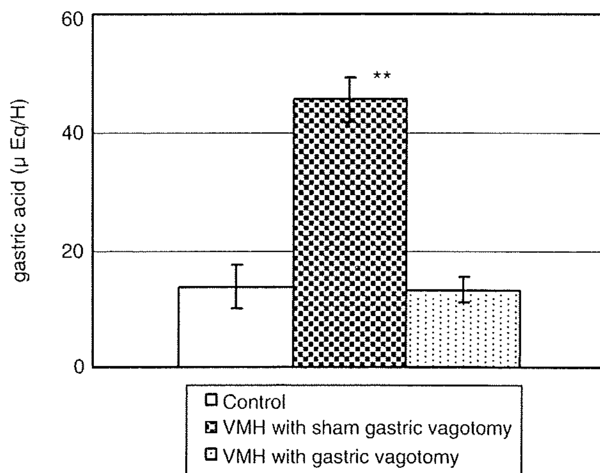

Figure 3 Comparison of basal gastric acid levels. $**P<0.01$ vs. Control. Abbreviations are similar to those of Fig. 2

in the proliferative zone were similar to those in control rat.

Exp.2

As shown in Fig. 5, the ulcer index in VMH-lesioned rats was higher than that in control rats. Consistent with the results of the first experiment, the thicknesses of gastric mucosa in VMH-lesioned rats were significantly greater than those in control rats (VMH-lesioned: 20.44±5.73 vs. sham VMH-lesioned: 13.66±1.72, $p<0.05$)

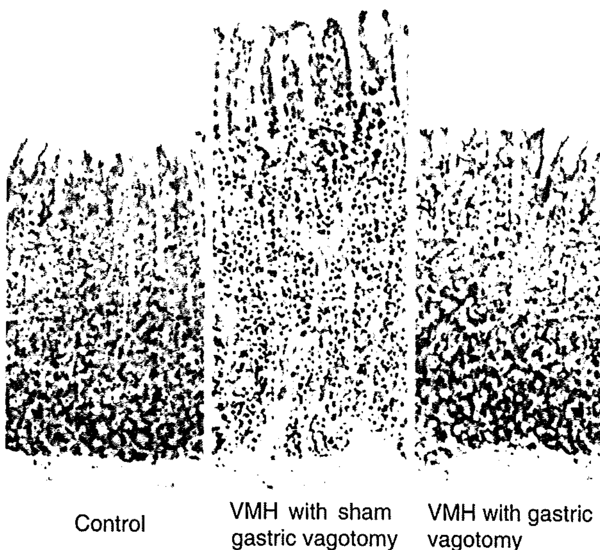

Figure 4 Typical images of the gastric mucosa after combined hematoxylin–eosin and PCNA staining. For details, see the "Results" section of the text. Abbreviations are similar to those of Fig. 2

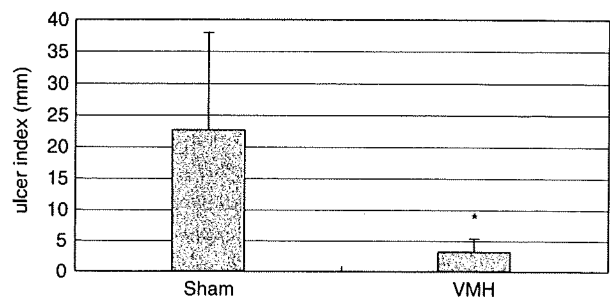

Figure 5 Ulcer indices in sham-VMH lesioned (control) and VMH-lesioned rats. $*P<0.05$. *Sham* sham-VMH-lesioned rats. *VMH* VMH-lesioned rats

Discussion

In this study, we found that selective gastric vagotomy diminished cell proliferation in the stomach but not in the other visceral organs, and induced gastric acid hypersecretion in the stomachs of VMH-lesioned rats. These results suggest that both cell proliferation in the gastric mucosa and secretion of gastric acid is induced hyperactivity of vagus nerve. It is possible that vagal hyperactivity promotes cell proliferation in the gastric mucosa, which induce hypersecretion of gastric acid in the stomach. Therefore, it may be reasonable to consider that cell hyperplasia of the visceral organs would induce hyperfunction of these organs in VMH-lesioned rats.

We also found that the gastric mucosa in VMH-lesioned rats was resistant to ethanol-induced AGML. It is possible that this resistance is associated with the increased thickness of the mucosal layer promoted by hyperactivity of the vagus nerve. Two principal mechanisms—decreased mucosal blood flow (MBF) and delayed mucosal cell proliferation—have been proposed to explain the basis for ethanol-induced AGML. Both ethanol-induced decrease of nitric oxide (NO) production and release, and a decrease of calcitonin gene-related peptide (CGRP) secretion from afferent nerves in the gastric mucosa lead to disturbances in MBF (Sibilia et al. 2007; Brzozowski et al. 2003, 2008; Szolcsányi and Barthó 2001). Ethanol-induced increased oxidative stress and decreased levels of PGs (COX-2 derived) delay mucosal cell proliferation (Hernández-Muñoz et al. 2000; Areche et al. 2008).

Endogenous prostaglandins (PGs) biosynthesized from cyclooxygenases (COXs) induce increased MBF. COXs have two isoforms, COX-1 and COX-2, with COX-1 expressed constitutively and found in most tissues. COX-2 on the other hand is usually expressed at low levels but is rapidly up-regulated in pathophysiological conditions such as inflammation, tissue damage, and malignant transformation (Kargman et al. 1996). COX-1-derived PGs induce an increase in MBF, whereas COX-2-derived PGs are crucial for the spontaneous healing of gastric ulcers by activating

COX-2 and PGE2 system with cell proliferation (Konturek et al. 2005; Hatazawa et al. 2007). Therefore, COX-derived PGs may also contribute to protection against ethanol-induced gastric damage.

In VMH-lesioned animals, which show vagal hyperactivity and decreased sympathetic activity (Inoue and Bray 1979; Yoshimatsu et al. 1984). Vagal hyperactivity can increase both nitric oxide (NO) production and release, and can increase carcitonin gene-related peptide(CGRP) release (Gyires 2005; Tanaka et al. 1993; Szolcsányi and Barthó 2001). Therefore, these effects may be involved in the resistance to ethanol-induced AGML in VMH-lesioned rats. On the other hand, we previously observed an increase in the gene expression of COX-1 and COX-2 in the jejunum of VMH-lesioned rats (Kageyama et al. 2003), which should have the stimulatory effect on cell proliferation. Consequently, it is likely that these factors together contribute to the manifestation of resistance to ethanol-induced AGML. The experiments in this study were performed 7 days after induction of the VMH lesions, when thickening of the gastric mucosal layer due to cell proliferation was well advanced. Consequently, we are inclined to believe that this increased gastric mucosal layer thickness primarily contributed to resistance against ethanol-induced AGML in the VMH-lesioned rats. While gastric acid hypersecretion was observed in the present study and in previous studies (Ridley and Brooks 1965; Tominaga et al. 1993), the results here suggest that this accentuating factor to ethanol-induced AGML was overcome by the aforementioned contributive factors.

There exists the promising possibility that mechanisms underlying the phenomenon of gastric cell proliferation induced by VMH lesions could be used to induce gastric ulcer healing. There are two known different repair mechanisms of mucous epithelia which cover different timescales; rapid repair of superficial lesions via cell migration—or so-called "restitution"—begins within minutes. Continuous regeneration via cell differentiation and proliferation and more commonly referred to as "regeneration" is responsible for self-renewal within days to months (Hoffmann 2008). Many growth factors such as epidermal growth factor (EGF), transforming growth factor-α (TGF-α), hepatocyte growth factor (HGF), basic fibroblast growth factor (bFGF), trefoil factor (TFF), and vascular endothelial growth factor (VEGF) are involved in both phases of gastric ulcer healing (Gyires 2005; Malara et al. 2005). Moreover, COX-1-derived PGs are involved in restitution, while COX-2-derived PGs are involved in regeneration (Konturek et al. 2005).

In this framework, if we could identify defined or undefined factors that contribute to restitution and regeneration of the gastric mucosa in VMH-lesioned rats, this information could be used to develop novel medicines to treat peptic ulcer diseases. To date, we have shown that both COX-1 and -2, but not EGF or TGF-α mRNA levels, are increased in the jejunal epithelial cells of VMH-lesioned rats (Kageyama et al. 2003). Further study is required to identify what molecules contribute to the cell proliferation process in the gastric mucosa of these animals.

