Study on factors that affect caries susceptibility in mice

（マウスにおける齲蝕感受性に影響を与える要因の検討）

日本大学大学院松戸歯学研究科歯学専攻*

日本大学松戸歯学部　小児歯科学講座**

遠藤　智佳*

山本　晴子**

清水　邦彦**

（指導：前田　隆秀教授）

Abstract

In our previous research, we discovered a C57BL/6CrSlc mouse strain (B6) with highly susceptible to caries and a C3H/HeSlc mouse strain (C3H) with highly resistant to caries, and reported that genetic factors play a role in caries susceptibility. In a study on the B6 and C3H mouse strains, a difference was found in their saliva flow rates, as well as their caries scores. In the present study, we focussed on saliva secretion, which may influence differences in caries susceptibility. We examined temporal changes in stimulated saliva secretion volume in B6 and C3H mice, and found that secretion volume was significantly higher in C3H mice than in B6 mice even at 30 min after stimulation, and that total saliva volume was significantly higher in C3H mice. In addition, histological comparisons of the submandibular gland in the two strains revealed the ratio of acinar cells area to be significantly higher in C3H mice than in B6 mice. We then examined the gene expression of the muscarinic acetylcholine receptors subtypes 1−3 (M1R, M2R and M3R), which are thought to be regulatory factors of water secretion from acinar cells in the parasympathetic nervous system, the gene expression of M3R in C3H mice was not high, in spite of high volume of salivary secretion. We also used a dynamic ultra-micro-hardness tester (DUH-211; Shimadzu, Kyoto, Japan) to measure the enamel hardness, which have been reported to be involved in caries development. The means differed significantly between the two strains.

The acinar cells ratio in the submandibular gland may be a factor associated with differences in salivary flow rate. The saliva secretion volume and the enamel hardness may be a determining factor of tooth decalcification- three factors that affect caries susceptibility.

Key words

Caries susceptibility, Saliva flow, Muscarinic acetylcholine receptor, Acinar cell, Enamel hardness

1. Introduction

Differences in caries susceptibility arise from complex interactions among environmental factors and genetic factors. Environmental factors can be standardised for conducting research on mice. In addition to the extent of enamel calcification, the saliva flow rate that greatly affects the oral environment is also a host factor affecting caries susceptibility.

In our previous research, we used mice in an experiment in which we induced caries with oral inoculation of *Streptcoccus mutans*, a bacterium that is cariogenic in humans [1−4]. We discovered a C57BL/6CrSlc strain (B6) with highly susceptible to caries and a C3H/HeSlc strain (C3H) with highly resistant to caries, and reported that genetic factors play a role in caries susceptibility [1−4]. When we conducted caries-inducing experiments on these mice and analysed their saliva secretion, we found the caries score to be significantly lower and pilocarpine-stimulated saliva flow rate higher in C3H mice than in B6 mice, but we did not assess temporal changes in saliva flow rate [5]. In a study on gene expression in the submandibular glands of these two strains, Kokubun *et al.* suggested that *Capn3* and *Tmem87a* may affect water secretion and transport in the salivary glands [6].

In the present study, we focussed on salivary secretion and enamel hardness, which appear to influence caries susceptibility. We compared stimulated saliva secretion over time, histology of the submandibular gland and muscarinic acetylcholine receptor subtypes 1−3 (M1R, M2R and M3R) gene expression in the B6 and C3H mouse strains. We also used a dynamic ultra-micro-hardness tester (DUH-211; Shimadzu, Kyoto, Japan) to measure the enamel hardness, which have been reported to be involved in caries development.

2. Materials and Methods

2.1. Mice

Two strains of mice (B6, C3H) were used in this study. Twenty-day-old B6 and C3H mice were

purchased from Sankyo Lab Service Co. (Tokyo, Japan). All mice were kept in clean racks at room temperature (25±1°C), with a relative humidity of 55±5% and a 12-h light/dark cycle. Mice were fed *ad libitum*on a commercial diet (MR Breeder; Nihon Nohsan Co, Kagawa, Japan), and were provided with pure bottled water. The animal-use protocols of this study were reviewed and approved by the Nihon University Institutional Review Board (AP12MD010).

2.2. Comparison of stimulated saliva secretion volume over time

At age 49 days, six male B6 mice and five male C3H mice were intraperitoneally administered pentobarbital diluted 1:10 with normal saline (1.0ml/100g body weight). After 5 min, they were intraperitoneally administered isoproterenol (0.20μg/100g body weight) and pilocarpine (0.05 μg/100 g body weight). For each 10-min period following administration (0−10 min, 10−20 min and 20−30 min), saliva was collected from the oral cavity of the mice with a pipette. The collected saliva was measured by weight.

