial sensory and motor nuclei without axotomy.

In this study, expression of ATF3, caspase-3 and CGRP was examined in the TG and brainstem after administration of prednisolone, a synthetic glucocorticoid. Immunohistochemistry for Iba1, glial fibrillary acidic protein (GFAP) and p38 MAPK was also performed to know effect of prednisolone on microglia and astrocytes in cranial nuclei of the brainstem (Suchorukova et al., 2010; Ichikawa et al., 2011).

Materials and methods

Tissue preparation

A total of 10 male Wistar rats (180-220 g) were used in this study. The animals were kept on a 12 h light/12 h dark cycle. Prednisorone (5 μg/h, n = 4; Shionogi Pharma. Chemicals Co., Ltd., Japan) or saline (n = 4) was subcutaneously administered by an osmotic minipump (model 2ML2; Alzet, USA) for 7 days. Then, these animals were deeply anesthetized by sevoflurane inhalation to the level at which respiration was markedly suppressed and perfused through the left ventricle with saline for 20–40s for exsanguination followed by 0.1 M sodium phosphate buffer containing 4% formaldehyde (prepared fresh from paraformaldehyde, pH 7.4). The TG and brainstem were dissected and further fixed in a fresh volume of the fixative overnight at 4℃. The materials were cryoprotected by immersing until sunken in 0.02 M phosphate-buffered saline containing 20% sucrose (pH 7.4). The TG and brainstem were horizontally and frontally, respectively, frozen-sectioned at 8μm. Two intact rats were also prepared as described above.

Immunohistochemistry

For evaluation of neuronal degeneration and cell death, and glial reaction, ABC (avidin-biotin-horseradish peroxidase complex) and indirect immunofluorescence methods were performed. Sections of the TG and brainstem were incubated with rabbit polyclonal antiserum against ATF3 (1:5000 for ABC, Santa Cruz Bio technology Inc., USA), cleaved caspase-3 (1:3000 for ABC, Cell Signaling Technology, USA), CGRP (1:100000 for ABC, Peninsula Laboratory Inc. , USA), Iba1 (1:15000 for ABC, 1:250 for immunofluorescence, Wako Pure Chemical Industries Ltd. , Japan), GFAP (1:40000 for ABC, 1:1000 for immunofluorescence, Dako, Japan) or phosphorylated p38 (p-p38) MAPK (1:1000 for ABC, Cell Signaling, USA). For ABC method, sections were incubated with biotinylated goat anti-rabbit IgG (1:200, Vector Laboratories, USA) and ABC-complex (1:25, Vector Laboratories). Following nickel ammonium sulfate (0.1%)-intensified diaminobenzidine (0.002%) reaction, these sections were dehydrated in a graded series of alcohols, cleared in xylene and cover-slipped with Entellan (Merck). For immunofluorescence method, sections were incubated with lissamine rhodamine B chloride-conjugated donkey anti-rabbit IgG (1:300,

Jackson ImmunoResearch Labs., USA). The sections were viewed with light or fluorescence microscopy (Nikon, Japan). The specificity of rabbit antiserum against ATF3 or caspase-3 has been described elsewhere (Sugimoto et al., 1997, 2004). In addition, the normal rabbit serum was used instead of primary antibodies. No immunoreaction was detected in the control.

Morphometric analysis

For analysis of distribution and morphology of Iba1-IR cells in the cranial sensory and motor nuclei, 3 ABC-stained sections were randomly selected in each animal. The mesencephalic nucleus, nucleus principalis, and subnuclei oralis, interpolaris and caudalis of the trigeminal sensory complex as well as the trigeminal motor, facial and hypoglossal nuclei were identified under a dark field microscopy. Light and dark field microscopic images were stored into a personal computer, and superimposed by Lumina Vision program (Mitani Corporation, Japan). For density of Iba1-IR cells, the number of Iba1-IR cells was counted and its proportion to the area of the cranial nucleus (unit/mm2) was calculated for each section (LuminaVision

program). For morphological analysis of Iba1-IR cells, 5 and 10 Iba1-IR cells in hypoglossal and other cranial nuclei, respectively, were randomly selected from each section and the length of Iba1-IR processes was measured. The average of density of Iba1-IR cells and length of their processes was recorded for each animal. All the difference between saline- and prednisolone-treated animals was analyzed by Student's t-test.