Helicobacter pylori and nonsteroidal anti-inflammatory drugs (NSAIDs) including aspirin are the major pathogenic factors for peptic ulcer diseases. Subsequent treatment options for *H. pylori* infection have seen a decrease in the number of patients with this peptic ulcer disease, but NSAID-related patients with peptic ulcer disease continue to increase in number. This increase is expected to continue with the aging of the population given the heavy use of NSAIDs by elderly patients with rheumatoid arthritis or chronic pain. The most important adverse effect of NSAIDs is the high risk of mucosal damage, especially ulcer disease, which is caused by decreased defense and repair systems, especially in relation to decreased mucosal PGs (Konturek et al. 2005). Moreover, NSAIDs are often associated with severe mucosal damage such as bleeding and perforation (Hawkey and Langman 2003). Hence, new drugs that augment the defense or repair of the gastric mucosa are much needed.

Regarding the interrelation between the central nervous system (CNS) and the gastric mucosa, several centrally induced effects have similar outcomes on the gastric mucosa to those induced by VMH lesions. For example, lateral hypothalamic lesions induce gastric mucosal damage that can be blocked by subdiaphragmatic vagotomy (Lindholm et al. 1975; Grijalva et al. 1980). Electric stimulation of the paraventricular nucleus (PVN) causes gastrointestinal damage that is reduced by subdiaphragmatic vagotomy (Ferguson et al. 1988) and aggravates stress-induced gastric damage (Zhang and Zheng 1997). In contrast, PVN lesions alleviate this stress-induced damage (Zhang and Zheng 1997). Intraventricular administration of adrenomedullin and peptide YY inhibits ethanol-induced gastric lesions (Kaneko et al. 1998; Yang et al. 1999), while intraventricular administration of thyrotropin-releasing hormone (TRH) at high doses causes gastric mucosal damage, but induces gastric protection against ethanol-induced mucosal damage at low doses (Kato et al. 1995). It was recently reported that stimulation of capsaicin-sensitive afferent fibers of the vagus nerve stimulate CGRP production in the gastric mucosa, which induces increased MBF resulting in heightened gastroprotection and enhanced gastric ulcer healing. This increase in the production of CGRP was mediated by efferent vagus nerve fibers since vagotomy blocked the effect (Evangelista 2006). Intraventricular administration of ghrelin reduced ethanol-induced AGML, which was inhibited by vagotomy (Sibilia et al. 2003). Thus, modulation within the hypothalamus plays a

crucial role in gastric protection via the vagus nerve. However, hyperplasia in the abdominal organs was observed only in VMH-lesioned animals but not observed in the lateral hypothalamus- and PVN-electrically lesioned or centrally administrated drug-stimulated animals (Lindholm et al. 1975; Grijalva et al. 1980; Ferguson et al. 1988; Kato et al. 1995; Zhang and Zheng 1997; Kaneko et al. 1998; Yang et al. 1999; Sibilia et al. 2003). As an interpretation of the results in the present study, we presume that VMH lesions stimulate different efferent fibers of the vagus nerve such as peptidergic or serotonergic fibers (Vanhatalo and Soinila 2001; Zhou et al. 2008) or stimulate different postsynaptic receptor systems (Hyde and Crook 2001).

In conclusion, VMH-lesions induced cell proliferation in the visceral organs (liver, stomach, small intestine, endocrine pancreas, and exocrine pancreas). Selective gastric vagotomy restored gastric cell proliferation and gastric acid secretion without affecting cell proliferation in the other visceral organs. These results suggest that vagal hyperactivity induces histological hyperplasia associated with hyperfunction of the visceral organs of these rats, and that VMH lesions provide resistance to ethanol-induced AGML, the main mechanism for this being thickening of the gastric mucosal layer due to gastric cell proliferation.

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Conflicts of interest None of authors have any conflict of interest.

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Gene Expression Profiling in Rat Liver After VMH Lesioning

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It has been reported that ventromedial hypothalamic (VMH) lesions induce hepatic cell proliferation and apoptosis and metabolic changes in the body. In the present study, we identified genes of which expression profiles showed significant modulation in rat liver after VMH lesions. Total RNA was extracted, and differences in the gene expression profiles between rats at day 3 after VMH lesioning and sham-VMH lesioned rats were investigated using DNA microarray analysis. The results revealed that VMH lesions regulated the genes that were involved in various types of metabolisms and cell proliferations in the liver. Real-time PCR also confirmed that gene expressions of ELOVL6 and SPC24 were upregulated, and that of SERPINA7 was downregulated. VMH lesions may change the expressions of multiple metabolism genes and cell proliferation–related genes in rat liver. Exp Biol Med 234:758–763, 2009

Key words: VMH; gene expression; cell proliferation and apoptosis; metabolism; liver; rat

Introduction

A focus of attention among researchers has been the pathways that connect the nervous system and the gastrointestinal organs (1, 2). The liver is one of the autonomic nerve–enriched organs (1, 2). The hypothalamus plays a vital role in the integration of neurohumoral information and possesses autonomic centers that are connected to the viscera via the autonomic nervous system (1, 2). It is well known that VMH lesions change various types of metabolisms in the body (3). We previously reported that VMH lesions induce hepatic cell proliferation and apoptosis (4, 5).

DNA microarray analysis is a powerful tool for detecting the characterization of mRNA expression pattern of a large number of genes. In the present study, we used DNA microarray analysis to identify genes for which expression profiles showed significant modulation and to investigate the cellular mechanisms of gene regulation in the rat liver at day 3 after VMH lesions, because it has been reported that cell proliferation in the liver increases and reaches a maximum at day 3 (4), and real-time polymerase chain reaction (PCR) also confirmed a part of the results obtained by DNA microarray analysis.

Materials and Methods

Female Sprague-Dawley rats weighing 230–250 g were used in this study. They were maintained in a constant-temperature environment (23 ± 2°C) in light-controlled cages with a 12-h light-dark cycle (lights on 7:00 AM) and were given free access to food and water. Tissue samples were taken from the liver of VMH-lesioned rats and sham VMH-lesioned rats at day 3 after the operation ($n = 2$ in each group for DNA chips and $n = 3$ in each group for real-time polymerase chain reaction).

VMH lesions or simulated operations were performed as previously described (4–6). The stereotaxic coordinates were at bregma anteriorly, 0.75 mm lateral to the midsagittal line, and 1.0 mm upward from the base of the skull, according to the atlas of De Groot (7). Sham operations were performed in an identical manner, except that no current was applied. After the operations, the rats were returned to their cages and given free access to food and water. Localization of the VMH lesions was verified by

All authors have stated that there is no conflict of interest to disclose regarding the information in this manuscript.

The accession numbers for information regarding the microarray are: 1367707_at; 1367856_at; 1367904_at; 1368160_at; 1368883_at; 1369455_at; 1369531_at; 1370067_a; 1370269_at; 1370334_at; 1371143_at; 1371237_a_at; 1373026_at; 1386637_at; 1387123_at; 1387391_at; 1387967_at; 1388108_at; 1388194_at.

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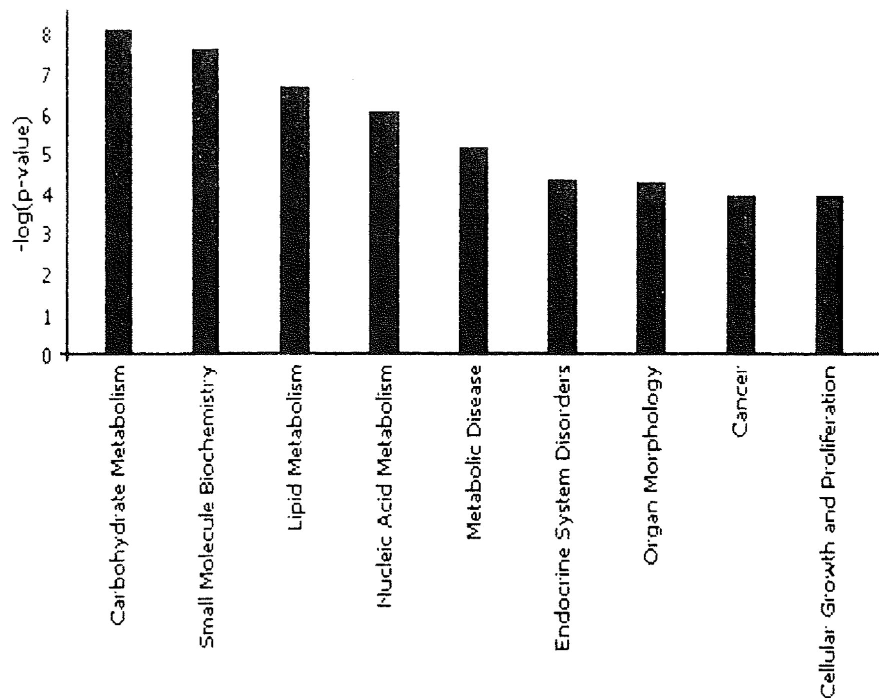

Figure 1. Changed biofunction after VMH lesioning identified in Ingenuity Pathways Analysis.

microscopic examination of the brain at the end of the experiment.

