Comparison of dentin apposition and dentinal caries progression in the mandibular and maxillary molars of the rat

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To show that the rate and the rate of reduction of dentin apposition are about the same in mandibular and maxillary molars, 23 Sprague–Dawley rat pups were randomized into 2 groups on the day of birth. During lactation half of their dams received a standard rodent diet; the other half, a diet containing 41% sucrose. At the age of 3 weeks the pups were weaned, weighed, given an intraperitoneal injection of oxytetracycline hydrochloride, and inoculated with oral *Streptococcus sobrinus*. During the experiment the pups received the same diet as their dams during lactation. After 5 weeks the pups were decapitated, their jaws sectioned sagittally, the first and second molars photographed, and the areas of dentin apposition and dentinal caries measured planimetrically. The area of dentin formation was about the same in maxilla and mandible in the first molars, but slightly smaller in the mandibular second molars of the control group. The sucrose diet reduced dentin apposition significantly in both jaws, although the areas were significantly smaller in the mandibles than in the maxillae. Caries did not affect the rate of dentin apposition. The areas of caries lesions were smaller in the maxillary molars of both diet groups. The results show that the hypothesis of equal rate of dentin apposition in mandible and maxilla was not valid because the reduction, caused by sucrose, was more prominent in mandibular molars for unknown reasons. The reduction of dentin apposition was reflected as acceleration of caries progression among the diet groups and the jaws. It was concluded that the response of the pulpodentinal complex to sucrose and dentinal caries during the primary dentinogenesis cannot be seen as a formation of reactionary or reparative dentin, as with adult rats. □ *Caries attack; morphology; primary dentinogenesis; sucrose diet*

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Our previous studies on rats have shown that the rate of caries progression in dentin is dependent on the developmental stage of the tooth, so that the younger the tooth is, the faster the caries progression (1). Clinical findings in humans support this: the eruption time is the most critical for caries initiation and progression, owing to enhanced plaque accumulation during the prefunctional period, which lasts from tooth emergence to full occlusion (2–4). The posteruptive maturation of enamel by salivary components explains the rapid posteruptive rate of progression of caries in immature enamel, but not in young dentin. During the primary dentinogenesis the rate of caries progression in dentin is faster than during the secondary dentinogenesis, when reparative or reactionary dentin formation is the outcome of any irritation, including caries (5), as a consequence of the immature response of original odontoblasts to the caries irritation.

A sucrose diet is known not only to enhance dental caries progression but also to significantly reduce the rate of dentin apposition during primary dentinogenesis (6–8). If the rate of dentin apposition is also one of the factors modulating the rate of caries progression in dentin, caries progression in teeth forming with the same speed should be affected similarly.

The sizes of the first and second molars are almost equal in the mandible and maxilla of the rat (9). Their development and eruption timetables are also similar (10, 11). This would suggest that the amounts of dentin formed per day during primary dentinogenesis should be of the same order of magnitude in maxilla and mandible, and thus the rates of dentin formation should also be about the same. However, it is known that the development of caries lesions is less rapid in the maxillary molars of the rat (9), which might reflect the rate of dentin formation.

Research on caries etiology has been concentrated on its initiation on enamel surface. We have expanded the approach, hypothesizing that inherent factors in the pulpodentinal complex regulate the rate of caries progression in dentin, but only after the initiation of the process. Factors regulating the rate of dentin formation may therefore also be involved in the regulation of dentinal caries progression. The aim of this experiment was to test the hypotheses that 1) the rates of primary dentin formation are similar in the maxillary and mandibular molars, 2) a sucrose diet reduces the rate of dentin apposition similarly in maxilla and mandible, and 3) the reduction of dentin apposition is reflected as the acceleration of dentinal caries progression.

