

Alloxan-induced hyperglycemia causes rapid-onset and progressive dental caries and periodontitis in F344 rats

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Summary. We have previously shown that diabetes increases dental caries, and periodontitis might be a secondary change resulting from dental caries in spontaneous diabetic rodent models. However, the lesions in these models were slow to manifest, and the intensity and frequency were mild and varied among individuals. The goal of this study was to confirm the reproducibility of caries development in chemically induced diabetic rats and investigate whether alloxan, which induces immediate and severe hyperglycemia in experimental animals, increases the lesions. Female F344 rats were examined 13 and 26 weeks after dosing of alloxan. Alloxan injection induced severe hyperglycemia in two-thirds of the rats. Progressive molar caries and periodontitis were already induced in all diabetic rats 13 weeks after dosing of alloxan, although the lesions were not observed in nondiabetic rats. Histopathologically, dental caries initially developed in the crown, then spread into the dental root, entered the periodontal connective tissue via the apical foramen, and progressed to periodontitis. In conclusion, alloxan-induced severe hyperglycemia is capable of causing rapid-onset and progressive dental caries and periodontitis in rats.

Key words: Diabetes, Dental caries, Periodontitis, F344 rats, Alloxan

Introduction

Epidemiological studies have suggested that diabetes and poor glycemic control may be important risk factors for periodontal disease (Loe, 1993; Ryan et al., 2003; Mealey and Oates, 2006). Many experimental studies

using spontaneous or chemical-induced diabetic animal models have indicated that diabetes increases the risk and severity of periodontitis (Liu et al., 2006; Pontes Andersen et al., 2006; Claudino et al., 2007; Tesseromatis et al., 2009). However, the relationship between caries and periodontitis has never been investigated in these diabetic animal models.

Meanwhile, several recent studies based on clinical observations have suggested that the teeth of diabetic patients are predisposed to dental caries (Twetman et al., 2002; Taylor et al., 2004; Miralles et al., 2006). In contrast, no significant difference was observed in caries susceptibility between diabetic and nondiabetic patients in other reports (Canepari et al., 1994; Collin et al., 1998; Iughetti et al., 1999; Hintao et al., 2007). There have been similar contradictory reports in experimental diabetic animals that did (Hartles and Lawton, 1958; Borghelli et al., 1966) or did not show caries susceptibility (Nichols and Shaw, 1957). Recently, we reported that dental caries were seen in *db/db* mice, an animal model of type 2 diabetes, and that gingivitis and periodontitis occurred as a result of severe periapical inflammation. These results suggest that the periodontal lesions may result from apical periodontitis secondary to dental caries (Sano et al., 2011). However, the onset of the lesions requires more than 20 weeks in *db/db* mice, and the intensity and frequency are mild and vary among individuals.

Alloxan is a chemical that induces loss of insulin-producing islet β -cells and causes a hypoinsulinemic condition and resultant diabetes mellitus in animals (Malaisse et al., 1982; Lenzen and Panten, 1988). Severe hyperglycemia develops immediately after dosing and lasts for more than 1 year without insulin treatment (Nichols and Shaw, 1957; Watanabe et al., 2004; Sano et al., 2009). Therefore, the alloxan-induced model is suitable for investigating the effects of persistent severe hyperglycemia.

The objective of this study was to confirm the

reproducibility of both 2 oral lesions, dental caries and periodontitis by using a chemically induced diabetic rodent model (type 1) and to investigate whether the lesions in alloxan-induced hyperglycemia are more severe than those in spontaneous diabetic animals.

Materials and methods

Animals and housing conditions

The animals were handled according to the principles for all experimental procedures outlined in the Guide for the Care and Use of Laboratory Animals prepared by our institution (Setsunan University) and the Japanese Association for Laboratory Animal Science. Six-week-old female F344 rats were supplied by Japan SLC, Inc. (Hamamatsu, Japan). The animals were housed in stainless-steel cages at a temperature of 20-26°C and a relative humidity of 40-70% under a 12/12 h light/dark cycle, and ventilated with filtrated fresh air. They were allowed free access to tap water and a widely used standard pelletized diet for experimental mice and rats (Charles River Formula 1; Oriental Yeast Co. Ltd., Tokyo, Japan).

Experimental design

The animals were randomly divided into 2 groups; intact group (17 untreated animals) and alloxan-treated group (30 animals). Seven-week-old rats were given a single dose (35 mg/kg body weight) of alloxan (Sigma-Aldrich Japan, Tokyo, Japan) via the tail vein. The dose of alloxan was decided as the given dose at which a rat survives for a long period after diabetes symptoms develop and demonstrates glucosuria continuously. Ten rats in the intact group and 16 rats (9 nondiabetic and 7 diabetic animals) in the alloxan-treated group were sacrificed at the 13th week after injection of alloxan. Furthermore, the remaining 7 rats in the intact group and 10 diabetic rats in the alloxan-treated group were sacrificed at the 26th week after injection of alloxan for comparison of progression of caries and periodontal lesions.

Moribund animals (a total of 4 rats) in the alloxan-treated group were sacrificed and necropsied within 1-8 weeks after alloxan treatment. The cause of the moribund condition or death was attributed to ketoacidosis and urinary tract infection resulting from a severe diabetic condition.