In 2 individual rats at day 3 after VMH lesioning and 2 sham-VMH lesioned rats, in order to circumvent RNA lysis by RNases that may be released in the rat liver when the animal is stressed, all procedures were conducted as swiftly as possible after each rat was sacrificed. The abdominal and chest cavities were opened. The samples of liver were quickly placed in 10 volumes of RNAlater® (Ambion, Inc., Austin, TX) at room temperature. The distance between the tissue surface, which is exposed to preservative, and the innermost regions of the fragment was minimized. We did this by cutting the tissues into 2-mm thick slices, thereby reducing the diffusion distance to 1 mm or less. RNA was isolated from the rat liver, using a commercially available kit (RNA easy Mini Kit, QIAGEN GmbH, Hilden, Germany). The RNA was quantified spectrophotometrically at 260/280 nm, and the quality of the isolated total RNA was determined by electrophoretic separation on anthodium bromide–containing 1% agarose gel. The preparation of cRNA was carried out by Ambion's MessageAmp® II-Biotin Enhanced and the target hybridization was performed according to the instructions provided in the Affymetrix GeneChip® technical manual. The double-stranded cDNA was synthesized from 5 μg of total RNA and hybridized Affymetrix GeneChip® arrays (Rat Genome 230 2.0, Affymetrix Japan Co., Tokyo, Japan) for 16 h at 45°C in a GeneChip® Hybridization Oven 640. After washing and staining in a GeneChip® Fluidics Station 450, hybridized cRNA was detected by a GeneChip® scanner 3000. The digitized image data were processed using the GeneChip® Operating Software 1.4. The amount of probe-specific transcripts was determined based on the average of the differences between the perfectmatch and mismatch intensities. As replicate assays were not performed, a very stringent cutoff point was selected for the detection of significant upregulation or downregulation of the genes in the mRNA amount between the arrays. Using the signal intensity of selected genes that were up- or downregulated compared to the sham-VMH lesioned control group, the analysis was performed using GeneSpring GX 7.3.1 (Agilent Technologies, Santa Clara, CA) and Ingenuity Pathway Analysis (http://www.ingenucity.com/) (Redwood City, CA). Using a computational tool, Ingenuity Pathway Analysis, we were able to build networks of interacting genes from protein-related genes lists.

Regarding the real-time PCR analysis, RNA was stored at −70°C until this analysis was performed. An aliquot (1 μg) of extracted RNA was reverse-transcribed into first-strand complementary DNA (cDNA) at 42°C for 15 min, using 200 U/μl reverse-transcriptase (Takara Biochemicals, Shiga, Japan) and 10 mM of oligo (dT)-adapter primer (Takara Biochemicals) in a 2.0-μl reaction mixture.

Real-time PCR was carried out with a Terminal Cycle Dice TP800 (Takara Biochemicals) using the DNA-binding dye SYBR Green I for the detection of PCR products. The reaction mixture (RT-PCR kit, Code RRO43A, Takara Biochemicals) contained 12.5 μl SYBR Premix Ex Taq (2x) (Code RRO41A, Takara Biochemicals), 10 μM PCR Forward Primer (0.5 μl), 10 μM PCR Reverse Primer (0.5

Table 1. Main Changed Gene Networks at 3 Days After VMH Lesioning Identified in Ingenuity Pathways Analysis[a]

Genes	Score	Focus genes	Top functions
AACS, ABCG5, ACACA, ACLY, AKR1C3, CSAD, ELOVL6, FABP5, FADS2, FASN, G6PD, GPAM, HMGCR, Insulin, IRS3, LSS, ME1, MVD, MYBL1, N-cor, NCOR-LXR-Oxysterol-RXR-9 cis RA, NFkB, ONECUT1, PFKFB1, Pka, PKLR, PP2A, SCD2, SLC2A5, SREBF1, T3-TR-RXR, TMEM97, TNFRSF9, TNFRSF13C, ZMYND1	56	28	Lipid Metabolism, Nucleic Acid Metabolism
ACAT2, Akt, AMPK, Ap1, ASCL1, BHLHB2, CAST, Creb, EFNA1, FGFR1, GPI, Gsk3, HSPB1, Igfbp, IGFBP1, IGFBP2, IL1, IRS2, Jnk, LDL, MEOX2, MT1E, NOV, Pdgf, PDGF BB, PER2, PI3K, Pkc(s), PPAP2B, PVR, S100G, Tgf beta, TGFBR1, VitaminD3-VDR-RXR, ZNF14	35	20	Endocrine System, Metabolism, Reproductive System
Ahr-aryl hydrocarbon-Arnt, AKT1, ASNS, Calmodulin, Caspase, CDKN1A, CISH, CYP1A1, DLAT, E2f, FAM33A, GADD45G, GPX2, Histone h3, HNF4A, ING5, JMJD2C, LDB3, Mapk, NFE2, NOC2L, NUSAP1, P38 MAPK, PFTK1, PTP4A3, RACGAP1, Rb, RNA polymerase II, SUPT16H, TESK1, TPX2, TRIP11, VAMP8, WNT3,WNT4	24	15	Cell Cycle, DNA Replication, Recombination, and Repair, Apoptosis
ALDH1A1, ANKRD25, ATAD2, C12ORF5, Ca2+, CASP3, CCNG1, CDH13, CDKN2A, CDKN2D, CHMP4C, CLCA1 (includes EG:1179), CTCF, CTNNB1, DDR1, dihydrotestosterone, ELL, EMILIN2, FZD7, GSTA3, HEMGN, HNRNPA2B1, IL10, ME1, PDCD6IP, PLA2G12A, progesterone, S100A11 (includes EG:6282), SPTBN1, STK11, TKT, TMEM97, TP53, TP53INP1, ursodeoxycholic acid	24	15	Apoptosis, Cell Growth and Proliferation
ALPL, ARF1, ATF4, BMF, CALR, CREB1, FGL2, GBP4 (includes EG:115361), GJA5,GTP, HACL1, HNF1A, IFNG, KRT19, LGALS8, MAPK8, MAPT, melatonin, MMP11, ODC1, PGD, PLEKHB1, PSPH, retinoic acid, RPL11, SCUBE1, SCYE1, SEC63, SERPINA7, SRP54, SYT9, TCF7L2, TGM1, thyroxine, TNFAIP6	22	14	Cell Growth and Proliferation, Apoptosis
ATAD2, ATP, ATPIF1, beta-estradiol, CCNE2 (includes EG:9134), CDC6, COMMD6, CTSD, ELN, estrone, IFI30, INHBE, KLF4, KLF10, KRT19, ORC1L, PKIB, PPCS, PRKCH, PTP4A1, RELA, RESP18, SKIP, SLC39A4 (includes EG:55630), SMARCA4, SOD2, sulfotransferase, SULT1A3, SULT1C2, SULT4A1, TERT, TMEM164, Ubiquitin, ZRANB1	20	13	Cell Growth and Proliferation, DNA Replication, Recombination, and Repair
BCL3, CAPRIN1, CDC42SE1, CTSC, DUSP22, E2F4, EVL, FGF2, GUSB, HRAS, HSD11B1, IL13, MAPK1, MKI67, NDC80, NUF2, ODC1, OPN3, PIR, POLA2, PRC1, RPL39, RSU1, SMC2, SMC4, SPC24, SPC25, SPINK1 (includes EG:6690), SRF, TGFB1, TNF, TNNT2, TPST1,TTK, ZWINT (includes EG:11130)	20	13	Cell Cycle, Cellular Development
2-methoxyestradiol, AGER, AP3D1, BUB1 (includes EG:699), CDC6, CDC25C, COPZ1, CTTN, CYP1A1, EDG7, EGFR, EZH1, FOXM1, GADD45B, GALE, GARNL1, hydrogen peroxide, ID3, IL6, IL1R1, INSR, LGALS1, MAD2L1, MCM2, MMP8, NLK, OSGIN1, POU2AF1, RUFY1, SDS, SNX4, TCF3, TMEM55A, TXN, XBP1	16	11	Cell Cycle, Cell Growth and Proliferation, Apoptosis
AGRN, ANGPT1, BZW2, CASP10, CCL2, CCND3, CHD2, Cyclin A, CYP17A1, DIXDC1, DUSP1, DVL2, FGF7, FOXO1, HGF, HMGA1, ID1, IL9, KITLG (includes EG:4254), MAP2K1, Mek, MUSK, NR4A1, NR5A1, NRG1, PDGF-AA, PIM1, PPP2R2A, PTTG1, RAF1, RB1, SPRY2, TSC22D3, USF2, VEGFC	6	5	Cell Growth and Proliferation, Cell Cycle

[a] Note: Ingenuity Pathways Analysis calculates a significance score for each network. (http://www.ingenuity.com/products/Monarch.NovelMolecularMechanisms_high.pdf). The score is generated using a P value calculation and is displayed as the negative log of that P value. This score indicates the likelihood that the assembly of a set of focus genes in a network could be random chance alone.