Materials and methods

Twenty-three Sprague–Dawley rats (11 males, 12 females) were weighed, marked, and randomly distributed in 2 diet

Table 1. Percentage distribution of the diets (%)

Nutritional value	Sucrose diet	Standard diet
	Analyzed values	Analyzed values
Crude protein	15.8	18.5
Fat	3.1	4.0
Carbohydrates	67.0	55.7
Crude fiber	3.1	3.5
Ash	4.6	6.3
Water content	6.4	<12.0
Energy value	Calculated values	Calculated values
Crude protein	18.1	22.8
Fat	6.8	9.5
Carbohydrates	74.5	66.9
Crude fiber	0.7	0.8

groups on the day of birth to obtain litters as similar in weight, relationship, and number as possible. The control group of 13 pups (6 males, 7 females) with the dam were fed a standard rodent diet (Lactamin C R36, Ewos, Stockholm, Sweden) containing 34% barley flour, 43% wheat flour, 5.0% wheat grains, 4.5% vitamins and trace elements, 5.0% soya, 4.0% fish powder, and 4.5% other ingredients (12.60 MJ/kg). The experimental group of 10 animals (5 males, 5 females) with the dam received a diet containing 41% sucrose, 10.1% barley flour, 10.3% wheat flour, and 5.8% casein, which was added to compensate for the loss of protein when the wheat and barley flour content was reduced (Ewos R 642, Ewos; 13.53 MJ/kg). The other ingredients of the sucrose diet were the same as in the standard diet, which was the base for the sucrose diet. Analyzed nutritional values of standard and sucrose diets with calculated energy values are presented in Table 1. The diets were in the form of powder. Both diet groups received distilled water. Food and drink were available *ad libitum*. The animals were weaned at the age of 21 days and housed 2–3 per cage under normal atmospheric conditions at 21°C. They were subjected to the same light:dark cycle (12 h of light and 12 h of dark), feeding and handling times, and noise level, as well as the same diet their dam had received during lactation. All animals were given an intraperitoneal injection of oxytetracycline hydrochloride (Terramycin®, Pfizer, Brussels, Belgium; 30 mg/kg) upon weaning, on day 21, and 2 days before the termination of the experiment, on day 54, to mark the areas of dentin apposition. For the cariogenic challenge all animals were inoculated with a fresh suspension of *Streptococcus sobrinus* (ATCC 27531 K 1 Fitzgerald) at the ages of 23 days and 37 days.

Five weeks after weaning the animals were weighed, anesthetized using a mixture of midazolam (Dormicum, Roche, Basel, Switzerland), fentanyl-fluanisone (Hypnorm®, Jansen Pharmaceutica, Brussels, Belgium), and aqua (1:1:2 at 0.3 mL/100 g, intraperitoneally), then killed by decapitation. The jaws were defleshed and preserved in absolute ethanol, and the maxillae and mandibles were sectioned sagittally (12).

In order to measure the amount of dentin apposition, the first and second molars were each examined under an Orthoplan Ploemopak microscope equipped with fluorescent light (detector wavelength, 460 nm) with which the tetracycline stripes surrounding the formed dentin could be seen (13). The main central transverse fissures of the first and second molars of both jaws were photographed with Kodak Ektachrome daylight film (400 ASA). The tetracycline-marked areas of dentin apposition were measured planimetrically from the video images by circumscribing their respective areas on a monitor (Salora 445 A RGB, Salo, Finland; camera: Hitachi VKM 96 E, Tokyo, Japan) using a serial 'mouse' connected to a PCVision Frame Grabber (Imaging Technology, Woburn, Mass., USA) (13). The size of the dentinal caries lesions, seen as a change of fluorescence (14) under the main fissures, was also measured planimetrically as described above.

The statistical analyses were done with SPSS for Windows, Release 7.5. The differences in mean weight and dentin apposition between the diet groups, as well as dentin apposition between the jaws, were determined using the independent samples *t* test. Statistically significant differences in dentinal caries as well as dentin appositions in carious and sound teeth were determined using the nonparametric Mann–Whitney U-test, because the distribution of the dentinal caries is strongly skewed to the right. The Mann–Whitney U-test is based on ranks of two compared groups and does not assume normal distribution, as does the independent samples *t* test.

Results

Table 2 presents the results regarding areas of dentin apposition and dentinal caries lesions. The areas of dentin apposition in the standard diet group were about the same in maxillary and mandibular molars. In second molars, however, dentin formation was significantly less ($P < 0.001$) in mandible than in maxilla.