Glucosuria and glycemia monitoring

Glucose levels in fresh urine were measured semiquantitatively with urine test paper (Wako Pure Chemical Industries, Osaka, Japan) daily from day 1 to day 3 after dosing, once every week for 1 month after the first week, and once every month thereafter. Blood glucose levels in tail vein samples were also measured semiquantitatively by the glucose oxidase method

(Glutest E; Sanwakagaku, Aichi, Japan) once every month from the fourth week after alloxan injection. Samples of blood from the tail vein and fresh urine were collected from 1:00 to 4:00 p.m. Hyperglycemia and glucosuria were defined as greater than 300 and 500 mg/dL, respectively (Nichols and Shaw, 1957).

Grading for caries and alveolar bone resorption by soft X-ray examination

The animals were euthanized by exsanguination under deep anesthesia at the end of the observation period. Subsequently, the mandible and maxilla were removed and fixed in 10% neutral-buffered formalin (pH 7.4). After a 24 h fixation, the occlusal, buccolingual, and proximal surfaces of all teeth were intensively observed under a binocular stereoscope. Following macroscopic examination, a soft X-ray examination was performed. Soft X-ray images of the mesiodistal plane were taken under conditions of 35 kV and 2 mA for 4 min. Teeth were classified into 5 groups according to caries characteristics by observation and measurement of the radiographs: no radiolucent change (grade 0), radiolucent area only on the occlusal surface of the crown (grade 1), radiolucent areas on occlusal surface and either of the mesiodistal surfaces of the crown (grade 2), radiolucent areas over the entire surface of the crown (grade 3), and radiolucent areas over most of the surface of the dental root (grade 4). Alveolar bone resorption of each tooth was evaluated by measurement of the radiolucent area around each molar root on the soft X-ray image. These were divided into 5 grades as follows: no radiolucent change (grade 0), 0.01-0.20 mm² (grade 1), 0.21-0.40 mm² (grade 2), 0.41-0.60 mm² (grade 3), and more than 0.61 mm² (grade 4).

Histopathological examination

After soft X-ray examination, a histopathological examination was performed on the mandible and maxilla in all rats. After fixation with 10% neutral-buffered formalin, the sample was decalcified in a 5% solution of ethylenediaminetetraacetic acid 4 Na (EDTA 4 Na) for 2 weeks at 4°C. After decalcification, the specimens were trimmed, then dehydrated in a sequential ethanol series by using an automated processor and embedded in paraffin wax. Serial 7- μ m-thick sections on the mesiodistal plane were made through the centers of all molars and then stained with hematoxylin and eosin for examination by light microscopy. The severity of a caries lesion was graded as follows: -, none; +, dentin caries localized in occlusal surface of dentin; ++, dentin caries extended into dental pulp; +++, decay of corona dentis. Gingivitis was scored on the basis of inflammatory cell infiltration and mucosal hyperplasia: -, no inflammatory cells and mucosal hyperplasia; +, scant inflammatory cells infiltrated into the mucosal epithelium with slight to mild mucosal hyperplasia; ++, inflammatory cells scattered throughout the gingival

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tissue with mild to moderate mucosal hyperplasia. Marginal periodontitis was evaluated according to inflammatory cell infiltration and alveolar bone resorption: –, no inflammatory cell infiltration and bone resorption; +, localized inflammatory cell infiltration in the cervical area and/or resorption of the alveolar bone crest; ++, inflammatory cell infiltration and alveolar bone resorption extended to the radicular area. Apical periodontitis was also evaluated on the basis of inflammatory cell infiltration and alveolar bone resorption: –, no inflammatory cells and bone resorption; +, localized inflammatory cell infiltration and/or alveolar bone resorption in the apical part of the molar root; ++, extensive inflammatory cell infiltration and alveolar bone resorption in the tissue around the molar root.

Statistical analysis

The Wilcoxon's rank-sum test was employed to compare the differences in the mean scores of caries lesions and alveolar bone resorption by soft X-ray examination between the groups. The chi-square test was used to determine the incidence of histopathological lesions by histological examination in each group of rats. A *p* value of less than 0.05 was regarded as statistically significant. Pearson's correlations were used to examine the associations between molar caries and alveolar bone resorption.

Results

Blood and urine glucose levels

Severe hyperglycemia (>400 mg/dL) and glucosuria (>500 mg/dL) persisted from the day after injection of alloxan to the last monitoring day in 17 rats of the alloxan-treated group. The remaining 9 rats in the alloxan-treated group showed transiently high values after alloxan treatment but an almost normal range of values (blood glucose <130 mg/dL, urine glucose <100 mg/dL) during the experimental period (Table 1). Meanwhile, blood glucose levels ranged from 67 to 125 mg/dL and urine glucose levels were less than 100 mg/dL in the intact rats.

Macroscopic feature of caries and caries scores by soft X-ray examination

The typical macroscopic features of carious molars in alloxan-treated diabetic rats are shown in Figure 1. Macroscopically, the dental caries developed mainly in occlusal fissures and was initially identified as a brown hole or partial coronal defect of the molar (Fig. 1B,C). The lesion expanded horizontally, until finally the crown of the carious molar was completely invisible (Fig. 1C). In contrast, intact and alloxan-treated nondiabetic rats showed no change in the molars (Fig. 1A).

Table 1. Blood and urine glucose levels in alloxan-treated F344 rats.