Table 2. Upregulated (>5.0 folds) and Downregulated (>4.5 folds) Genes Identified by DNA Microarray Analysis at 3 Days After VMH Lesioning

Accession No.	Gene name	Fold change[a]	Categories	Functions
1) Upregulated genes				
1370269_at	CYP1A1	25.31	Metabolism	Cytochrome P450 enzyme
1388108_at	ELOVL6	17.76	Metabolism	Elongase of long chain fatty acids
1373026_at	SPC24	14.93	Migration	NDC80 kinetochore complex component
1387123_at	CYP17A1	10.41	Metabolism	Cytochrome P450 enzyme
1388194_at	DLAT	6.48	Enzyme	Putative dihydrolipoamide acetyltransferase
1370067_at	ME1	6.33	Metabolism	Cytosolic, NADP-dependent enzyme that generates NADPH for fatty acid biosynthesis
1387967_at	SPINK1	6.10	Metabolism	Trypsin inhibitor to protect the pancreas from premature activation of pancreatic juice; monitor peptide activity to induce cholecystokinin release in the intestine
1367707_at	FASN	6.05	Metabolism	Fatty acid synthase
1367856_at	G6PD	5.23	Metabolism	Glucose-6-phosphate dehydrogenase
2) Downregulated genes				
1371143_at	SERPINA7	−58.08	Enzyme	Serpin peptidase inhibitor
1368160_at	IGFBP1	−12.51	Growth	Insulin-like growth factor binding
1387391_at	CDKN1A	−10.62	Differentiation	Potent cyclin-dependent kinase inhibitor
1371237_a_at	MT1E	−7.10	Binding	Capacity to bind both physiological and xenobiotic heavy metals through the thiol group of its cysteine residues
1369531_at	SULT1C2	−6.68	Enzyme	Sulfotransferase for categorizing sulfate conjugation of many hormones, neurotransmitters, drugs, and xenobiotic compounds
1370334_at	PLEKHB1	−5.80	Morphology	Pleckstrin (a protein found in platelets) homology domain containing
1368883_at	NOV	−5.00	Differentiation	Regulating the proliferation, differentiation, and adhesion
1367904_at	RESP18	−4.95	Signaling	Regulated endocrine-specific protein 18 (a role as an intracellular signaling molecule expressed exclusively in peptidergic neurons and endocrine cells)
1386637_at	FGL2	−4.89	Secretion	Secreted protein that is similar to the beta- and gamma-chains of fibrinogen
1369455_at	ABCG5	−4.70	Transporter	ATP-transporter family member that regulates absorption of dietary cholesterol

[a] Note: Fold changes in average difference values were calculated using Ingenuity Pathway Analysis.

μl), and cDNA (2.0 μl) to give a final reaction volume of 25 μl. The sequences were obtained using Perfect Real Time support system (http://www.takara-bio.co.jp/prt/imtro.htm). The PCR settings were as follows: the initial denaturation for 10 s at 95°C was followed by 40 cycles of amplification for 5 s at 95°C and 30 s at 60°C, with the subsequent melting curve analysis increasing the temperature from 60°C to 95°C. Relative quantification of gene expression with real-time PCR data was calculated relative to GAPDH.

In the present study, 3 representative genes related to metabolism and cell proliferation were investigated by real-time PCR: 1) ELOVL6 (elongation of very long chain fatty acids–like 6); 2) SPC24 (spindle pole body component 24); and 3) SERPINA7 (serpin peptidase inhibitor, clade A, member 7).

Results are expressed as the mean ± SEM. The mRNA levels were analyzed by the Mann-Whitney *U* test. Statistical analysis was conducted with SPSS version 11.0 statistical software. The differences between the groups were considered significant if the *P* value was <0.05 (2-tailed).

Results

Among 31,099 probes, the expression of 203 probes (0.7%) showed at least a 2-fold upregulation (127 probes) or downregulation (76 probes) at day 3 after VMH lesioning as compared with sham-VMH lesioning. Figure 1 shows the changed biofunction after VMH lesioning identified in Ingenuity Pathways Analysis. The result revealed that VMH lesions regulated some genes that are involved in carbohydrate metabolisms, lipid metabolisms, nuclear acid metabolisms, organ morphology, cell growth and proliferation, and so on. Table 1 shows the main changed gene networks at 3 days after VMH lesioning identified in Ingenuity Pathways Analysis. Moreover, Table 2 shows the upregulated and downregulated genes identified by DNA microarray analysis. As to the gene expressions regarding metabolism, VMH lesions upregulated Cytochrome P450, family 1, subfamily A, polypeptide 1 (CYP1A1), elongation

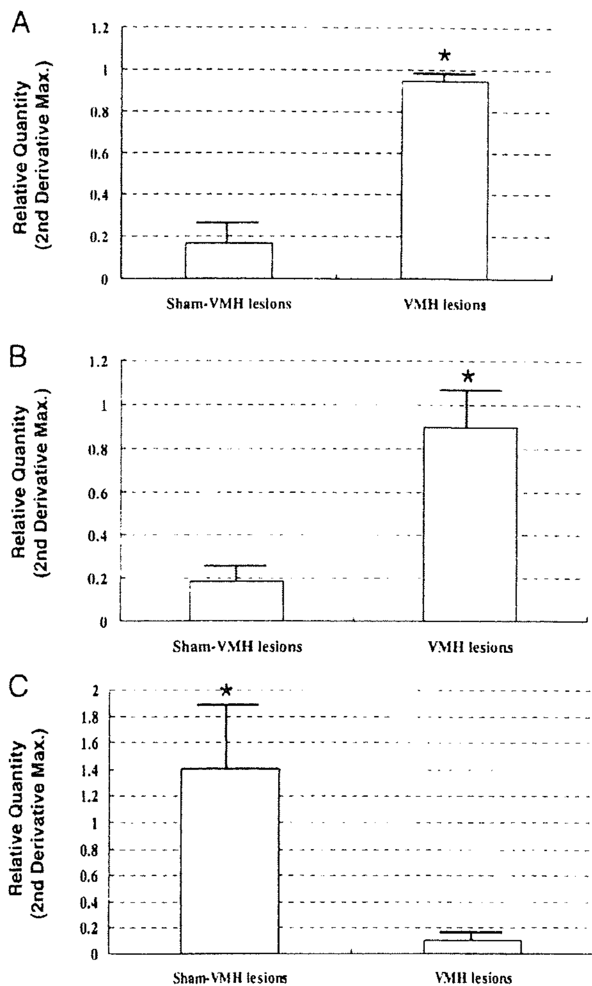

Figure 2. VMH lesion-induced gene expression in real-time PCR analysis. Real-time PCR analysis of total RNA extracts was described in Materials and Methods. A. ELOVL6, elongation of very long chain fatty acids–like 6; B. SPC24, spindle pole body component 24; C. SERPINA7: serpin peptidase inhibitor, clade A, member 7. Values are the means ± SEM of 3 different experiments. * < 0.05 compared with sham-VMH lesioned rats.

of very long chain fatty acids–like 6 (ELOVL6), Cytochrome P450, family 17, subfamily A, polypeptide 1 (CYP17A1), Dihydrolipoamide S-acetyltransferase component of pyruvate (DLAT), Malic enzyme 1, NADP(+)-dependent, cytosolic (ME1), serine peptidase inhibitor, Kazal type 1 (SPINK1), fatty acid synthase (FASN), and glucose-6-phosphate dehydrogenase (G6PD), and downregulated serpin peptidase inhibitor, clade A, member 7 (SERPINA7), sulfotransferase family, cytosolic, 1C, member 2 (SULT1C2), fibrinogen-like protein 2 (FGL2), and ATP-binding cassette, subfamily G, member 5 (ABCG5). Meanwhile, as to gene expressions regarding cell proliferation, VMH lesions upregulated spindle pole body component 24 (SPC24), and downregulated insulin-like growth factor binding protein 1 (IGFBP1), cyclin-dependent kinase inhibitor 1A (CDKN1A), nephroblastoma overexpressed gene (NOV), and regulated endocrine-specific protein 18 (RESP18). The expressions of EVOVL6, SPC24, and SERPINA7 were also examined by the real-time quantitative analysis (Fig. 2). The gene expressions of EVOVL6 and SPC24 were upregulated ($P < 0.05$ and $P < 0.05$, respectively), and that of SERPINA7 was downregulated ($P < 0.05$).

Discussion

In the present study, we used the DNA microarray technique for mRNA expression profiling of rat liver mixtures to investigate cellular responses in response to VMH lesions. The DNA microarray analysis revealed that VMH lesions regulated some genes that are involved in the functions, such as carbohydrate metabolisms, lipid metabolisms, nuclear acid metabolisms, organ morphology, cell growth and proliferation, and apoptosis (Figs. 1 and 2). This result by DNA microarray technique confirmed the previous results that VMH lesions change various types of metabolisms, and that they also induce cell proliferation and apoptosis (4, 6).