2.3. Histological comparison of submandibular gland

At age 49 days, three male B6 mice and three male C3H mice were anesthetised with pentobarbital diluted 1:10 with normal saline (0.1 ml/10 g body weight) and fixed by perfusion with heparin solution in 1X PBS (300U/ml) and 4% paraformaldehyde. The left submandibular glands were extracted and paraffin-embedded blocks were prepared. Blocks were sliced into 4-μm sections with a microtome and stained with hematoxylin-eosin (HE). Slices were examined and photographed with an all-in-one fluorescence microscope (BIOREVO BZ-9000 Generation II®; KEYENCE, Osaka, Japan). Ducts were traced on the histological images. A scanner was used to re-digitise the tracings with an image analysis resolution of 300 dpi, and Image J image analysis software was used to take measurements. For measurement, the area of a given region on the image was measured as (a), and

the area of the ducts in (a) was measured as (b). The ratio of ducts cells in the given region was calculated as (b/a). The ratio of acinar cells in the given region was calculated as ((a−b)/a) (Figure 1). In one mouse of each strain, the area was measured at 10 sites on histological images and the average was calculated..

2.4. Comparison of muscarinic acetylcholine receptor gene expression in submandibular gland

At age 49 days, the submandibular glands were extracted from three male B6 mice and three male C3H mice using the same methods as for histological comparisons. The extracted submandibular glands were immersed in RNA*later*® solution and stored at 4°C, after which RNA was extracted. Expression of M1R, M2R and M3R was analysed by real-time PCR. The primers used are shown in Table 1. Primers were synthesised as previously reported [14]. Amplification was induced for 30 cycles with TaKaRa Thermal Cycler Dice mini® (TaKaRa, Shiga Japan) at 94°, 56 and 72°C. Relative quantitation was performed based on the quantitation results obtained.

2.5. Comparison of enamel hardness

At age 49 days, the mandibles were extracted from three male B6 mice and three male C3H mice and were immersed in 2% KOH. They were incubated for 24 h at 42°C and the soft tissue was then removed. Mandibles were cut and the left mandible was fixed to slide glass with composite resin (Figure 2). The enamel hardness of the lingual side of the first mandibular molar on the left side was measured with a dynamic ultra-micro-hardness tester (DUH-211; Shimadzu, Kyoto, Japan). This instrument uniquely measures dynamic indentation depth using Berkovich indenters [7]. In one mouse of each strain, enamel hardness was measured at 5 sites on the left first molar in each mice were measured, and the average was calculated.

2.6. Statistical analysis

Statistical differences were determined by Student's -t test or Whelch's -t test. *P* values bellow 0.05 are regarded as statistically significant and indicated by asterisk.

3. Results

3.1. Comparison of simulated saliva secretion volume over time (Figure 3)

The saliva secretion volume was 0.14 (SD ±0.09) g in B6 mice and 0.2 (SD ±0.06) g in C3H mice at 0−10 min after pilocarpine administration and 0.08 (SD ±0.04) g in B6 mice and 0.14 (SD ±0.07) g in C3H mice at 10−20 min after administration. Although the secretion volume was higher in B6 mice, the difference was not significant. The saliva secretion volume differed significantly between the strains at 20−30 min after administration, at 0.05 (SD ±0.03) g in B6 mice and 0.1 (SD ±0.04) g in C3H mice. The total saliva secretion volume also differed significantly between the strains, at 0.27 (SD ±0.14) g in B6 mice and 0.46 (SD ±0.18) g in C3H mice.

3.2. Histological comparison of the submandibular gland

Ratios of ducts area and acinar area in given regions in the histological images of submandibular glands from B6 and C3H mice are shown in Figures 4. The ratio of ducts area was 41.6 (SD ±5.0) % in B6 mice and 35.6 (SD ±8.0) % in C3H mice. The ratio of acinar area was 58.4 (SD ±5.0) % in B6 mice and 64.4 (SD ±8.0) % in C3H mice. The means differed significantly between the two strains.

3.3. Comparison of muscarinic acetylcholine receptor gene expression in submandibular gland (Table 2)

The gene expression of M1R was 9.84 (SD ±0.51) in B6 mice and 9.83 (SD ±0.26) in C3H mice, that of M2R was 3.18 (SD ±3.90) in B6 mice and 4.51 (SD ±3.96) in C3H mice, and that of M3R

was 8.65 (SD ±0.30) in B6 mice and 8.58 (SD ±0.60) in C3H mice. No differences were observed between the B6 and C3H strains with regard to the gene expression of M1R, M2R or M3R.