Animals	Period	Blood glucose (mg/dL) and urine glucose			
		Week 1	Week 4	Week 13	Week 26
Alloxan 1	13 weeks after dosing	102 (–)	91 (–)	95 (–)	//
Alloxan 2		118 (–)	106 (–)	114 (–)	//
Alloxan 3		106 (–)	103 (–)	121 (–)	//
Alloxan 4		81 (–)	93 (–)	106 (–)	//
Alloxan 5		388 (++++)	108 (–)	111 (–)	//
Alloxan 6		90 (–)	97 (–)	112 (–)	//
Alloxan 7		94 (–)	111 (–)	114 (–)	//
Alloxan 8		89 (–)	102 (–)	108 (–)	//
Alloxan 9		299 (+++)	104 (–)	94 (–)	//
Alloxan 10		420 (+++)	494 (++++)	409 (++++)	//
Alloxan 11		484 (++++)	518 (++++)	448 (++++)	//
Alloxan 12		575 (++++)	406 (++++)	380 (++++)	//
Alloxan 13		488 (++++)	600 (++++)	460 (++++)	//
Alloxan 14		509 (++++)	>600 (++++)	>600 (++++)	//
Alloxan 15		514 (++++)	>600 (++++)	>600 (++++)	//
Alloxan 16		403 (++++)	436 (++++)	404 (++++)	//
Alloxan 17	26 weeks after dosing	>600 (++++)	>600 (+++)	>600 (++++)	482 (++++)
Alloxan 18		454 (++++)	344 (+++)	>600 (++++)	446 (++++)
Alloxan 19		559 (++++)	>600 (++++)	505 (++++)	462 (++++)
Alloxan 20		600 (++++)	>600 (++++)	>600 (++++)	498 (++++)
Alloxan 21		>600 (++++)	>600 (++++)	>600 (++++)	556 (++++)
Alloxan 22		>600 (++++)	>600 (++++)	373 (++++)	>600 (++++)
Alloxan 23		>600 (++++)	470 (++++)	549 (++++)	502 (++++)
Alloxan 24		505 (++++)	>600 (++++)	>600 (++++)	494 (++++)
Alloxan 25		579 (++++)	488 (++++)	498 (++++)	354 (++++)
Alloxan 26		>600 (++++)	455 (++++)	482 (++++)	339 (++++)

Grade sign: (–), <100 mg/dL; (+), >100 mg/dL; (++) , >250 mg/dL; (+++), >500 mg/dL; (++++), >2000 mg/dL. Abbreviation: //, not examined.

The ratio of teeth with caries in the mandibular and maxillary molars based on each scoring is shown in Table 2. In all diabetic rats, dental caries were detected as radiolucent lesions in the maxillary and mandibular molars after 13 and 26 weeks of dosing (Fig. 2B). The lesions gradually progressed with age, and most mandibular molars (91.7%) and more than half of the maxillary molars (66.7%) were affected 26 weeks after dosing (Table 2). The severity worsened with age, and the mean caries score after 26 weeks of dosing (maxilla, 1.05; mandible, 2.13) was significantly higher (maxilla, $p<0.05$; mandible, $p<0.01$) than after 13 weeks of dosing (maxilla, 0.69; mandible, 1.19) (Table 2, Fig. 3). The mean caries score in the mandibular molars was significantly higher ($p<0.05$ and 0.01) than that in the maxillary molars, both after 13 and 26 weeks of dosing. In contrast, no radiolucent lesions were observed in any molars of intact or alloxan-treated nondiabetic rats (Table 2, Fig. 2A).

Change of alveolar bone resorption by soft X-ray examination

The ratio of teeth with alveolar bone resorption in the mandible and maxilla based on each scoring is shown in Table 3. Bone resorption was also remarkably increased in alloxan-induced diabetic rats, and almost half of the mandibular molars (46.7%) and almost a quarter of the maxillary molars (23.3%) were affected at 26 weeks after treatment. The severity tended to progress with age both in the mandible and maxilla, although there was no significant difference between 13 and 26 weeks after alloxan treatment. Bone resorption in the mandibles tended to be more severe than that in the

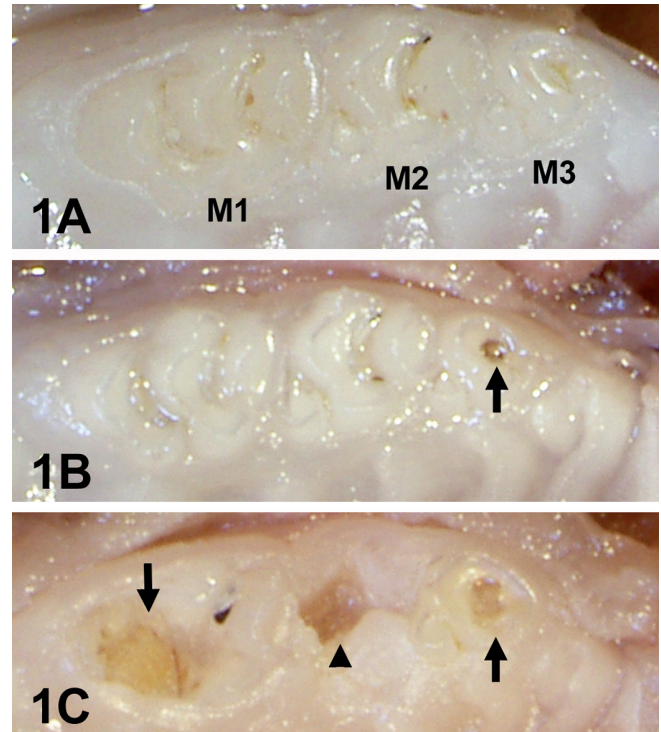


Fig. 1. Macroscopic features of molar caries in the maxillae of female F344 rats. M1: the first molar, M2: the second molar, M3: the third molar. **A.** Normal molars. **B.** Partial coronal defect (arrow) of M3. **C.** Partial coronal defects (arrows) of M1 and M3, and complete defect (arrowhead) of M2 crown.