Compared with the standard diet, the sucrose diet resulted in smaller dentin appositions in both maxillary and mandibular molars. Dentin appositions of the sucrose group in mandibular first molars were about 54% and in second molars about 31% of the dentin appositions of the controls. The corresponding percentages in maxillary first and second molars were 33% and 17%, respectively. In the sucrose group dentin apposition in maxillary molars was greater, and the differences between the jaws were significant in both molars.

The sucrose diet induced larger caries lesions than the control diet in both jaws. The differences between the sucrose and the control group in maxilla and mandibula ($P = 0.001$) were statistically significant.

An order of magnitude and fewer and smaller caries lesions were developed in the maxilla. In the sucrose group the differences between the mandibular and maxillary first ($P = 0.001$) and second molars ($P = 0.001$) were statistically

Table 2. Areas of dentin appositions (means ± standard deviations (*s*)) and dentinal caries lesions in the mandibles and maxillae

	Dentin apposition ($\mu m^2 \times 10^3$)			Dentinal caries ($\mu m^2 \times 10^3$)			
	Mean	*s*	*P**	Q1	Median	Q3	*P**
Standard diet group ($n = 10$)							
Maxillary first molar	150.9	32.4[a]		0	0[A]	0	
Mandibular first molar	150.1	27.9[b]	NS	0	0[B]	7.2	0.034
Maxillary second molar	134.9	16.1[c]		0	0[C]	0	
Mandibular second molar	106.4	20.3[d]	<0.001	0	0[D]	5.3	0.015
Sucrose diet group ($n = 13$)							
Maxillary first molar	101.3	21.9		0	2.0	3.4	
Mandibular first molar	69.1	24.6	<0.01	7.4	18.3	26.4	0.001
Maxillary second molar	112.5	21.2		0	0	2.1	
Mandibular second molar	64.9	24.7	<0.001	9.9	17.1	22.4	0.001

Q1 = 25% quartile; Q3 = 75% quartile; NS = no significant difference.
Significant differences from corresponding teeth of sucrose diet group: [a, b, d, A, D] $P < 0.001$; [c] $P < 0.01$; [B] $P = 0.003$; [C] $P = 0.049$ ([a–d]: independent samples *t* test; [A–D]: nonparametric Mann–Whitney U-test).
* Comparison of respective teeth between the jaws.

significant. In the control group the areas of dentinal caries lesions were also smaller in maxillary molars. The differences between the mandibular and maxillary first ($P = 0.034$) and second molars ($P = 0.015$) were significant.

Regardless of the induction by high-sucrose cariogenic challenge, the effect of caries on dentin apposition was minimal. In the control group the areas of dentin apposition ($\mu m^2 \times 10^3$) were not significantly smaller in carious than in sound mandibular first molars (mean carious, 129.8; $n = 4$ vs. mean sound, 159.1; $n = 9$) and second molars (mean carious, 107.6; $n = 5$ vs. mean sound, 105.6; $n = 8$). In maxilla all the measured molars of the control group were sound ($n = 13$), so comparison of carious and sound teeth was not possible. In the maxilla of the sucrose group, dentin appositions of carious molars did not differ from sound first molars (mean carious, 90.8; $n = 4$ vs. mean sound, 111.8; $n = 6$) and second molars (mean carious, 92.3; $n = 2$ vs. mean sound, 117.5; $n = 8$). All mandibular first molars of the sucrose group were carious ($n = 10$), and only one of the second molars was sound.

Dentin apposition in sound maxillary molars of the sucrose group was reduced when compared with the sound first molars (mean sucrose, 111.8; $n = 6$ vs. mean control, 150.9; $n = 13$; $P = 0.027$) and second molars (mean sucrose, 117.5; $n = 8$ vs. mean control 134.9; $n = 13$) of the control group. Dentin appositions in carious mandibular molars of the sucrose group were significantly smaller than the areas in the carious mandibular first molars (mean sucrose, 69.1; $n = 10$ vs. mean control, 129.8; $n = 4$; $P = 0.013$) and second molars (mean sucrose, 62.8; $n = 9$ vs. mean control, 107.6; $n = 5$; $P = 0.014$) of the control group.