The experiments were carried out under the control of the Animal Research Control Committee in accordance with The Guidelines for Animal Experiments of Tohoku University, Government Animal Protection and Management Law (No. 105), and Japanese Government Notification on Feeding and Safekeeping of Animals (No. 6). All efforts were made to minimize the number of animals used and their suffering.

Results

ATF3, CGRP and caspase-3 in the TG and brainstem

In intact and saline-treated animals, the TG contained few ATF3-IR neurons (Fig. 4A). The IR was restricted to the nucleus within sensory

neurons. ATF3-IR neurons were also rare in the TG after prednisolone treatment (Fig. 4B). Less than 1 % of TG neurons showed ATF3-IR in saline- or prednisolone-treated animals. The cranial sensory and motor nuclei were devoid of ATF3-IR profiles in intact, and saline- or prednisolone-treated animals. In intact animals, many CGRP-IR motor neurons were observed in the trigeminal motor, facial and hypoglossal nuclei. CGRP-IR nerve fibers were also abundant in the subnuclei caudalis and oralis of the trigeminal sensory complex. The distribution of CGRP-IR in the brainstem appeared to be unchanged by saline or prednisolone treatment (Fig. 4C, D). The TG and brainstem were devoid of caspase-3-IR profiles in all examined animals.

Iba1, GFAP and p-p38 MAPK in the brainstem

In intact and saline-treated animals, Iba1- and GFAP-IR were detected in numerous cells in the cranial sensory and motor nuclei (Fig. 4E-H, M, N). These cells were distributed throughout the trigeminal sensory and motor, facial and hypoglossal nuclei. They had small cell bodies and some processes (Fig. 4E-H, M, N). The number of Iba1-IR cells in the

brainstem was barely affected by prednisolone treatment. As a result, density of Iba1-IR cells was similar in cranial sensory and motor nuclei of saline- and prednisolone-treated animals (Fig. 5). However, prednisolone treatment had an effect on morphology of Iba1-IR cells in the subnucleus caudalis of the trigeminal sensory complex. In superficial and deep layers of the subnucleus, their processes extended many branches with repeated ramification (Fig. 4E-L). Compared to saline-treated animals, the length of Iba1-IR processes significantly increased in the subnucleus caudalis of prednisolone-treated animals (Fig. 6). The morphology of Iba1-IR cells was similar in other portions of the brainstem of intact, and saline- or prednisolone-treated animals. Prednisolone treatment scarcely influenced distribution or morphology of GFAP-IR cells in the brainstem (Fig. 4M-P). The expression of p-p38 MAPK-IR was very rare in the brainstem of salineand prednisolone-treated animals (figure not shown).

Discussion

The present study demonstrated that ATF-3 and caspase-3 were absent or very infrequent in the TG and brainstem of intact, and saline- or

prednisolone-treated animals. In addition, distribution of CGRP-IR was similar in the cranial motor nuclei of these animals. Therefore, we could not obtain the data that prednisolone induced neuronal degeneration or cell death in the TG or brainstem. Prednisone and dexamethasone are known to cause neuronal degeneration in the prefrontal cortex and hippocampus (Ramos-Remus et al., 2002; Tan et al., 2002). Dexamethasone induces neuronal cell death in the hippocampus of adult animals (Haynes et al., 2001). In the neonatal hippocampus, neuronal apoptosis is increased by methylprednisolone (Schubert et al., 2005). However, the prefrontal cortex and hippocampus are devoid of caspase-3-IR in saline- or prednisolone-treated animals (our own unpublished data). It is unlikely that prednisolone causes severe damages to neurons in the cortex, hippocampus and brainstem. The variety of degenerative and apoptotic effects of GCs on brain neurons may depend on their molecular structures.