Table 2. Incidence and grading of the caries in molars in nondiabetic and diabetic F344 rats.

Groups	Intact		Alloxan			
	Nondiabetic (13W) (n=10)	Nondiabetic (26W) (n=7)	Nondiabetic (13W) (n=9)	Diabetic (13W) (n=7)	Diabetic (26W) (n=10)	
No. of animals affected with caries	0	0	0	7 (100%)	10 (100%)	
Maxillary molars	No. of examined	60	54	42	60	
	No. of teeth with caries	0	0	0	17 (40.5%)**	40 (66.7%)** †
	Grade 0	60 (100%)	42 (100%)	54 (100%)	25 (59.5%)	20 (33.3%)
	Grade 1	0	0	0	6 (14.3%)	19 (31.7%)
	Grade 2	0	0	0	10 (23.8%)	19 (31.7%)
	Grade 3	0	0	0	1 (2.4%)	2 (3.3%)
Grade 4	0	0	0	0	0	
Mandibular molars	No. of examined	60	54	42	60	
	No. of teeth with caries	0	0	0	26 (61.9%)**	55 (91.7%)** ††
	Grade 0	60 (100%)	42 (100%)	54 (100%)	16 (38.1%)	5 (8.3%)
	Grade 1	0	0	0	8 (19.0%)	3 (5.0%)
	Grade 2	0	0	0	13 (31.0%)	33 (55.0%)
	Grade 3	0	0	0	4 (9.5%)	17 (28.3%)
Grade 4	0	0	0	1 (2.4%)	2 (3.3%)	

Significant difference from age-matched intact group (**: $p<0.01$). Significant difference from diabetic 13 weeks group (†: $p<0.05$; ††: $p<0.01$).

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Table 3. Incidence and grading of alveolar bone resorption in nondiabetic and diabetic F344 rats.

Groups	Intact		Alloxan			
	Nondiabetic (13W) (n=10)	Nondiabetic (26W) (n=7)	Nondiabetic (13W) (n=9)	Diabetic (13W) (n=7)	Diabetic (26W) (n=10)	
No. of animals affected with caries	0	0	0	7 (100%)	10 (100%)	
Maxillary molars	No. of examined	60	42	54	42	60
	No. of teeth with ABR	0	0	0	5 (11.9%)**	14 (23.3%)**
	Grade 0	60 (100%)	42 (100%)	54 (100%)	37 (88.1%)	46 (76.7%)
	Grade 1	0	0	0	2 (4.8%)	7 (11.7%)
	Grade 2	0	0	0	1 (2.4%)	5 (8.3%)
	Grade 3	0	0	0	2 (4.8%)	2 (3.3%)
Grade 4	0	0	0	0	0	
Mandibular molars	No. of examined	60	42	54	42	60
	No. of teeth with ABR	0	0	0	12 (28.6%)**	28 (46.7%)**
	Grade 0	60 (100%)	42 (100%)	54 (100%)	30 (71.4%)	32 (53.3%)
	Grade 1	0	0	0	4 (9.5%)	14 (23.3%)
	Grade 2	0	0	0	5 (11.9%)	8 (13.3%)
	Grade 3	0	0	0	2 (4.8%)	4 (6.7%)
Grade 4	0	0	0	1 (2.4%)	2 (3.3%)	

Significant difference from age-matched intact group (**: $p < 0.01$). Abbreviation: ABR, alveolar bone resorption.