The general health of the animals during the experiment period was good. The differences in mean weight (g) between the diet groups during the experiment were not permanent, although the male rats weighed more than the female rats by the end of the experiment (Table 3), a result of the higher daily growth rate of male rats (Fig. 1).

Discussion

The working hypothesis that the rates of dentin apposition in mandible and maxilla are about the same holds true for the first, but not the second, molars in rats fed a standard rodent diet. This difference might be due to different mesiodistal and buccolingual dimensions of maxillary and mandibular second molars (9), which resulted in a smaller amount of dentin apposition in mandibular molars (Table 2).

The sucrose diet reduced dentin apposition and induced dentinal caries progression in the molars of both jaws, but the rate of reduction was more prominent in mandibular molars for unknown reasons. Thus, the second hypothesis of our study turned out to be incorrect. An explanation for

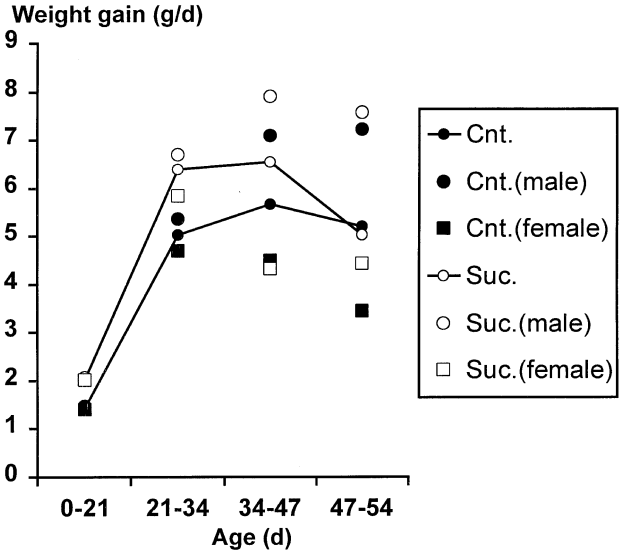

Fig. 1. The daily mean weight gains (g/d) of the groups. Cnt. = standard diet group; Suc. = sucrose diet group. Age of weaning, 21 days.

Table 3. Mean weights (*g*) (± standard deviations (*s*)) of the groups during the experiment

	21 days			34 days			47 days			54 days		
	Mean	*s*	*P**	Mean	*s*	*P**	Mean	*s*	*P**	Mean	*s*	*P**
Standard diet group	37.9	2.5†		103.3	7.5†		177.0	23.9‡		213.4	37.2	
Male ($n = 6$)	38.7	2.0		108.3	5.6		200.5	5.8		251.1	6.6	
Female ($n = 7$)	37.3	2.9	NS	98.3	6.3	0.018	156.9	9.2	<0.001	181.0	8.9	<0.001
Sucrose diet group	50.9	4.7		133.9	9.2		219.1	31.7		254.3	45.3	
Male ($n = 5$)	51.9	5.9		139.0	8.9		241.7	14.2		294.7	19.9	
Female ($n = 5$)	50.8	3.6	NS	126.7	4.5	0.033	182.8	5.3	<0.001	213.9	12.0	<0.001

* Comparison between male and female rats of the same age and diet group.
Significant differences from pups of the sucrose diet group at same age (independent samples *t* test): † $P < 0.001$; ‡ $P = 0.003$.
NS = no significant difference.

the close connection between determined dentin apposition and large dentinal caries lesions is that a sucrose diet itself not only induces caries but also changes odontoblast response and causes a reduction in dentin apposition during the primary dentinogenesis (15). A threshold value of sucrose in the diet is known to be more than 30%, which is a critical point for the reduction of dentin apposition as well as the frequent appearance and fast progression of dentinal caries lesions (16). In addition, the sucrose diet may disturb the mineralization or collagen synthesis of predentin (8). Glucose is known to induce hypercalciuria in the rat (17), as does xylitol (18). Therefore, the effects of carbohydrates on the host side of odontoblasts should be reconsidered.