Iba1 and GFAP were expressed by small cells in the brainstem of intact animals. The density of Iba1- and GFAP-IR cells was similar in the cranial sensory and motor nuclei of animals with and without prednisolone treatment. Iba1 and GFAP are considered to be markers for microglia and

astrocytes, respectively (Suchorukova et al., 2010; Ichikawa et al., 2011). Thus, it is suggested that prednisolone has little or no effect on the number of microglia and astrocytes in the cranial nuclei. The morphology of these glial cells was also similar in the cranial motor nuclei of saline- and prednisolone-treated animals. In the trigeminal sensory complex, however, prednisolone treatment increased the length of processes of Iba1-IR cells. Prednisolone probably increases ramification of microglial processes in the cranial sensory nucleus. The GC may be unable to activate p38 MAPK in glial cells, because expression of p-p38 MAPK was infrequent in saline- and prednisolone-treated brainstems.

Capsaicin can activate the transient receptor potential, subfamily V, member 1 (TRPV1) receptor in primary nociceptors with small cell bodies (Caterina et al., 1997; Ichikawa and Sugimoto, 2001). A previous study has demonstrated that injection of capsaicin into the whisker pad activates microglia in the subnucleus caudalis of the trigeminal sensory complex (Kuroi et al., 2012). The treatment causes increase of ramified microglia and decrease of ameboid microglia. These findings are similar to the present observations that prednisolone increased ramification of microglial processes

in the subnucleus caudalis. In the TG, primary nociceptors such as TRPV1- and CGRP-containing neurons project to the subnucleus caudalis (Sugimoto et al., 1997, Bae et al., 2004). In the subnucleus, microglial activation is associated with development of oro-facial pain after nerve injury and inflammation (Lee et al., 2010). Therefore, microglial activation in the subnucleus caudalis by prednisolone treatment may suggest that the GC affects primary nociceptors in the TG and nociceptive transmission in the subnucleus. This suggestion may be supported by the previous finding that CGRP-IR nociceptors in the TG also showed GR-IR (DeLeón et al., 1994). On the other hand, prednisolone is known to cross the blood brain barrier (Bannwarth et al., 1997). Thus, the possibility that the GC directly acts on neurons and/or microglia within the subnucleus caudalis cannot be excluded.

In intact brains, microglial processes make contacts with neuronal synapses at an interval of one hour (Wake et al., 2009). The duration of the microglia-synapse contacts is markedly prolonged after cerebral ischemia. In this study, relationship between ramified processes of microglia and nociceptive neurons in the subnucleus caudalis after prednisolone treatment is unclear. Further studies will be necessary to know mechanism and

significance of prednisorone-induced microglial activation.

Conclusion

In the subnucleus caudalis of the trigeminal sensory complex, prednisolone increased ramification of microglial processes. The glucocorticoid may affect nociceptive transmission in the brainstem.

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Figure legends

Fig.1 Relationship between the steroid therapy and SD-toothache

The patients treated with steroid pulse therapy more frequently felt the SD-toothache than the patients without steroid pulse therapy. *p<0.05

Fig.2 Relationship between dose of prednisolone and the VAS of SD-toothache

Toothache magnitude was measured using a visual analogue scale (VAS).

Fig.3 The features of SD-toothache

The time point when SD-toothache appeared after onset of steroid therapy (A). The appearance within 3 months was the most frequent (mean: 94.8 days). Types of trigger of SD-toothache (B). The most of patients (approximately 84%) felt SD-toothache by cold water and approximately 24% of patients felt pain even by hot water. The degree of SD-toothache (C). “Tolerable” was the most frequent (approximately 64%), but 31% of the patients complained of “intolerable” symptoms. The patients who answered “hardly anxious” were few.