As to the genes' expressions regarding metabolism, Table 2 shows the dynamic biofunctional changes of gene expressions. Especially, cytochrome P450 is noted to be changed massively after VMH lesioning. Cytochrome P450 is primarily membrane-associated proteins, located either in the inner membrane of mitochondria or in the endoplasmic reticulum of cells (8). Most cytochrome P450 can metabolize multiple substrates, and many can catalyze multiple reactions, which accounts for their central importance in metabolizing the extremely large number of endogenous and exogenous molecules (8). Consistent with this, Inui et al. (9) previously reported that the content of cytochrome P450 per mg microsomal protein in VMH lesioned rats was significantly higher than that in sham-lesioned rats. Moreover, in the present study, the gene expressions of ELOVL6 and SPC24 were upregulated after VMH lesioning. The upregulation of ELOVL6 gene expression may be involved in developing hepatosteatosis by VMH lesioning (10), because ELOVL6 gene protein is long-chain fatty-acyl elongase and is involved in fatty acid biosynthesis (11). Meanwhile, because the 4-subunit NDC80 complex, which is included in SPC24 protein, directly connects kinetochores to spindle microtubules, SPC24 plays a major role in regulating cell division (12, 13). We previously reported that VMH lesions induce hepatic cell proliferation (4, 5). The changes of SPC24 expression after VMH lesioning confirmed this fact.

We previously reported that VMH lesions induce cell proliferation, but also stimulate Fas/Fas ligand system–mediated apoptosis through the activation of caspase 3 in the rat liver (6). Therefore, VMH lesions may suppress mainly the caspase-dependent type I pathway for apoptosis in the liver. Consistent with this, in the present study, DNA microarray analysis indicated VMH lesions induced the

increased Fas, caspase 3, glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) gene expression in liver tissue mixture, but the expression of these probes showed <2-fold upregulation. However, in the present study, the gene expression of SERPINA7, a serpin peptidase inhibitor, was downregulated after VMH lesioning. Because serpins protect hepatocytes from apoptosis mediated by the granzyme-perforin mechanisms present in the abundant natural killer cell found in normal liver (14), and serpins also inhibit metacaspase-like proteases *in vivo* and control cell death pathways (15), the downregulation of SERPINA7 gene expression may protect hepatocytes from apoptosis, and therefore induce cell proliferation in the liver.

In conclusion, many genes related to the changed metabolism and cell proliferation and apoptosis based on VMH lesions were detected by the DNA microarray analysis. Although the networks of these genes involved in this process have not yet been elucidated sufficiently, this study is the first report to demonstrate that VMH lesions may cause changes of various gene expressions regarding metabolism and cell proliferation and apoptosis in the liver.

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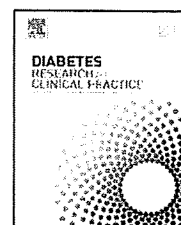

Multi-center intervention study on glycohemoglobin (HbA1c) and serum, high-sensitivity CRP (hs-CRP) after local anti-infectious periodontal treatment in type 2 diabetic patients with periodontal disease

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ABSTRACT

The purpose of this study was to examine whether periodontal treatment incorporating topical antibiotic therapy affects on levels of glycohemoglobin (HbA1c) and serum high-sensitivity C-reactive protein (hs-CRP) in type 2 diabetic patients with periodontal disease, and to explore the relationship between CRP and glycemic control. The whole intervention group ($n = 32$), which underwent anti-infectious periodontal treatment, showed only transient reduction in HbA1c levels without any change in hs-CRP, while the control group ($n = 17$) did not show any changes in HbA1c or hs-CRP. Multiple regression analysis of all subjects revealed that BMI and change in hs-CRP correlated significantly with the reduction of HbA1c at 6 months after the periodontal treatment. Based on the results of multiple regression analysis, the intervention group was subdivided into two groups: those in which hs-CRP levels decreased (CRP-D group), and those in which hs-CRP levels unchanged or increased (CRP-N group) ($n = 16$, respectively), and re-analysis was conducted based upon these subgroups. In the CRP-D subgroup, HbA1c was significantly reduced at the end of the study, but it did not decrease in the CRP-N subgroup. The decrease of HbA1c in the CRP-D subgroup following periodontal treatment was significantly greater than that in the CRP-N

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subgroup. BMI of each group remained unchanged in this study at the end of the study. Thus, the results suggested that periodontal treatment with topical antibiotics improves HbA1c through reduction of CRP, which may relate to amelioration of insulin resistance, in type 2 diabetic patients with periodontal disease.

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1. Introduction

Periodontal disease is a chronic infectious disease, triggered by the bacterial biofilm of dental plaque, which results in an inflammatory loss of bone, and both soft and hard tooth-supporting structures [1,2]. It is very often associated in diabetic patients following microangiopathies and macroangiopathies [3]. The prevalence of periodontal disease among individuals with inadequately controlled type 2 diabetes is generally higher than that of non-diabetic individuals [4]. Thus, it is recognized that diabetic patients with poor glycemic control are at increased risk for the incidence and progression of periodontal disease. However, few reports indicated that glycemic control in diabetic patients improved periodontal disease.

On the contrary, there are a large number of reports that have addressed the effects of periodontal disease on glycemic control. A 2-year longitudinal study demonstrated a six-fold increased risk of worsening glycemic control in type 2 diabetic patients with severe periodontitis compared with those with no periodontitis [5]. Intervention studies focusing on the effects of periodontal treatment on glycemic control in diabetic patients with periodontal disease have shown that periodontal treatment with or without adjunctive antibiotic therapy improved metabolic control of diabetes [6–9]. However, other studies found no improvement in glycemic control after periodontal treatment [10]. The reason for the discrepancy regarding efficacy remains unclear at present.

Although type 2 diabetes is a multifactorial metabolic disease, recent evidence suggests that chronic subclinical inflammation plays an important role in the development of type 2 diabetes [11,12]. Elevated circulating levels of subclinical inflammatory markers, such as C-reactive protein (CRP) and interleukin-6 (IL-6) are reported to be significant risk indicators not only for the development of cardiovascular disease, but also for the development or progression of type 2 diabetes [13]. Several reports have demonstrated a significant elevation of serum CRP levels in patients with periodontitis compared with periodontally healthy controls [14–18]. Some intervention studies have suggested that successful anti-infective periodontal treatment can reduce CRP with TNF-α and IL-6 levels in systemically healthy subjects [19–22]. Thus, the possibility exists that reduction of CRP may contribute to the improvement of glycemic control in diabetic patients after periodontal treatment. However, to the best of our knowledge, there have been no studies that investigated the effects of periodontal treatment on the levels of CRP and HbA1c simultaneously in type 2 diabetic patients with periodontal disease.

The purpose of this study was to examine whether anti-infectious periodontal treatment with topical antibiotic therapy affects levels of HbA1c and serum CRP in type 2 diabetic patients with periodontal disease without changes of the treatment of diabetes (drug, diet and exercise) for glycemic control, and to explore the relationship between the CRP and HbA1c.

2. Subjects and methods

2.1. Subjects

A total of 63 type 2 diabetic patients were recruited from diabetic clinics at 5 institutes: Tokyo Medical and Dental University Hospital, Kagoshima University Medical and Dental Hospital, Aichi Gakuin University Dental Hospital, Tokyo Medical University Hospital and Kyoto Prefecture Medical University Hospital. At each institute, patients were selected based on the medical condition inclusion criteria: age of 39–75 years, HbA1c 6.5–10.0%, without severe diabetic complications, no evidence of systemic diseases other than diabetes as a risk factor for periodontitis, no systemic antibiotics during the preceding 3 months, no pregnancy or lactation, no allergy to tetracycline, no smoking, and no modifications in the treatment of diabetes during the preceding 2 months. Then, the patients were transferred to the dental clinic in each institute. Patients for the study were selected based on the dental condition inclusion criteria: at least 11 remaining teeth, at least two pocket sites with probing depth 4 mm or more (indicated as mild to severe periodontitis), no periodontal treatment during the preceding 6 months. Finally, 49 participants fulfilled both medical and dental inclusion criteria.

2.2. Study design

Forty-nine subjects compatible with the medical and dental criteria were randomly allocated to either the periodontal treatment (intervention) or non-periodontal treatment (control) groups by the envelope method. The distribution of subjects to the groups was assigned as 2 in the intervention group and 1 in the control group.

In the intervention group, mechanical debridement of the sub-gingival plaque and calculus was performed using piezo-electric ultra sonic scalers, and 10 mg of minocycline ointment (Periofil®, Showa Yakuhin Co., Tokyo, Japan) was administered topically in every periodontal pocket at the end of each visit. The intensive periodontal treatment was completed over the course of four visits within 2 months. Control group participants received instructions for brushing their teeth, including the use of inter-proximal cleaning aids, such as floss and inter-dental brushes, depending on their individual needs. After the completion of 2 months of intensive period-

ontal treatment, all participants visited the respective medical and dental clinics at 1, 3 and 6 months. In the intervention group, additional periodontal treatment including instructions for brushing, supra- and sub-gingival debridement without topical administration of antibiotics were performed, if necessary. The doses and kinds of anti-diabetic drugs, including oral hypoglycemic drugs and insulin injections and methods of diet and exercise were not changed to assess the real effects of periodontal treatment on blood glucose control. The study protocol was approved by the Ethics Committee for each university hospital, and written informed consent was obtained from each of the participants.