3.4. Comparison of enamel hardness

Enamel hardness measurements are shown in Figure 5. Enamel hardness was 55.1 (SD ±22.4) GPa and 77.6 (SD ±12.2) GPa in the left first molars of B6 and C3H mice, respectively, and this difference was significant.

4. Discussion

Orino*et al.* [5] collected pilocarpine-stimulated saliva, and found that the flow rate was higher in C3H mice than in B6 mice. They also found the caries score to be higher in the B6 mice. They concluded that salivary flow rate may affect caries susceptibility [5]. In the present study, we examined saliva collected at 10-min intervals for 30 min and found that C3H mice have higher saliva secretion volume than B6 mice at 0−10 minutes and at 10−20 min after administration of pilocarpine, although the differences between strains was not significant. The difference was significant, however, at 20−30 min after administration, with C3H mice showing a higher saliva secretion volume than B6 mice. Moreover, the total saliva secretion volume was significantly higher in C3H than B6 mice. These results concur with those of previous studies [5], and the transition in salivary flow rate over time for 30 min after stimulation was the same.

Saliva has numerous functions, such as cleaning, antibacterial action and pellicle formation. Saliva also acts as a buffer. It adjusts the pH of plaque and performs useful actions to enhance buffering capacity, making it an important factor for determining caries susceptibility. The pH of plaque reflects the balance between acid and base production, and a higher pH translates to lower caries susceptibility [8, 9]. As shown in the Stephan Curve, plaque pH drops below 5.0 about 2−3 minutes

after rinsing with sucrose and returns to normal levels after 30−60 min [10, 11]. When there was no salivary access, plaque pH levels were similar in the caries-resistant and caries-susceptible groups. As access to saliva increased, the observed pH minima increased to a greater degree in the caries-resistant group than in the caries-susceptible group [12].

A comparison of patients with reduced saliva secretion and patients with normal saliva secretion showed a difference in pH recovery in the Stephan Curve, indicating that low saliva secretion results in a pronounced drop in plaque pH levels [13]. In the present study, total saliva secretion volume during the 30-min period after pilocarpine stimulation was 0.27(SD ±0.14) g for B6 mice and 0.46(SD ±0.18) g for C3H mice, showing a significant difference between the two mouse strains. Secretion volume was higher in C3H mice, and remained higher than in B6 mice even 30 min after stimulation with pilocarpine. This suggests that cleansing of the oral cavity and early functioning of the buffering capacity promotes recovery of plaque pH and reduces decalcification time.

Salivary flow rate was proposed as contributing to the buffering capacity of saliva. Salivary buffering capacity is another important factor that may be involved in dental caries. This property depends mainly on the carbonate secretion rate [8]. Therefore, low salivary flow rates may be associated with both low carbonate secretion rate and low salivary buffering capacity [14]. As C3H mice have a higher salivary flow rate, they may have stronger self-cleansing and buffering capacities. This may inhibit tooth decalcification and lead to differences in caries susceptibility.

Saliva is secreted from the major and minor salivary glands, and the submandibular gland within the major salivarygland is the highest saliva producer. In the two strains of mice with differing susceptibility, it is plausible that the submandibular glands differ histologically. We therefore calculated the ratio of duct cells and acinar cells per unit area to histologically compare the submandibular glands in the two strains. Comparing the B6 and C3H mice revealed a significant difference in the ratio of duct cells, with 41.6 (SD ±5.0) % for B6 mice and 35.6 (SD ±8.0) % for

C3H mice. Submandibular glands with reduced saliva secretion have a higher ratio of duct cells and a low ratio of acinar cells [15, 16]. In the present study, C3H mice that exhibited a low ratio of duct cells had a higher ratio of acinar cells, at 58.4 (SD ±8.0) %, than B6 mice, with an acinar cell ratio of 64.4 (SD ±5.0) %. C3H mice have a higher salivary flow rate than B6 mice and therefore a greater ratio of acinar cells, which may lead to a greater volume of macromolecular substances, water and electrolytes secreted from acinar cells.

Although various cascades have been reported for saliva secretion, the main cascade involves control of the salivary glands by the parasympathetic and sympathetic nerves to regulate salivary flow rate. Parasympathetic stimulation elicits the release of acetylcholine from the nerve endings to the surface of the acinar cells, which regulates water secretion. By contrast, sympathetic stimulation causes noradrenaline to be released from the nerve endings that regulates secretion of macromolecular substances [17, 18].