Fig.4 Microphotographs of the TG and brainstem of the rats

Microphotographs for ATF3 (A, B), CGRP (C, D), Iba1 (E-L) and GFAP (M-P)

in the TG (A, B) and brainstem (E-P) after saline (A, C, E-H, M, N) and prednisolone (B, D, I-L, O, P) treatments. ATF3-IR neurons (arrows in A, B) are very rare in saline (A)- and prednisolone (B)- treated TGs. The distribution and morphology of CGRP-IR motoneurons are similar in the facial nucleus of saline (C)- and prednisolone (D)- treated animals. Panels E, I, M and O were obtained under dark field microscopy. Panels E and F, I and J, M and N, and O and P are the same fields of view, respectively. In superficial (SL in E, F, I, J, and G, K) and deep layers (DL in E, F, I, J, and H, L) of the subnucleus caudalis of the trigeminal sensory complex, dendritic processes of Iba1-IR cell develop after treatment of prednisolone(I-L) but not saline (E-H). Saline (M, N) or prednisolone (O, P) treatment has a little or no effect on GFAP-IR cells in superficial (SL in M, N) and deep layers (DL in M, N) of the subnucleus caudalis. Bras = 200 μm (A, E) and 50 μm (G). Panels A-D, E, F, I and J, and G, H, K and L are at the same magnification, respectively.

Fig. 5 The density of microglial cells

Bar graphs showing mean density± S.D. (unit/mm^2) of Iba1-IR cells in the cranial sensory and motor nuclei after saline (open column) and prednisolone

(gray column) treatment. The data were obtained from 4 saline-treated and 4 prednisolone-treated animals.

Fig. 6 The length of dendritic prosesses of the microglial cells

Bar graphs showing mean length ± S.D. (μm) of dendritic processes of Iba1-IR cells in the cranial sensory and motor nuclei after saline (open column) and prednisolone (gray column) treatment. The data were obtained from 4 saline-treated and 4 prednisolone-treated animals. Differences are statistically analyzed by Student's t-test (*$p < 0.01$)

Fig.1

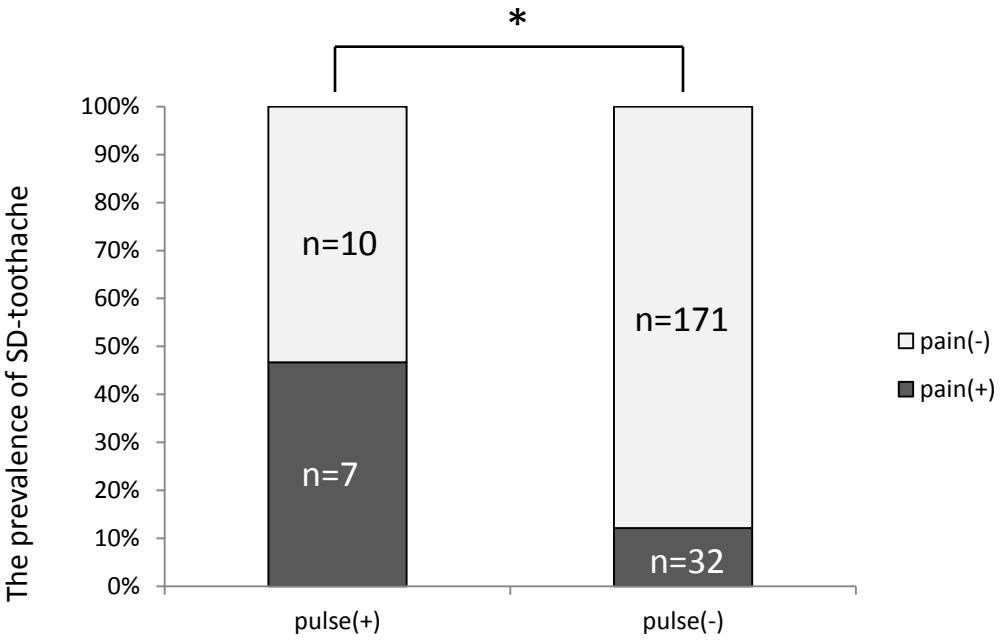

*
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
The prevalence of SD-toothache
n=10
n=7
n=171
n=32
pain(-)
pain(+)
pulse(+)
pulse(-)