2.3. *Periodontal examination*

All participants underwent a full-mouth clinical periodontal examination at the dental clinic. The periodontal parameters were recorded at baseline, 1, 3 and 6 months following the intensive phase of periodontal treatment. Full-mouth clinical measurements of the probing pocket depth (PPD) and bleeding on probing (BOP) were recorded using a manual probe (PCP-UNC 15, Hu-Friedy Manufacturing Co., Inc., Chicago, USA) at all six sites of each tooth.

2.4. *Detection of serum peripheral parameters*

At the medical clinic, venous blood samples were taken from each patient and analyzed for fasting plasma glucose (FPG), glycohemoglobin (HbA1c) and high-sensitivity C-reactive protein (hs-CRP). FPG was determined by a hexokinase assay. HbA1c was determined by high-performance liquid chromatography (Kyotokagaku Co., Ltd., Kyoto, Japan). Serum was immediately stored at −70 °C. Serum hs-CRP was measured using a latex particle-enhanced immunonephelometric method. FPG, HbA1c and hs-CRP measurements were performed at baseline, and 1, 3 and 6 months following the intensive phase of periodontal treatment.

2.5. *Statistical analysis*

Statistical analysis was performed using the JMP® (SAS Institute Inc., Cary, NC, USA) software program. Changes of all parameters from baseline to 1, 3 and 6 months were compared using Wilcoxon signed-rank test. Mann–Whitney U-test was used to compare the changes of parameters between the intervention and control groups, and the CRP-decreased (CRP-D) and CRP-unchanged or increased (CRP-N) groups of subanalysis for the intervention group. Multiple regression analysis was performed to examine the effect of age, gender, baseline body mass index (BMI), duration of diabetes mellitus, baseline HbA1c, baseline hs-CRP, number of teeth, baseline PPD, baseline BOP, change in hs-CRP at 1 month (Delta hs-CRP), change in PPD at 1 month (Delta PPD), change in BOP at 1 month (Delta BOP) and intervention of periodontal treatment on the change in HbA1c at 6 months.

3. Results

3.1. *Patient profile*

The analyzed participants included 32 patients in the intervention group and 17 patients in the control group. Table 1A presents the demographics of the participants at

Table 1 – Demographics of the participants at baseline.

	A. All participants		B. Intervention group	
	Intervention[a] (n = 32)	Control[a] (n = 17)	CRP-D[a] (n = 16)	CRP-N[a] (n = 16)
Age (years)				
Mean ± S.D. (median)	60.3 ± 9.9 (60)	59.0 ± 4.8 (59)	59.3 ± 10.6 (58)	61.3 ± 9.0 (63)
Gender (n)				
Male/female	21/11	6/11	13/3	8/8
BMI				
Mean ± S.D. (median)	24.1 ± 4.1 (23.5)	25.8 ± 4.8 (24)	24.3 ± 4.9 (22.8)	23.8 ± 3.0 (23.6)
Duration (years)				
Mean ± S.D. (median) [range]	11.3 ± 6.4 (6) [3–25]	8.8 ± 7.5 (9.0) [1–30]	8.8 ± 4.9 (6) [3–22]	12.4 ± 7.2 (6) [5–25]
Diabetic treatment				
Diet only/oral hypoglycemic/insulin	1/15/16	2/12/3	0/7/9	1/8/7
Number of teeth (n)				
Mean ± S.D. (median)	23.6 ± 5.0 (25)	24.1 ± 2.8 (24)	22.9 ± 5.7 (24)	24.2 ± 4.0 (25)
HbA1c (%)				
Mean ± S.D. (median)	7.2 ± 0.9 (7.1)	6.9 ± 0.9 (6.7)	7.1 ± 0.9 (7.0)	7.4 ± 0.8 (7.2)
FPG				
Mean ± S.D. (median)	152 ± 53 (149)	133 ± 35 (128)	146 ± 56 (149)	159 ± 53 (149)
hs-CRP (ng/ml)				
Mean ± S.D. (median)	1858 ± 2531 (725)	2278 ± 2593 (2025)	2523 ± 3141 (1150)	1192 ± 1569 (400)

There are no significant differences between intervention and control groups, and between CRP-D and CRP-N groups in all parameters.
[a] Groups.

Table 2 – Periodontal status at baseline and follow-up period.

Group	Baseline	1 month	3 months	6 months
A. All participants				
Control group ($n = 17$)				
PPD (mm)	2.8 ± 0.9	2.6 ± 0.6	2.6 ± 0.7	2.6 ± 0.7*
BOP (%)	24.9 ± 14.7	23.3 ± 16.3	20.1 ± 17.1	18.8 ± 18.1*
Intervention group ($n = 32$)				
PPD (mm)	3.0 ± 0.9	2.3 ± 0.6§,‡	2.2 ± 0.5§,‡	2.2 ± 0.5§,‡
BOP (%)	36.5 ± 22.5	11.5 ± 10.8§,‡	13.4 ± 12.5§,‡	12.0 ± 11.4§,‡
B. Intervention group				
CRP-D group ($n = 16$)				
PPD (mm)	3.3 ± 1.1	2.5 ± 0.7§	2.3 ± 0.5§	2.5 ± 0.6§
BOP (%)	46.6 ± 23.7†	15.5 ± 12.3§,†	17.4 ± 14.4§	14.5 ± 13.5§
CRP-N group ($n = 16$)				
PPD (mm)	2.7 ± 0.6	2.1 ± 0.4§	2.1 ± 0.4§	2.0 ± 0.3§
BOP (%)	26.4 ± 15.8	7.5 ± 6.9§	8.8 ± 7.6§	9.5 ± 8.3§

Values are given as mean ± S.D. PPD: probing pocket depth. BOP: bleeding on probing.
* Statistically significant decrease compared with baseline ($p < 0.05$).
§ Statistically significant decrease compared with baseline ($p < 0.01$).
‡ Statistically significant decrease compared with control group ($p < 0.05$).
† Statistically significant decrease compared with CRP-N group ($p < 0.05$).

baseline. Age, gender, BMI, duration of diabetes mellitus, method of diabetic treatment, number of teeth, HbA1c, FPG and hs-CRP values showed no significant differences between the intervention and control groups.

As shown in Table 2A, which presents the periodontal parameters of the participants, there were no significant differences in the periodontal status, including PPD and BOP, between the intervention and control groups at baseline.

3.2. *Effect of periodontal treatment on the periodontal status*

In the whole intervention group, both PPD and BOP improved significantly at 1 month following the intensive periodontal treatment compared with those at baseline, and these improvements were maintained throughout the study period (Table 2A). In the control group, PPD and BOP were slightly reduced at 6 months compared with those at baseline (Table 2A). PPD and BOP were significantly lower in the intervention group compared with the control group at 1 month after the intensive periodontal treatment and these significant differences continued until the end of the study, which indicated that periodontal treatment in the intervention group was effective (Table 2A).

3.3. *Metabolic assessments*

The levels of HbA1c in the whole intervention and control groups are shown in Fig. 1a. The HbA1c levels in the intervention and control groups were not significantly different at baseline (7.2 ± 0.9% in the intervention group vs. 6.9 ± 0.9% in the control group). One month after the intensive periodontal treatment, a significant reduction in the HbA1c was observed in the intervention group (Fig. 1a). The reduced HbA1c levels in the intervention group tended to continued thereafter. However, the HbA1c levels at 3 or 6 months were not statistically significant compared with the baseline HbA1c level (Fig. 1a). In the control group, HbA1c levels did not show any significant changes in HbA1c levels during the follow-up period.

Serum hs-CRP levels in the whole intervention and control groups are shown in Fig. 1b. There were no significant differences in serum hs-CRP levels between the intervention and control groups at baseline 1, 3 and 6 months. No significant changes were observed in both groups during the follow-up period. There were no significant differences between the intervention and control group at each point during the follow-up period.

BMI and FPG remained unchanged significantly during the whole study in the control and intervention groups.

3.4. *Multiple regression analysis*

To further analyze the effect of periodontal treatment on glycemic control, multiple regression analysis was applied to examine the correlation of the decrease of HbA1c levels from the baseline to the 6-months, with the 13 variables of age, gender, baseline BMI, duration of diabetes mellitus, baseline HbA1c levels, baseline hs-CRP levels, number of teeth, baseline PPD, baseline BOP, change in hs-CRP between baseline and 1 month after, change in PPD between baseline and 1 month after, change in BOP between baseline and 1 month after and intervention of periodontal treatment. As shown in Table 3, baseline BMI and change in hs-CRP were correlated significantly with the change in HbA1c levels during 6 months in all participants ($p = 0.03$ for BMI, $p = 0.03$ for change in hs-CRP).