Kokubun *et al.* [6] examined glycoprotein levels in the saliva of B6 and C3H mice, and did not find any significant differences between the strains, suggesting that there is a weak relationship between glycoproteins in saliva and caries. Furthermore, they also reported a difference in the expression of *Capn3* and *Tmem87a*, which affect water secretion and transport in the submandibular gland [6]. For the present study, we decided to focus on expression of muscarinic acetylcholine receptor, which may be an important factor affecting water secretion regulation.

The muscarinic acetylcholine receptor is a G protein-coupled receptor with four extracellular domains and seven transmembrane domains. It has five subtypes and is expressed, for example, in the central nervous system, exocrine gland, smooth muscle and cardiac muscle. M2R is expressed in smooth muscle. Myoepithelial cells are found between the acinar epithelium and basement membrane that cover the lumen of the acinus and ducts in the exocrine gland. Myoepithelial cells are packed with alpha-smooth muscle actin, and their contraction stimulates saliva secretion [21, 22].

High M2R expression levels in the submandibular gland indicate the presence of a large number of myoepithelial cells in the submandibular gland. A high level of gene expression of M2R was observed for B6 mice. Myoepithelial cells are present in salivary acinar cells as well as in salivary ducts. Since the ratio of salivary ducts to acinar cells was higher in B6 mice than that in C3H mice, B6 mice showed a high expression of M2R in this study. The increased expression of M2R in B6 mice was not related to the volume of salivary secretion. M3R is expressed in salivary glands. Acetylcholine is an endogenous agonist that couples with M3R on the acinar cells to activate M3R. M3R activation elicits an increase in intracellular Ca concentration via G proteins, phospholipase C, inositol 1,4,5-trisphosphate (IP_3) and IP_3 receptor. As intracellular Ca levels rise, the luminal chloride channel is activated and saliva is secreted [19]. Tsuboi *et al.* [20] suggested that M3R antibodies may reduce the rise in intracellular Ca concentration to effectively reduce saliva secretion. High M3R expression levels may therefore play a role in increasing saliva flow rate. In the present study, there was no difference in gene expression level of M3R between two strains that have gap in amount of saliva. We suggest that the expression of M3R did not affect the difference in caries susceptibility. The relationship between the expression level of the muscarinic acetylcholine receptors and the differences in caries susceptibility was not remarkable.

Essig *et al*. [23] reported that changes in both plaque pH and enamel microhardness were in agreement as indicators of substrate cariogenicity. This suggests that either of these parameters would be appropriate for the evaluation of the cariogenicity of substrates. We therefore investigated enamel hardness in the two strains of mice. Hardness was significantly higher in C3H mice than in B6 mice, demonstrating that enamel hardness influences caries susceptibility.

5. Conclusion

We examined four factors of caries susceptibility between B6 and C3H mice. These results

suggest that acinar cells ratio in the submandibular gland may be associated with differences in salivary flow rate. And the enamel hardness and the saliva secretion volume may be associated with tooth decalcification. Thus, suggesting that these three factors affect with caries susceptibility.

Disclosure

None of the authors have any conflicts of interest that should be disclosed.

Acknowledgments

The authors are grateful to Prof. Takahide Maeda for his helpful advice in this study. We would like to thank Dr. Miwako Fukushima and Dr. Yasuhiro Tanimoto for the invaluable assistance. We would like to thank also to various teachers of Department of Pediatric Dentistry, Nihon University School of Dentistry at Matsudo.

References

[1] Obayashi K. Caries susceptibility of Balb/c mice to caries induction by *Streptococcus mutans* serotype *c*, *d*, *g* strains. Jpn J Ped Dent1984;22:87-95 [in Japanese].

[2] Suzuki T, Caries Susceptibility of Mice of Different Strains Infected with *Streptococcus mutans*. Jpn J Ped Dent 1985; 23: 198-203[in Japanese].

[3] Kurihara Y, Naito T, Obayashi K, Moriwaki K. Caries susceptibility in inbred mouse strains and inheritance patterns in F_1 and backcross(N_2) progeny from strains with high and low caries susceptibility. Caries Res 1991;25:341-346.

[4] Maeda T, Ogiwara K, Kurihara Y. Study on the inheritance patterns of caries susceptibility in Inbred Mice. Jpn J Ped Dent 1992;30:618-623 [in Japanese].