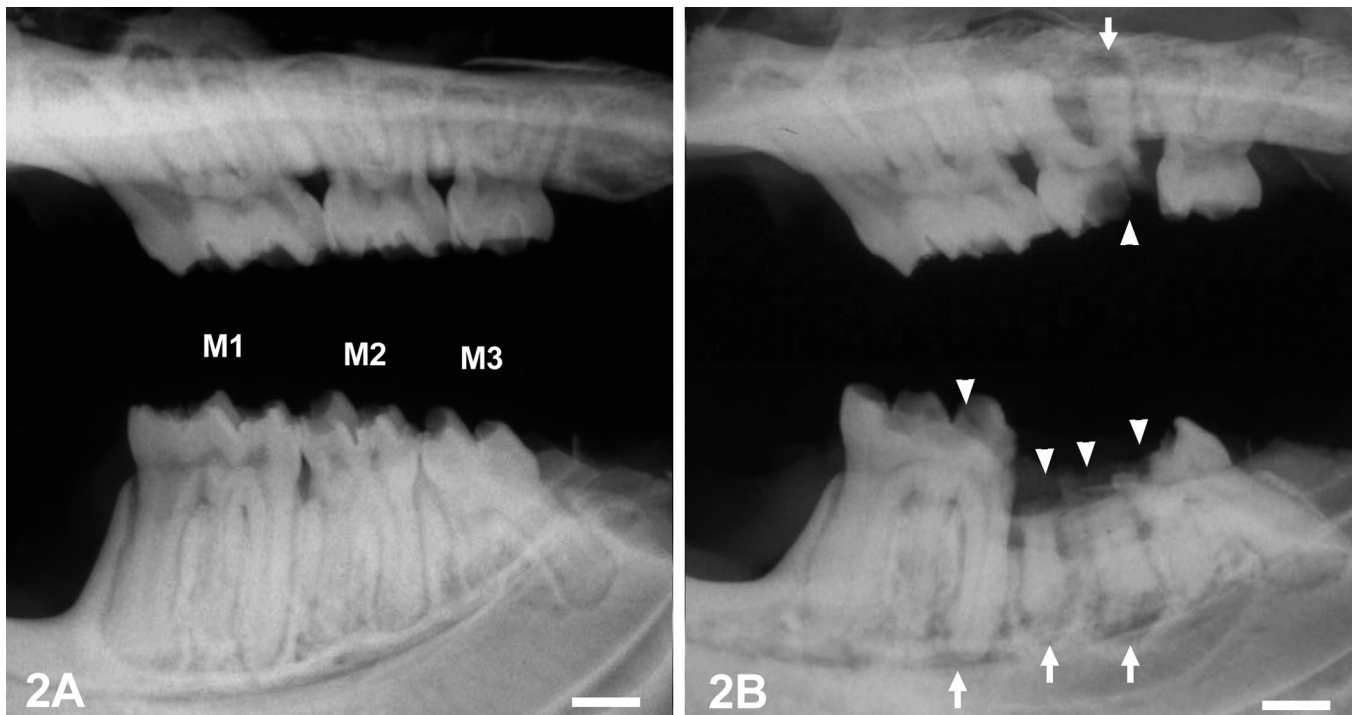


Fig. 2. Soft X-ray images of molar caries and alveolar bone resorption in the maxillae and mandibles of female F344 rats. M1: the first molar, M2: the second molar, M3: the third molar. **A.** Normal maxilla and mandible of a nondiabetic rat 26 weeks after dosing of alloxan. **B.** Maxilla and mandible of a diabetic rat 26 weeks after dosing of alloxan. Corresponding to the macroscopic observation, severe caries with a focal or an extensive radiolucent area in the dental crown is observed (arrowheads). The caries scores are 0 (M1), 2 (M2), and 0 (M3) in the maxilla and 2 (M1), 4 (M2), and 1 (M3) in the mandible. In the alveolar bone surrounding the carious molar, focal radiolucency at the apical part of the dental root is detectable (arrows). Scale bar: 2 mm.

maxillae, and the mean score in the mandibles (0.83) was significantly higher ($p<0.01$) than that in the maxillae (0.38) after 26 weeks of dosing (Table 3 and Fig. 4). On the other hand, no radiolucent areas surrounding the molars were seen in any intact or alloxan-treated nondiabetic rats (Table 3, Fig. 2A). In diabetic rats, alveolar bone resorption was frequently observed in the area adjacent to the carious molars, but there was no radiolucent change in the alveolar bone surrounding the noncarious molars (Fig. 2B). There was a high positive correlation between the alveolar bone resorption and caries scores (mandible: $r=0.72$, $p<0.01$; maxilla: $r=0.64$, $p<0.01$).

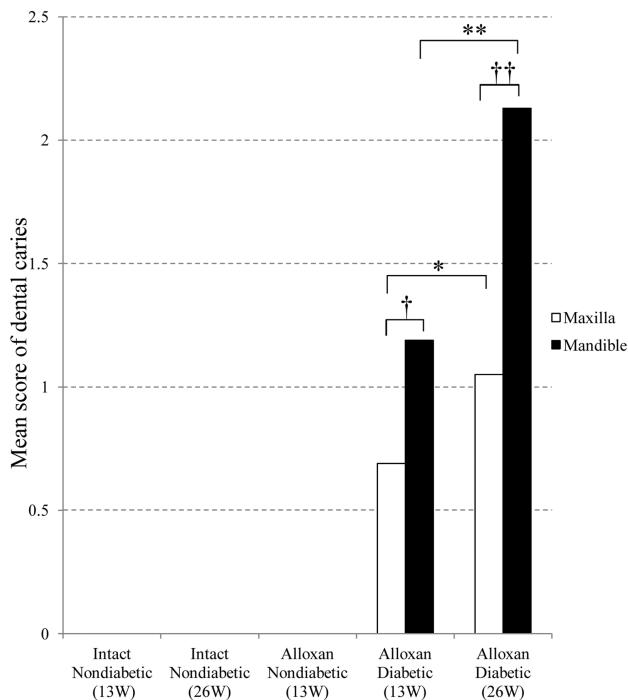


Fig. 3. Mean caries scores in maxillary and mandibular molars. Significant difference from diabetic rats after 13 weeks of alloxan treatment (*: $p<0.05$; **: $p<0.01$), and from the maxillae in diabetic rats after 13 and 26 weeks of alloxan treatment (†: $p<0.05$; ††: $p<0.01$).

Histopathological findings

Mild dental caries were initially detected in the crown as a partially eroded dentin surface (Fig. 5B). In moderately affected teeth, the dental caries reached the dental pulp from the dentin surface and developed into pulpitis and/or pulp necrosis with bacterial colonization and neutrophil infiltration (Fig. 5C). In more severely affected teeth, the dentin caries expanded through the entire crown or to the dental root (Fig. 5D).