3.5. *Effect of periodontal treatment on the periodontal status in the CRP-decreased (CRP-D) and the CRP unchanged or increased (CRP-N) groups*

Based on the results of regression analysis, the intervention group was subdivided into two groups by the change in hs-CRP, and sub-analysis was conducted. Out of 32 patients in the

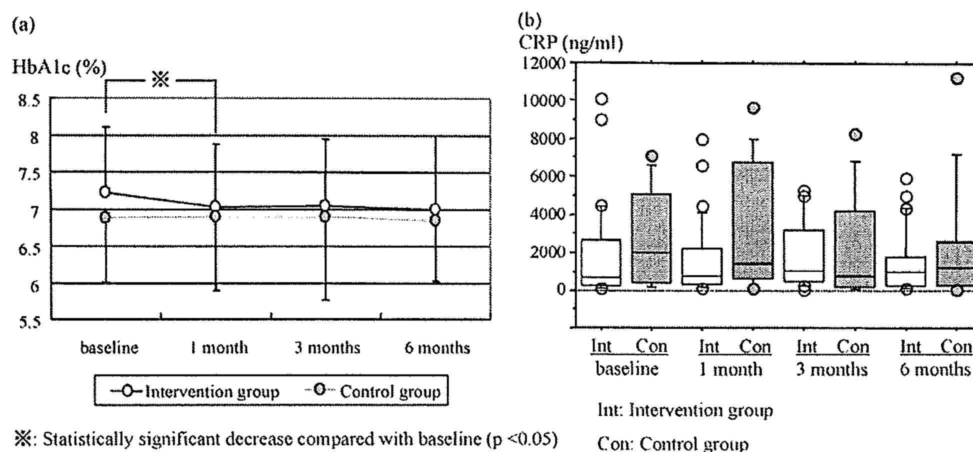

Fig. 1 – The levels of HbA1c and serum CRP at baseline and during the follow-up period in the intervention and control groups. (a) Shows the mean HbA1c levels of the intervention and control groups at baseline and during the follow-up period. There were no significant differences between the intervention and control groups at baseline, 1, 3 and 6 months. No significant differences were observed in HbA1c between the baseline and follow-up period in the control group. There were significant differences in HbA1c between the baseline and 1 month after periodontal treatment in the intervention group. (b) Shows the serum concentration of the high-sensitivity C-reactive protein (hs-CRP) of the intervention and control groups at baseline and during the follow-up period. Box plots show the medians, the 25th and 75th percentiles as boxes, and the 10th and 90th percentiles as whiskers. Values outside this range are shown as open circles. There were no significant differences in hs-CRP concentration between the intervention and control groups. No significant difference was observed in hs-CRP concentration between the baseline and the follow-up period in the intervention or control groups.

intervention group, serum hs-CRP was decreased in 16 patients (CRP-D group), whereas the other patients showed unchanged or increased their serum hs-CRP at 1 month following periodontal treatment (CRP-N group). We re-analyzed the results by comparing the results between the CRP-D and CRP-N groups. As shown in Table 1B, there were no differences in age, gender, baseline BMI, duration of diabetes mellitus, method of diabetic treatment, number of teeth, HbA1c, FPG and hs-CRP at baseline between the CRP-D and CRP-N groups. In addition, PPD showed no differences between the CRP-D and CRP-N groups at baseline. However, BOP was significantly higher in the CRP-D group compared with the CRP-N group at baseline, indicating that active inflammation was more prominent in the CRP-D group than in the CRP-N group (Table 2B). PPD was decreased significantly in both the CRP-D and CRP-N groups at 1 month and continued throughout follow-up period. There were no significant differences in PPD between the CRP-D and CRP-N groups during the follow-up period (Table 2B). BOP significantly decreased in both CRP-D and CRP-N groups at 1 month and it continued during the follow-up period. Although significant differences in BOP were observed at 1 month between the CRP-D and CRP-N groups ($p < 0.05$), but the differences disappeared at 3 and 6 months (Table 2B).

As shown in Fig. 2b, serum hs-CRP in the CRP-D group decreased significantly compared with baseline hs-CRP at 1 and 6 months following periodontal treatment. On the other hand, serum hs-CRP in the CRP-N group remained unchanged during the follow-up period.

Serum HbA1c at baseline was not different between the CRP-D and CRP-N groups. The serum HbA1c in the CRP-D group significantly decreased at 6 months, but it remained unchanged in the CRP-N group during the follow-up period (Fig. 2a). A significant difference between the CRP-D and CRP-N groups was observed at 6 months.

Any significant differences in BMI and FPG were not observed in both subgroups (CRP-D and CRP-N groups) of the intervention group between baseline and the follow-up periods.

Table 3 – Multiple regression model for changes of HbA1c levels between baseline and 6 months.

Standardized partial correlation coefficient	All participants	
	Estimates	p
Age	0.05	0.75
Gender	0.23	0.12
Baseline BMI	0.33	0.03
Duration	−0.27	0.09
Baseline HbA1c	−0.24	0.15
Baseline ln [hs-CRP]	0.28	0.08
Number of teeth	0.03	0.87
Baseline PPD	0.09	0.78
Baseline BOP	−0.04	0.89
Change in hs-CRP	0.33	0.03
Change in PPD	0.40	0.22
Change in BOP	0.004	0.99
Intervention of periodontal treatment	−0.19	0.42

Model $r^2 = 0.65$.

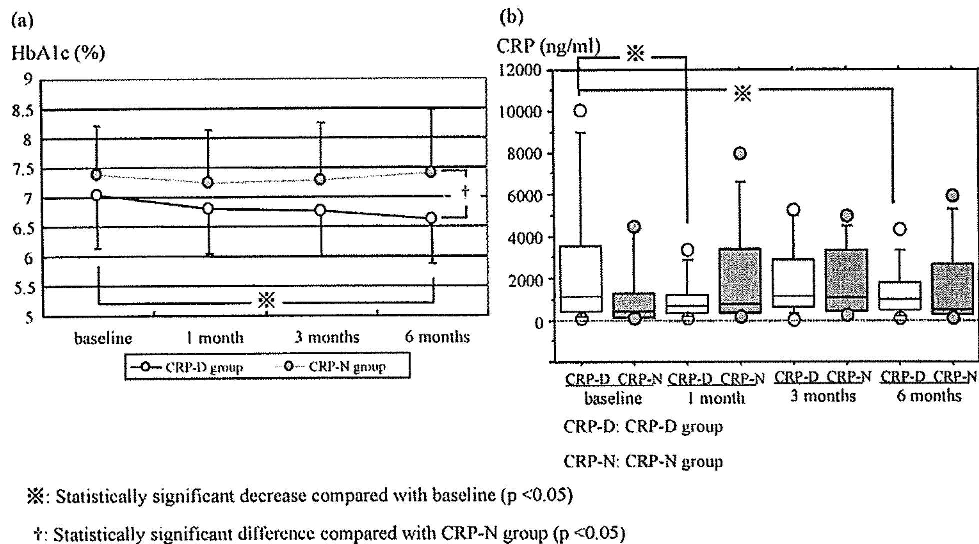

Fig. 2 – The levels of HbA1c and serum CRP at baseline and during the follow-up period in the CRP-decreased (CRP-D) and CRP-unchanged or increased (CRP-N) groups. (a) Shows the HbA1c levels of the CRP-D and CRP-N groups at baseline and during the follow-up period. There were no significant differences in HbA1c between the CRP-D and CRP-N groups at baseline, 1, 3 and 6 months. In the CRP-D group, there were significant differences in HbA1c level between the baseline and 6 months follow-up periods. No significant differences were observed between the baseline and follow-up period in the CRP-N group. (b) Shows the serum concentration of hs-CRP of the CRP-D and CRP-N groups at baseline and during the follow-up period. Box plots show the medians, the 25th and 75th percentiles as boxes, and the 10th and 90th percentiles as whiskers. Outside values are shown as open circles. In the CRP-D group, there were significant differences in hs-CRP between the baseline and the 1- and 6-month follow-up period. No significant changes were observed in concentration of hs-CRP during the whole study in the CRP-N group.

4. Discussion

The main results of the present study are summarized as follows: without any changes in hypoglycemic treatments, including drugs, diet and exercise; (1) periodontal treatment with local antibiotics administration was able to improve glycemic control in type 2 diabetic patients, (2) the improvement of glycemic control was significant when associated with a reduction of hs-CRP, (3) patients with hs-CRP associated with high BOP responded well to periodontal treatment and exhibited reduction of HbA1c, and (4) CRP contributed to the improvement of glycemic control by the treatment of periodontal disease.