[5] Orino D, Shimizu K. Reduced dental caries susceptibility in chromosome 2-substituted consomic mice. Int J Oral-Med Sci 2011;9:234-240.

[6]Kokubun T, Simizu K. ComprehensiveAnalysis of Gene Expression Associated with Caries Susceptibility in Chromosome 2-substituted Mice.Int J Oral-Med 2013;Sci 11;242-248.

[7]HeLH, Fujisawa N, Swain MV. Elastic modulus and stress-strain response of human enamel by nano-indentation. Biomaterials 2006;27:4388-4398.

[8] Edgar M, Dawes C, O'Mullane D. (Translation Supervisor: Watanabe S) Saliva and Oral Health-3rd edition. Ishiyaku Publishers Inc; 2008. p. 27-40, p.72-86. [in Japanese]

[9]VanWuyckhuyse BC, Perinpanayagam HER, BevacquaD, RaubertasRE, BillingsRJ, BowenWH, TabakLA. Association of Free Arginine and Lysine Concentrations in Human Parotid Saliva with Caries. J Dent Res 1995;74:686-690.

[10] Stephan RM. Changes in Hydrogen-Ion concentration on tooth surfaces and in carious lesions. JADA1940;27:718-723.

[11] Stephan RM, Miller BF. A Quantitative Method for Evaluating Physical and Chemical Agents

which Modify Production of Acids in Bacterial Plaques on Human Teeth. J Dent Res 1943;22:45-51.

[12] AbelsonDC, MandelID. The Effect of saliva on plaque pH in vivo. J Dent Res 1981;60:1634-1638.

[13] Lingstoröm P, Birkhed D. Plaque pH and oral retention after consumption of starchy snack products at normal and low salivary secretion rate. ActaOdontolScand 1993;51:379-388.

[14] SánchezGA, Fernandez de Preliasco MV. Salivary pH changes during soft drinks consumption in children. Int J Ped Dent 2003;13: 251-257.

[15] Matsui-Inohara H, Uematsu H, Narita T, Sato K, Yonezawa H. Kuroda K, Ito T, Yoneda S, Kawarai T, Sugiya H, Watanabe H, Senpuku H. E2F-1deficient NOD/SCID mice developed showing decreased saliva production. ExpBiol Med (Maywood) 2009;234:1525-1536.

[16] Satoh K, Narita T,Matsuki-Fukushima M,Okabayashi K, Ito T,Senpuku H,Sugiya H. E2f1-deficient NOD/SCID mice have dry mouth due to a change of acinar/duct structure and the down-regulation of AQP5 in the salivary gland. Pflugers Arch-Eur J Physiol 2013;465:271-281.

[17] Baum BJ. Neurotransmitter control of secretion. J. Dent. Res 1987;66:628-632.

[18] Sugita M. A study on Ca^{2+}-dependent fluid secretion from rat salivary glands monitored by a fluorescent probe, calcein. J Hiroshima Univ Dent Soc1996;28:193-209 [in Japanese].

[19] Dawson L, Tobin A, Smith P, Gordon T. Antimuscarinic antibodies in Sjogren's syndrome. Arthritis Rheum 2005;52:2984-2995.

[20]Tsuboi H, Iizuka M, Asashima H, Sumida T. Anti-M3 muscarinic acetylcholine receptor antibodies and Sjögren's syndrome. Jpn J ClinImmunol 2013;36:77-85 [in Japanese]

[21] Nelson DA, Manhardt C, Kamath V, Sui Y,Santamaria-Pang A, Can A, Bello M, Corwin A, DinnSR, Lazare M, Gervais EM,Sequeira SJ, Peters SB,Ginty F, Gerdes MJ, Larsen M. Quantitative single cell analysis of cell population dynamics during submandibular slivary gland development and differentitation.Biol Open 2013;18:439-447.

[22] Ogawa Y. Immunocytochemistry of myoepithelial cells in the salivary glands. ProgrHistochemCytochem 2003;38: 343-426.

[23]EssigME, BoddenWR, BradleyJr EL, KoulouridesT, HouschT. Enamel Microhardness Change and Plaque pH Measurements in an Intra-oral Model in Humans.J Dent Res 1985;64:1065-1068.