In the gingival tissue, mucosal epithelium adjacent to the tooth with caries showed thickening and downward proliferation with neutrophil infiltration and impaction of the hair shafts (Fig. 6B,C). In severely affected lesions, gingival inflammation involved the alveolar crest, and marginal periodontitis developed (Fig. 6D). In contrast, no abnormality was observed in the gingival tissue adjacent to the normal molars (Fig. 6A). Moderate to severe caries were usually accompanied by moderate to severe gingivitis, marginal periodontitis, or apical periodontitis. In the mild periapical lesions, small numbers of neutrophils

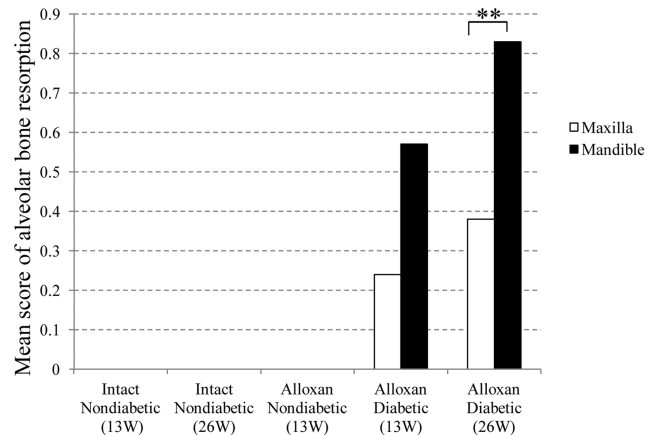


Fig. 4. Mean scores of alveolar bone resorption in maxillary and mandibular molars. Significant difference from the maxillae in diabetic rats after 26 weeks of alloxan treatment (**: $p<0.01$).

Table 4. Incidence of histopathologic lesions in maxilla and mandible.

Groups	Intact		Alloxan		
	Nondiabetic (13W) (n=10)	Nondiabetic (26W) (n=7)	Nondiabetic (13W) (n=9)	Diabetic (13W) (n=7)	Diabetic (26W) (n=10)
Caries	2/120 (1.7%)	2/84 (2.4%)	2/108 (1.9%)	48/84 (57.1%)**	98/120 (81.7%)**
Pulpitis/ pulp necrosis	1/120 (0.8%)	0/84	1/108 (0.9%)	19/84 (22.6%)**	66/120 (55.0%)**
Gingivitis	39/120 (32.5%)	30/84 (35.7%)	35/108 (32.4%)	72/84 (85.7%)**	107/120 (89.2%)**
Marginal periodontitis	0/120	0/84	0/108	11/84 (13.1%)**	37/120 (30.8%)**
Apical periodontitis	1/120 (0.8%)	0/84	1/108 (0.9%)	15/84 (17.9%)**	47/120 (39.2%)**

Significant difference from age-matched intact group (**: $p<0.01$).

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accumulated in the apical foramen area (Fig. 6F), coincident with pulpitis and pulp necrosis. In the severe lesions, the suppurative inflammation expanded into the surrounding alveolar bone, and the lesion was usually accompanied with alveolar bone resorption with widening of the periodontal connective tissue space (Fig. 6G,H), corresponding to the radiolucent area in the soft X-ray analysis (Fig. 2B).

The incidence of dental and periodontal lesions is summarized in Tables 4 and 5. The incidence of lesions in diabetic rats was significantly higher than that in intact or alloxan-treated nondiabetic rats (Table 4). The incidence of periodontal lesions accompanying a noncarious tooth is shown in Table 5. No dental lesions other than surface gingivitis were detected around any teeth without caries. The incidence of surface gingivitis near the noncarious molars was comparable in all groups (Table 5), but that of surface gingivitis with accompanying dental caries significantly increased in diabetic rats (Table 4).

Discussion

In experimental animals, 2 reports have emphasized

the close relationship between diabetes and dental caries (Hartles and Lawton, 1958; Borghelli et al., 1966), whereas another study denies such a correlation (Nichols and Shaw, 1957). Recently, in 2 studies using a noncariogenic diet, we found dental caries developing in *db/db* mice, an animal model of type 2 diabetes (Sano et al., 2011), and more pronounced caries formation in male WBN/KobSlc rats with chronic diabetes than in nondiabetic female rats of the same strain (Kodama et al., 2011). The present study revealed that alloxan-induced hyperglycemia predictably resulted in dental caries in rats. Also, no carious lesions were observed in intact or alloxan-treated nondiabetic rats fed with the same diet. These results suggest that dental caries is not triggered by alloxan toxicity, and hyperglycemia may cause dental caries both in naturally and chemically induced diabetic animals.