Fig.2

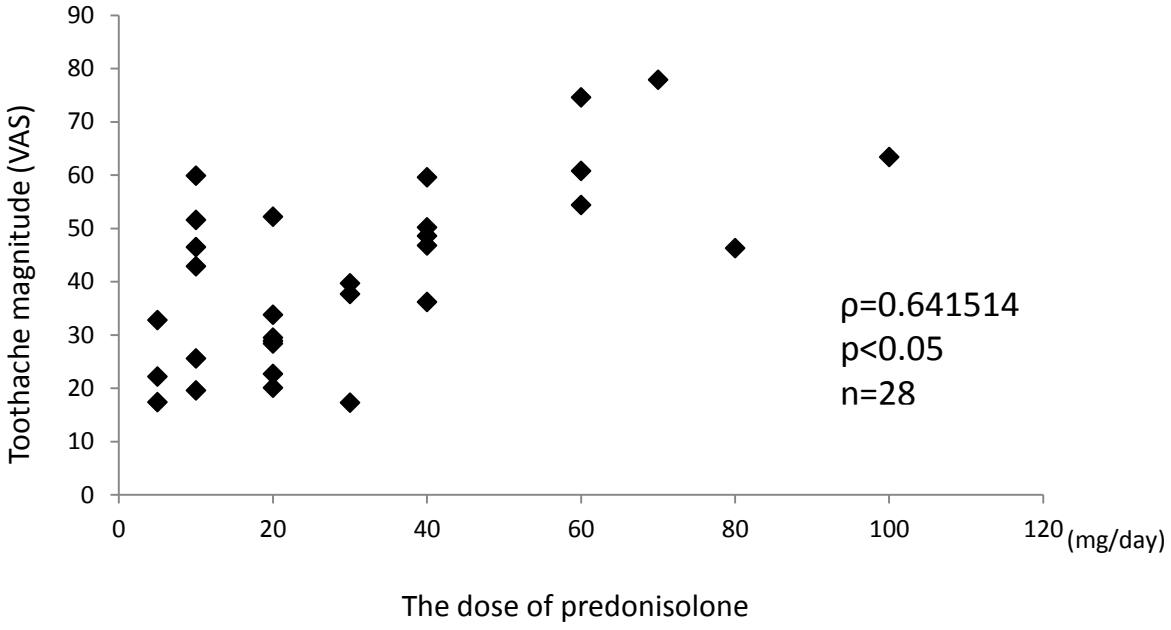

Toothache magnitude (VAS)
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
(mg/day)
The dose of predonisolone
ρ=0.641514
p<0.05
n=28