Recent evidence has indicated that patients with severe periodontitis exhibit increased serum levels of hs-CRP, as well as proinflammatory cytokines, such as TNF-α and IL-6 [21,23] and these levels decrease after periodontal treatment [21,22]. These results supported that local infection contributes to systemic inflammation [21,24]. In diabetic patients with periodontal disease, Lalla et al. also reported that periodontal treatment with antibiotics reduced hs-CRP [25]. However, it has not been determined whether reduced hs-CRP may relate to reduce HbA1c in diabetic patients since the effects of anti-infectious periodontal treatment on HbA1c, have been inconsistent [6–9,26,27]. Meta-analysis of 10 reliable intervention studies with more than 456 type 2 diabetic patients with periodontal disease demonstrated an average decrease in actual HbA1c, an indicator of glycemic control, of 0.57%, but the reduction was not significant [28].

In this study, a remarkable improvement of periodontal status was observed in all patients after periodontal treatment. However, significant reduction of HbA1c was observed only in diabetic patients in the CRP-D group. Moreover, multiple regression analysis of all subjects revealed that Delta hs-CRP level at 1 month correlated significantly with the reduction of HbA1c at 6 months after periodontal treatment in this study. It suggests that change in hs-CRP level is a contributing factor relating to the changes in HbA1c level. These results suggested that anti-infectious periodontal treatment is effective when associated with a reduction in hs-CRP, and the results may, in part, explain the inconsistent effects of periodontal treatment on glycemic control in diabetic patients.

It is noteworthy that patients in CRP-D group associated with higher bleeding on probing, which indicated active inflammation, responded well to anti-infectious treatment with antibiotics, resulting in reduction of HbA1c in this study. It is consistent with the results that periodontal treatment with antibiotics reduced the levels of hs-CRP [29] and HbA1c [8,30,31] more than that without antibiotics.

What caused the reduction of HbA1c by anti-infectious periodontal treatment in type 2 diabetic patients in this study? Some reports have suggested that improvement of insulin resistance was associated with improvement of glycemic control in type 2 diabetic patients with periodontal disease after periodontal treatment [9,23]. It has been reported that hs-CRP directly induces insulin resistance in type 2 diabetic patients [11,32]. Periodontal diseases also release proinflam-

matory cytokines such as TNF-α and IL-6 [18]. TNF-α and IL-6 have been reported to induce insulin resistance [33,34].

Anti-infectious periodontal treatment ameliorates local inflammation, which reduces production of CRP as well as TNF-α and IL-6, resulting in improvement of insulin resistance [9,23].

In the present study, supportive periodontal treatment including additional periodontal debridement was performed in follow-up period after the intensive periodontal treatment, if necessary. As a result, improved periodontal parameters were maintained during the period of study. This may suggest that appropriate supportive periodontal treatment is needed to maintain improved glycemic control obtained after the intensive periodontal treatment.

The regression analysis in this study revealed that another contributing factor to the effect of anti-infectious periodontal treatment on reduced HbA1c was BMI. However, a mean BMI of each group remained unchanged at the end of this study. Consequently, the result suggested that BMI did not contribute to reduce HbA1c level after the periodontal treatment in this study.

In conclusion, periodontal treatment with topical antibiotics was effective towards the improvement of glycemic control in type 2 diabetic patients with periodontal disease when associated with a decrease in serum hs-CRP. This report indicated the possibility that CRP may play independent contributing role on the reduced HbA1c in diabetic patients by local periodontal treatment with antibiotics.

Conflict of interest

There is no conflict of interest in this study.

Acknowledgements

We are grateful to all of the study participants. We thank Dr. Yoshiyuki Sasaki at Tokyo Medical and Dental University for his excellent statistical processing. This work was supported by Grants-in Aid from the Ministry of Health and Welfare of Japan (H16-Iryo-020) and the Mitsui Sumitomo insurance foundation.

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エビデンスに基づく
全身の健康と口腔との関係

花田信弘

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■本文23ページのサイエンス欄を参照

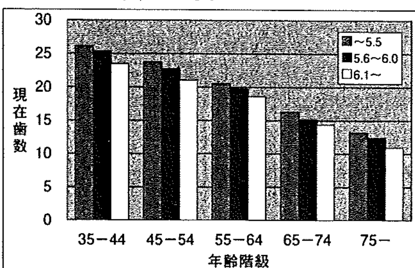
エビデンスに基づく全身の健康と口腔との関係

～厚生労働科学研究「口腔保健と全身のQOL」報告書から抜粋～

花田信弘（鶴見大学歯学部探索歯学講座教授）

●国民健康・栄養調査

分担研究者安藤雄一室長の報告[3]では，口腔状態と食品・栄養摂取は密接な関連を有しており，糖尿病の指標であるHbA1cと現在歯数にも違いが出ている。

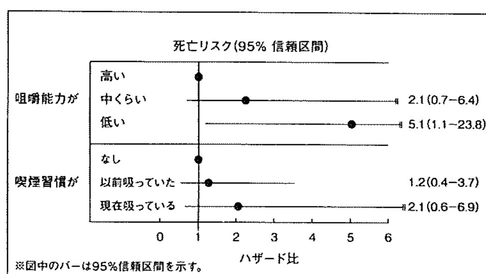


❶ HbA1c別にみた現在歯数（歯の数）～2004年国民健康・栄養調査～
〔厚生労働科学研究「口腔と全身」報告書：安藤雄一分担報告書[3]から引用〕

※年齢階級，性，自治体規模，仕事，歯ぐきの自覚症状，歯科保健行動（歯間部清掃，歯石除去・歯面清掃，歯磨き個別指導，歯科健康診査），喫煙状況を調整して現在歯数の平均値を算出

●80歳福岡県地域住民におけるコホート研究

分担研究者安細敏弘准教授の報告書では，4年間の追跡期間中に108名が死亡し，そのうち心血管疾患による死亡が27名であった。咀嚼能力が高い方を基準とすると，中くらいの者で2.1倍，低い者では5.1倍心血管疾患により死亡するリスクが高かった。



❷ 80歳における咀嚼能力が心血管疾患による死亡リスクに及ぼす影響
〔厚生労働科学研究「口腔と全身」報告書：安細敏弘分担報告書から引用[6]〕

●吹田研究と栄養

分担研究者小野准教授の「吹田研究」[4]では，歯数が20歯未満となった場合にメタボリックシンドロームのリスクが増加することが報告された。

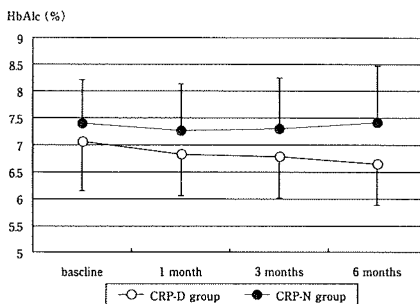
	調整済みオッズ比	95%信頼区間	P-value
血　圧	1.15	1.0－1.3	0.08
血糖値	1.61	1.3－2.0	0.01
HDLC	1.34	1.1－1.7	0.01
中性脂肪	1.27	1.0－1.6	0.02
腹　囲	1.02	0.9－1.2	0.83
メタボリックシンドローム	1.22	1.0－1.5	0.03

年齢，既往歴，飲酒・喫煙の有無を調整

❸ 歯数20本未満とメタボリックシンドロームの関係
〔小野高裕分担研究者の報告書から引用[4]〕

●糖尿病と歯周病の介入研究

歯周病治療介入群を，hs-CRPが低下した被験者群（CRP-D群）と上昇もしくは変化しなかった群(CRP-N群)に分けると，CRP-D群では，HbA1cが介入研究期間を通して低下したが，CRP-N群では低下しなかった。

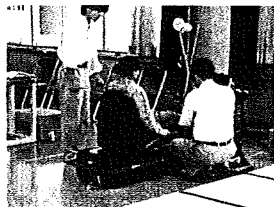


❹ 糖尿病と歯周病の介入研究
〔厚生労働科学研究「口腔と全身」報告書：井上修二分担研究者の報告書から引用[5]〕

●厚生労働科学研究「口腔と全身」では岩手県，新潟県，福岡県，愛知県において80歳の健診を実施した。その結果，8020達成者は，達成できなかった人に比べて全身的な健康，QOLが優れていることが証明された。

❺ 岩手県における80歳の健診風景（窓の外は，神戸市歯科医師会，兵庫県歯科医師会および兵庫県から借用したレントゲンバス：阪神・淡路大震災の時に使用されたもの）

❻ 80歳の健診の前に行った歯科医師のキャリブレーション風景（岩手県歯科医師会）



❼ 80歳の健診で初めて行った脚力検査

❽ 80歳の健診に引き続き実施された新潟スタディーの健診風景（新潟スタディーでは70歳の住民が80歳になるまで毎年健診が行われ，この調査により高齢者の口腔機能と脚力やバランス，機敏性などの身体能力が関連することが実証された）