Progressive molar caries were already induced at higher frequency in all alloxan-treated diabetic F344 rats at 13 weeks after dosing of alloxan, and some animals showed severe dental caries where the crown was completely obliterated. Thus, dental caries may develop earlier than 13 weeks after dosing of alloxan in our study rats. Moreover, almost all mandibular molars showed

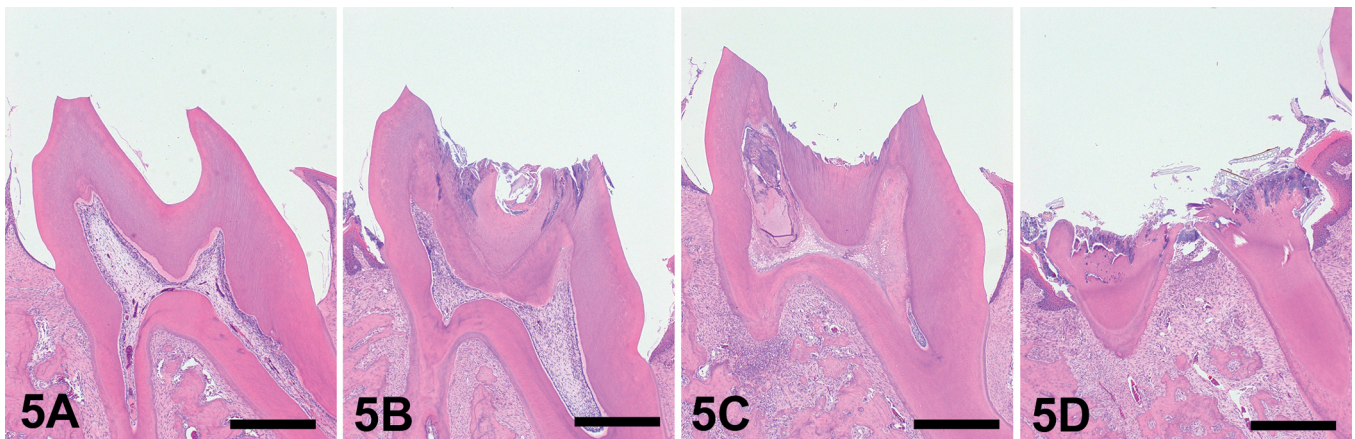


Fig. 5. Histopathological features of molar caries (M3) in the mandibles of female F344 rats. **A.** Normal molar. **B.** Mild caries lesion. Dentin caries localizes in the occlusal surface of the dentin. **C.** Moderate caries lesion. Dentin caries penetrates into the dentin and results in pulp necrosis. **D.** Severe caries lesion. Dentin caries expands through the entire crown or to the dental root, resulting in a decayed crown. HE stain. Scale bar: 400 μ m.

Table 5. Incidence of histopathologic lesions in surrounding noncarious molars in maxilla and mandible.

Groups	Intact		Alloxan		
	Nondiabetic (13W) (n=10)	Nondiabetic (26W) (n=7)	Nondiabetic (13W) (n=9)	Diabetic (13W) (n=7)	Diabetic (26W) (n=10)
Pulpitis/ pulp necrosis	0/118	0/82	0/106	0/36	0/22
Gingivitis	38/118 (32.2%)	28/82 (34.1%)	3/106 (31.1%)	12/36 (33.3%)	8/22 (36.4%)
Marginal periodontitis	0/118	0/82	0/106	0/36	0/22
Apical periodontitis	0/118	0/82	0/106	0/36	0/22

No significant difference from age-matched intact group.

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dental caries 26 weeks after dosing of alloxan. In *db/db* mice, caries development required more than 20 weeks, and less than 40% of molars were carious even at 50 weeks of age in both sexes. Lesion development appears to be more uniform and progressive in alloxan-induced diabetic rats compared to that in spontaneous diabetic animals.

Diabetes is becoming an epidemiologically important problem as a risk factor for periodontal disease in humans (Loe, 1993). Marginal periodontitis, gingivitis, and alveolar bone resorption are pathognomonic lesions in diabetic patients (Ryan et al., 2003; Mealey and Oates, 2006). Similar lesions are induced in diabetic rodents and considerable research on periodontal disease in diabetic rodent models has been carried out (Liu et al., 2006; Pontes Andersen et al., 2006; Claudino et al., 2007; Tesseromatis et al., 2009). However, detailed morphological analyses on dental

lesions, including caries, have never been performed in those diabetic animals. In our alloxan-induced diabetic rats there was a high positive correlation between the alveolar bone resorption score and that for caries in soft X-ray examination, and inflammation surrounding the apical part of the molar root was associated with pulpitis or pulp necrosis extending through the apical foramen on histopathological examination. Furthermore, periodontitis was notably nonexistent in tissues around the normal molars but frequently observed in tissues adjacent to the carious molars. These characteristics closely resemble those in spontaneous diabetic *db/db* mice (Sano et al., 2011) and WBN/KobSlc rats (Kodama et al., 2011). Thus, we conclude that the inflammatory changes of crown-caries origin extend across the apical foramen, and the resultant apical periodontitis secondary to dental caries might be transmitted to the periodontal tissue. Additionally, advanced gingivitis and marginal