Fig.3

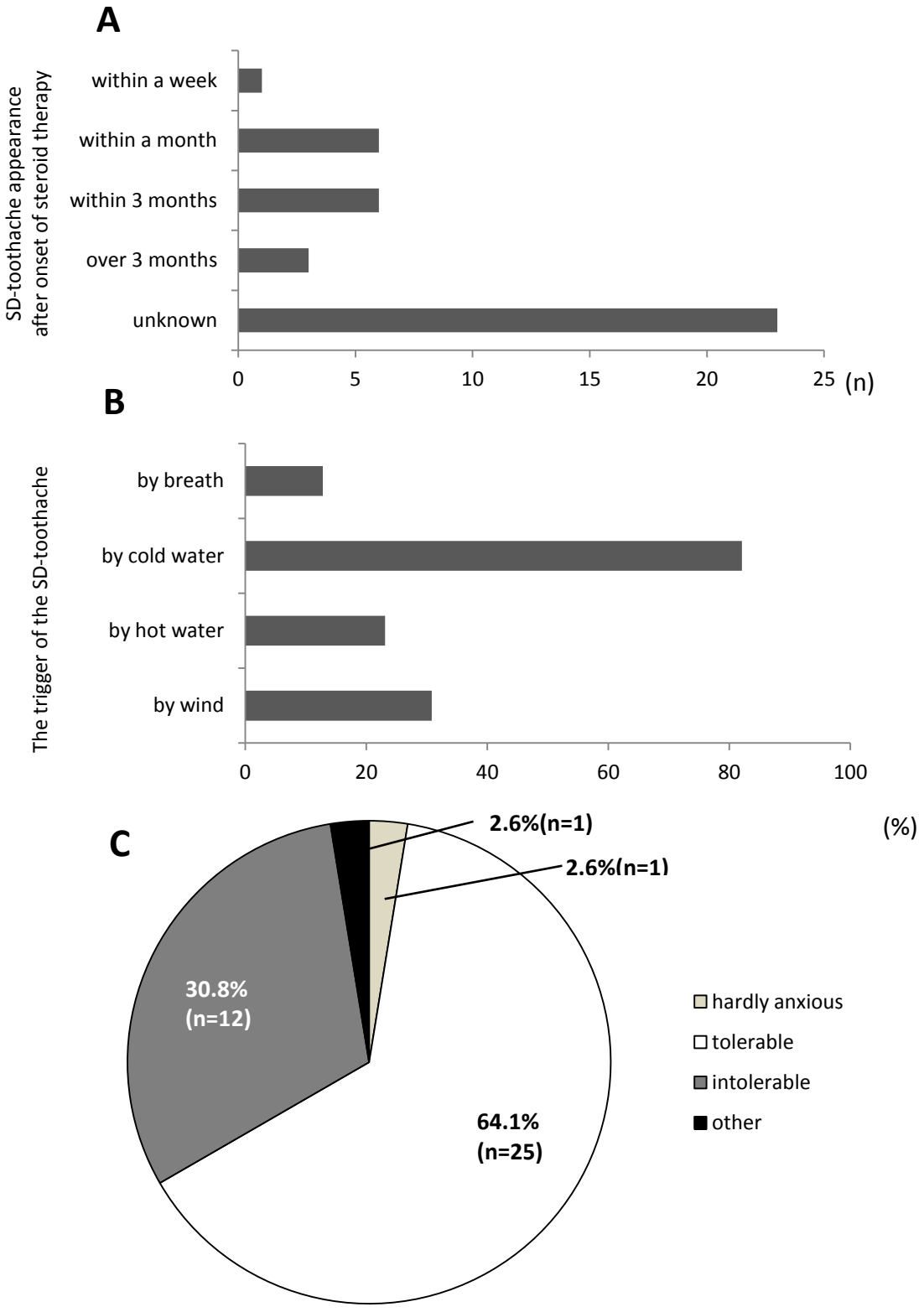

A
SD-toothache appearance after onset of steroid therapy
within a week
within a month
within 3 months
over 3 months
unknown
0
5
10
15
20
25
(n)
B
The trigger of the SD-toothache
by breath
by cold water
by hot water
by wind
0
20
40
60
80
100
C
(%)
2.6%(n=1)
2.6%(n=1)
30.8%
(n=12)
64.1%
(n=25)
hardly anxious
tolerable
intolerable
other