Table 1 The primers used in real-time PCR

Gene	Sequence
M1R	sense: AGAAGAGGCTGCCACAGGTA
	antisense: CAGACCCCACCTGGACTTTA
M2R	sense: GAATGGGGATGAAAAGCAGA
	antisense: GCAGGGTGCACAGAAGGTAT
M3R	sense: CACAGCCAAGACCTCTGACA
	antisense : ATGATGTTGTAGGGGGTCCA
GAPDH	sense: TCCACCACCCTGTTGCTGTA
	antisense: ACCACAGTCCATGCCATCAC

GAPDH:glyceraldehyde-3-phosphate dehydrogenase

Table2 Gene expression level of muscarinic acetylcholine receptors in the submandibular gland

B6			
	mAch R Average Ct	GAPDH Average Ct	⊿Ct mAch R－ GAPDH
M1R	29.22±0.92	19.38±1.32	9.84±0.51
M2R	29.12±0.77	25.94±3.52	3.18±3.90
M3R	26.58±0.89	17.93±0.61	8.65±0.30
C3H			
	mAch R Average Ct	GAPDH Average Ct	⊿Ct mAch R－ GAPDH
M1R	28.12±0.47	18.29±0.53	9.83±0.26
M2R	27.60±3.92	23.08±4.95	4.51±3.96
M3R	25.93±0.43	17.35±0.63	8.58±0.60