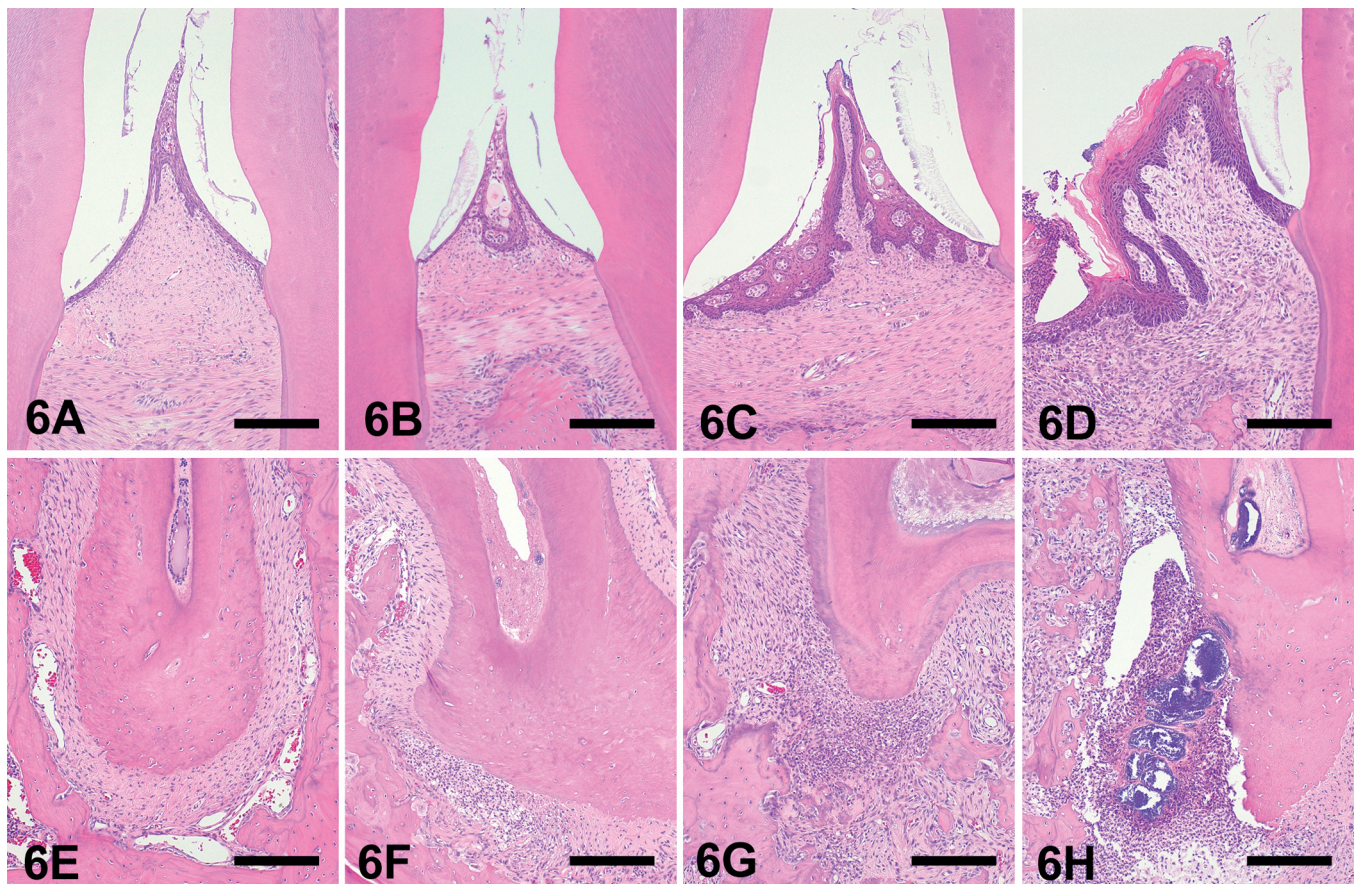


Fig. 6. Histopathological features of periodontal lesions of female F344 rats. **A.** Normal gingival tissue. **B.** Mild gingivitis. Scant neutrophils infiltrate into the mucosa with impaction of hair shafts. **C.** Moderate gingivitis. Gingival mucosal hyperplasia with mild neutrophil infiltration and impaction of hair shafts. **D.** Moderate gingivitis and marginal periodontitis. Gingival inflammation extends to the periodontal ligament. Alveolar bone resorption in the alveolar crest is also observed. **E.** Normal periradicular tissue. **F.** Mild apical periodontitis. Scant neutrophils accumulate in the apical foramen area. **G.** Moderate apical periodontitis. Extensive neutrophil infiltration and mild alveolar bone resorption in the apical area. **H.** Moderate apical periodontitis and marginal periodontitis. Apical inflammation with abscess formation, which expands into the surrounding alveolar bone and connects to marginal periodontitis. HE stain. Scale bar: 140 μ m.

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periodontitis may result from multiple stimuli, such as impaction of foreign materials (hair shafts or food particles), microorganism accumulation, and mechanical irritation of the gingival and periodontal tissues in diabetic animals.

The precise pathogenesis of dental caries in diabetic animals has not been determined. However, increased prevalence of caries has been attributed to reduced salivary flow rates in diabetic patients (Saadoun, 1980; Moore et al., 2001). Reduction of salivary flow also plays an important role in reduced wound healing and plaque formation (McNamara et al., 1982; Nagy et al., 2001). Altered components of salivary secretions may affect profiles of oral microflora in rats and humans (Saadoun, 1980; Belazi et al., 1998; Mahay et al., 2004; Mata et al., 2004). Meanwhile, diabetic animals typically have higher food intakes than nondiabetic animals (Lee and Bressler, 1981; Tesseromatis et al., 2009). Thus, all these factors may contribute to the onset of dental caries and severity of caries-related periodontitis in diabetic animals.

In conclusion, alloxan-induced hyperglycemic conditions are capable of causing rapid-onset and progressive dental caries and periodontitis in rats. It is considered that the alloxan-induced diabetic model is useful for analysis of dental caries.

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