Fig.4

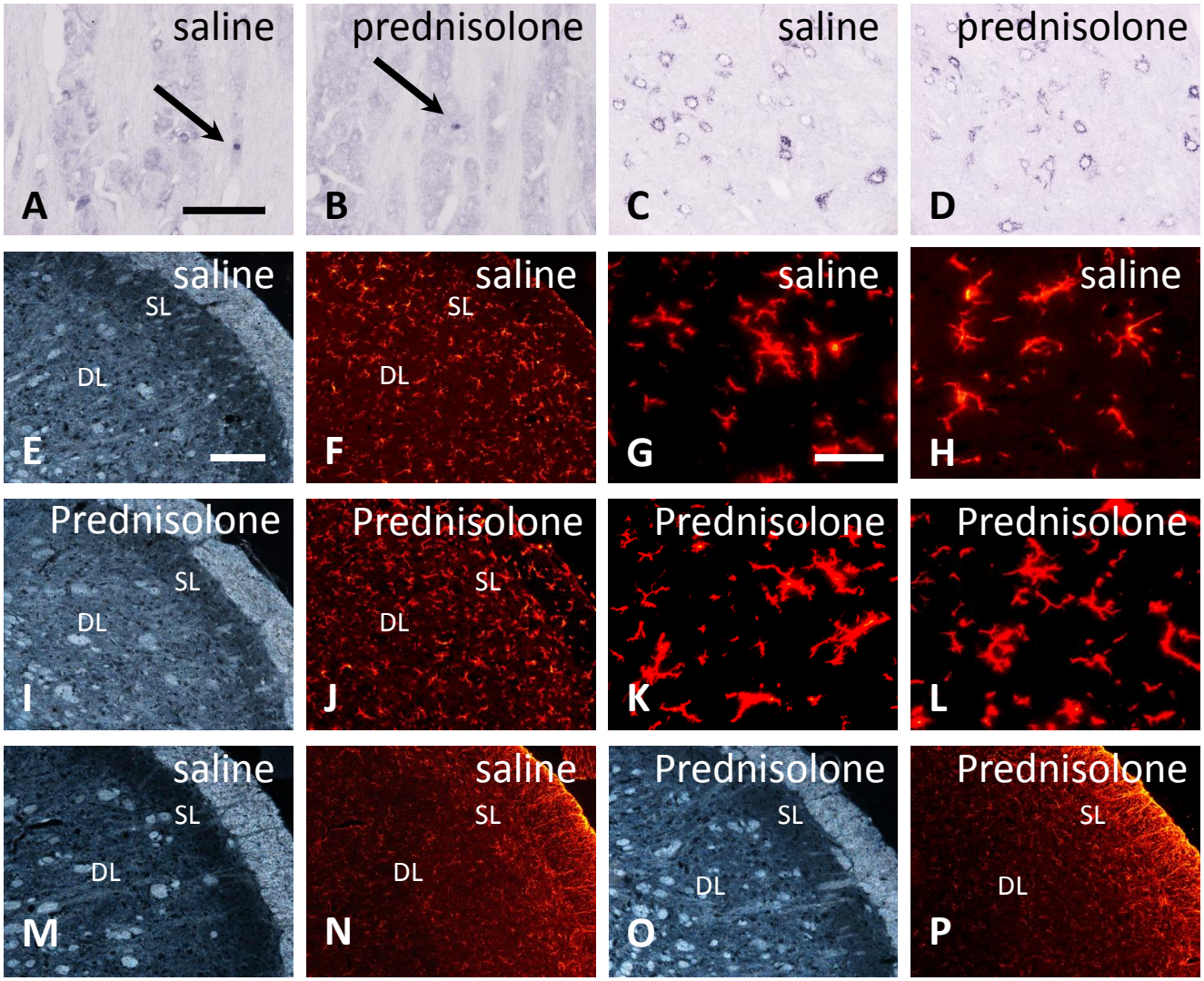

saline
prednisolone
saline
prednisolone
A
B
C
D
saline
SL
DL
E
saline
SL
DL
F
saline
G
saline
H
Prednisolone
SL
DL
I
Prednisolone
SL
DL
J
Prednisolone
K
Prednisolone
L
saline
SL
DL
M
saline
SL
DL
N
Prednisolone
SL
DL
O
Prednisolone
SL
DL
P