mAch R: Muscarinic acetylcholine receptor

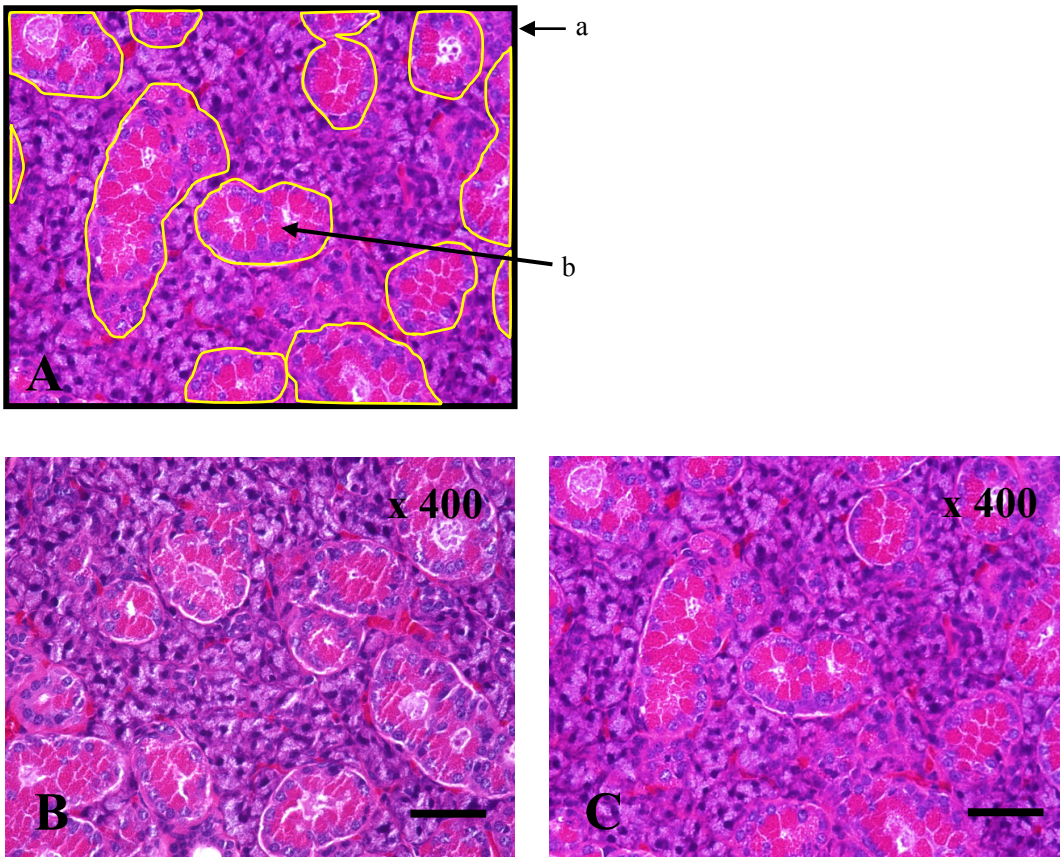


Fig.1 Histological analysis of submandibular gland

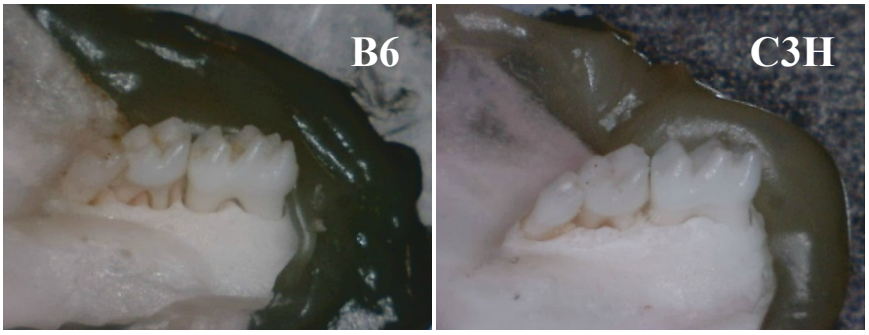


Fig.2 Comparison of enamel hardness

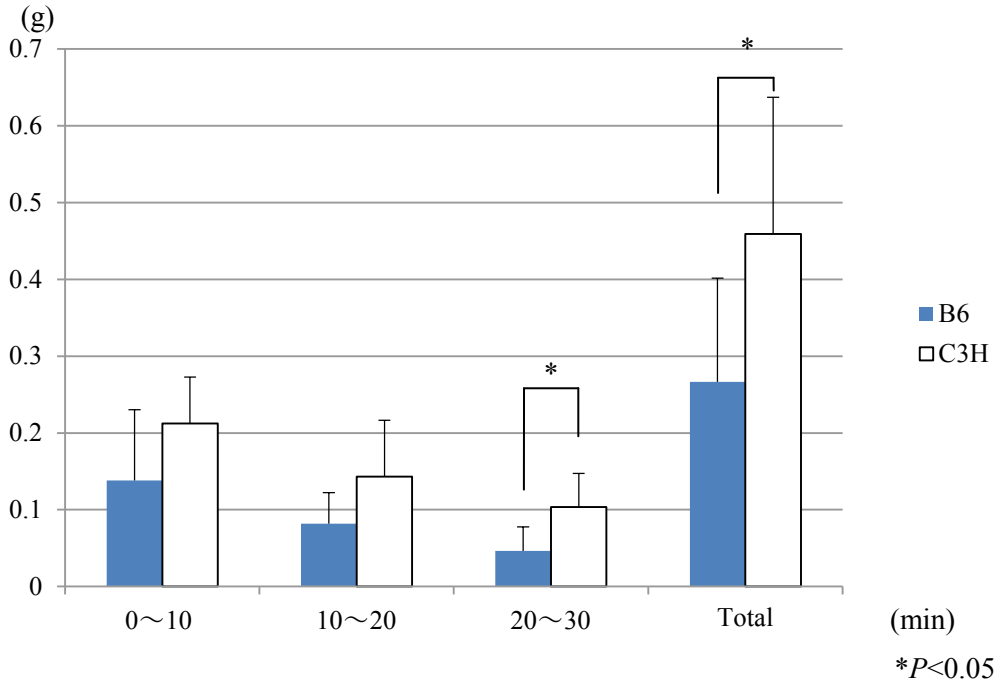


Fig.3 Comparison of stimulated saliva secretion volume over time

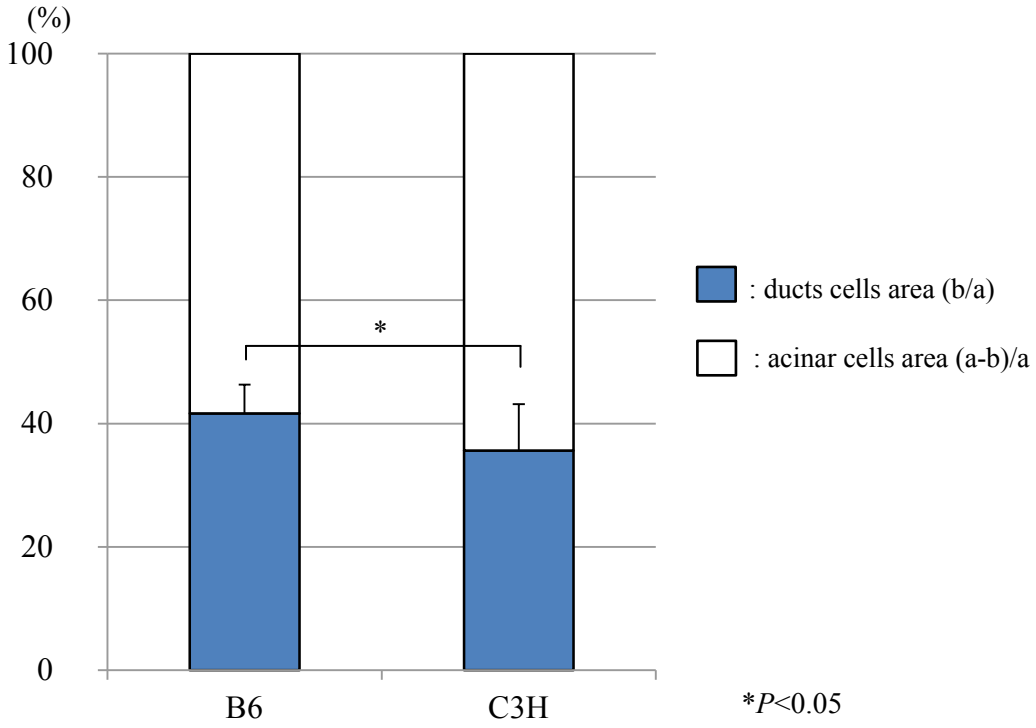
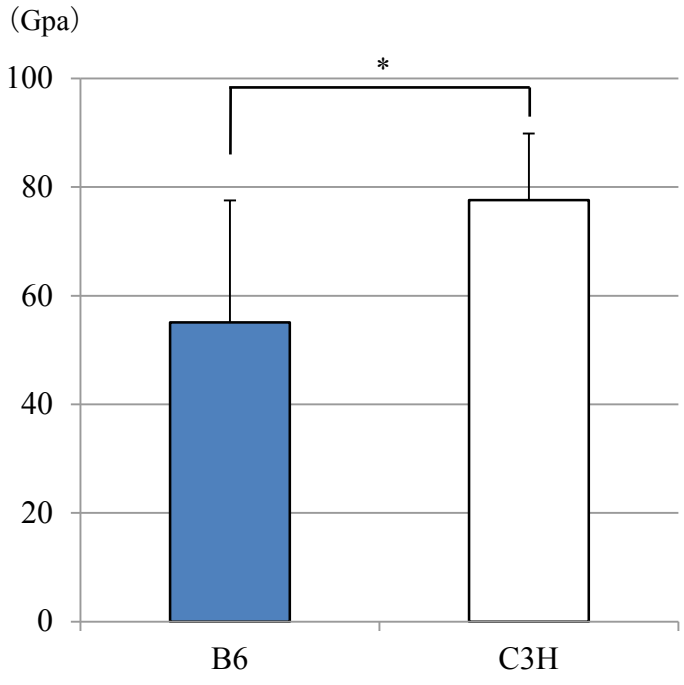


Fig.4 Histological comparison of ducts cells area and acinar cells in submandibular glands



*P<0.05

Fig.5Comparison of enamel hardness

Figure legends

Fig.1 Histological analysis of submandibular gland

A: Submandibular gland histology (HE: x 400). Trace on the organization's image.

a: The area of a given region on the image.

b: The area of ducts in (a).

Scale bar was indicated 25μm

B: Submandibular gland histology of B6 strain.

C: Submandibular gland histology of C3H strain.

Fig.2 Measurement point of enamel hardness

Photograph of mouse teeth prepared.（Magnification x 50）

Fig.3 Comparison of stimulated saliva secretion volume over time