Fig.5

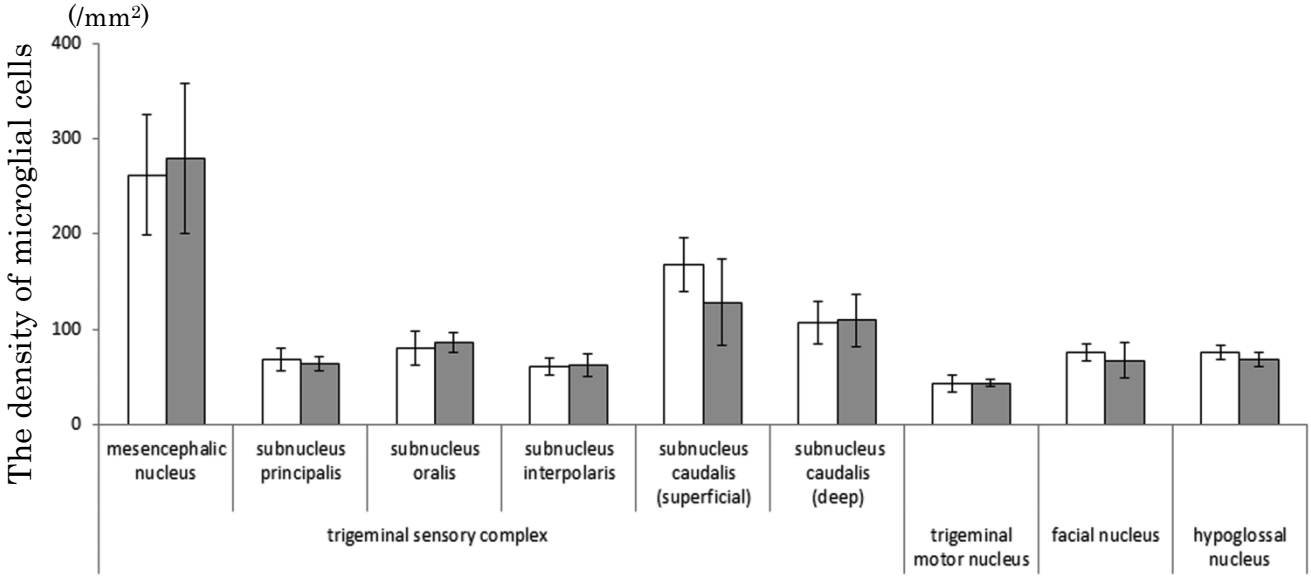
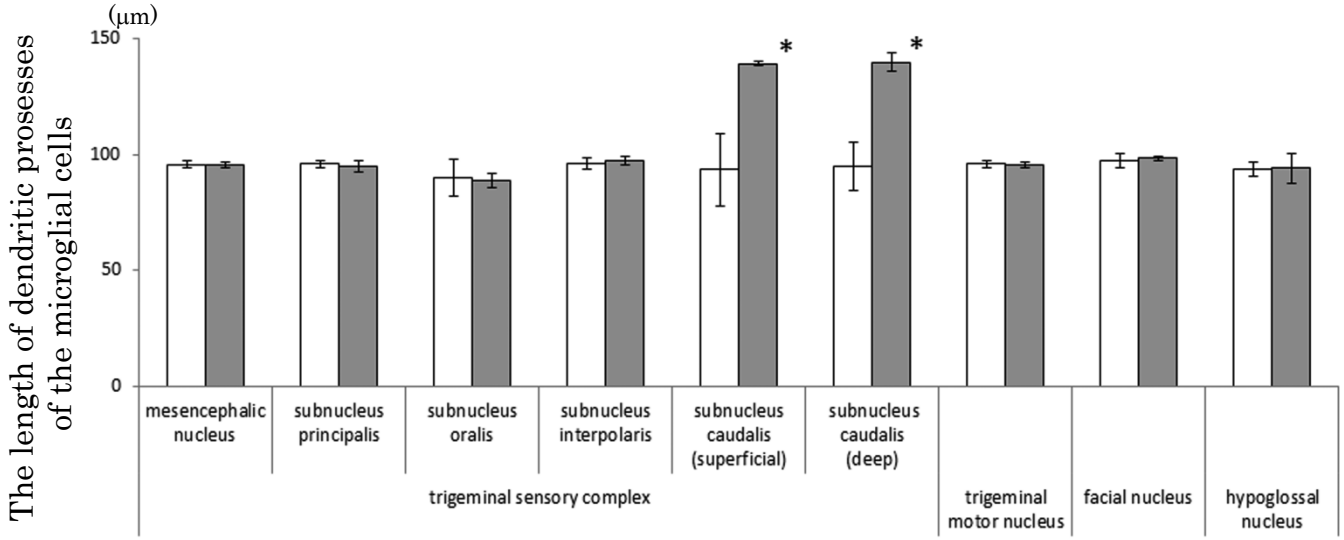

(/mm2)
The density of microglial cells
400
300
200
100
0
mesencephalic nucleus
subnucleus principalis
subnucleus oralis
subnucleus interpolaris
subnucleus caudalis (superficial)
subnucleus caudalis (deep)
trigeminal sensory complex
trigeminal motor nucleus
facial nucleus
hypoglossal nucleus

Fig.6


(μm)
150
100
50
0
The length of dendritic prosesses of the microglial cells
*
*
mesencephalic nucleus
subnucleus principalis
subnucleus oralis
subnucleus interpolaris
subnucleus caudalis (superficial)
subnucleus caudalis (deep)
trigeminal sensory complex
trigeminal motor nucleus
facial nucleus
hypoglossal nucleus

謝辞

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