The saliva secretion volume was 0.14 (SD ±0.09) g in B6 mice and 0.2 (SD ±0.06) g in C3H mice at 0-10 min after pilocarpine administration and 0.08 (SD ±0.04) g in B6 mice and 0.14 (SD ±0.07) g in C3H mice at 10-20 min after administration. The saliva secretion volume was 0.05 (SD ±0.03) g in B6 mice and 0.1 (SD ±0.04) g in C3H mice at 20-30 min after administration. The total saliva secretion volume was 0.27 (SD ±0.14) g in B6 mice and 0.46 (SD ±0.18) g in C3H mice.

Fig.4 Histological comparison of ducts cells area and acinar cells in submandibular glands

The blue bar indicates the ratio of the area of ducts cells. The white bar indicates the ratio of the area of ducts cells. The ratio of ducts cells was 41.6 (SD ±5.0) % in B6 mice and 35.6 (SD ±8.0) % in C3H mice. The ratio of acinar cells was 58.4 (SD ±5.0) % in B6 mice and 64.4 (SD ±8.0) % in C3H

mice.

Fig.5 Comparison of enamel hardness

Enamel hardness were measured in B6 and C3H mice of left first molar by Berkvich indenters. Enamel hardness was 55.1 (SD ±22.4) GPa in B6 mice and 77.6 (SD ±12.2) GPa in C3H mice and this difference was significant.