It has been observed earlier that carbohydrates, such as xylitol (15) and potato starch (8), reduce dentin apposition without caries induction. Therefore, a direct relation between dentin apposition and caries progression does not hold indisputably true in all cases. Because the caries-inducing effect of the sucrose diet was higher in mandible than in maxilla by an order of magnitude, but the reducing effect of caries on the dentin apposition was not noticed, the presumption that dentinal caries itself would reduce dentin apposition could not have been correct. Regardless, as less dentin and more and larger areas of dentinal caries lesions were formed in the sucrose diet group, we concluded that the third hypothesis, regarding reduction of dentin formation and acceleration of dentinal caries, was valid among the diet groups. The hypothesis also turned out to be defensible in relation to the jaws, because larger areas of dentin appositions together with smaller areas of dentinal caries lesions were noticed in maxilla as a result of similar physiologic exposure of odontoblasts to sucrose in the mandibular and maxillary molars.

Moreover, the rate of dentin formation of adult rats is at least 10 times slower than that of young rats (1, 19). The dentin formation in sound as well as carious molars of the adult rat takes place via secondary dentinogenesis as a reparative or reactionary dentin, which is synthesized by replacement/secondary or original odontoblasts (20). Reparative dentin is synthesized by replacement or secondary odontoblasts (odontoblast-like cells) as the normal response of fully formed tooth to carious attacks (21) or operative procedures when dentin is exposed and original odontoblasts injured or destroyed (22). Therefore, it is presumptive that during the primary dentinogenesis there are no possibilities for reparative responses in dentin against caries because the genetically determined rate of dentinogenesis takes all the capacity of the original odontoblasts. In fully formed teeth reactionary dentin is synthesized by original odontoblasts, and its formation can also be due to a stimulus other than caries (5, 20), for example attrition. It was surprising that dentinal caries, which should induce reactionary or reparative dentin formation, did not significantly affect the areas of dentin appositions in the carious teeth of the animals, whereas the sucrose diet as such did so even in sound molars. This gave us reason to believe that reduction of dentin apposition was not a consequence of secondary dentinogenesis, but actually a consequence of a reduced, cell-mediated response of pulpodentinal complex to the sucrose irritation during the primary dentinogenesis.

Nevertheless, the explanations above do not totally explain the differences in the progression of dentinal caries lesions among maxillary and mandibular first molars of the control group (Table 2). In the Sprague–Dawley strain, the first mandibular and maxillary molars erupt into the oral cavity around the 16th day of age. The second mandibular and maxillary molars erupt by the 20th day of age (10), so the differences in caries progression in maxilla and mandible could not be caused by different eruption schedules.

Because the rate of tooth eruption does not explain the differences in caries progression between the mandibular and maxillary molars, it is presumptive that the extrinsic cariogenic challenge should differ between these teeth. The environmental factors enforced by plaque accumulation during the prefunctional (2–4) or the posteruptive maturation of enamel do not completely explain the differences between maxilla and mandible. Therefore, local factors should be considered, in addition to tooth morphology and salivary factors, as additional explanations.

It is known that there are morphologic differences between the mandibular and maxillary molars of the rat (9, 10), which may cause alterations in the cariogenic

challenge to these teeth. In the rat mandibular first molar, there are two transverse fissures, which divide the teeth into three lobes. These lobes are bisected by a deep longitudinal sulcus, which also appears in the mandibular second molar, with the exception that in the second molar there is only one deep transverse fissure. Maxillary molars lack longitudinal sulci and deep transverse fissures (10); thus, in maxillary molars the fissure system is shorter and less deep than in mandibular molars. These differences might decrease the level of dental plaque and food debris in the fissures of maxillary molars (23) and explain the differences in the progression and areas of dentinal caries lesions between the mandibular and maxillary first molars of the control group.

The difference in mean weight between the diet groups at the beginning of the experiment period could not be caused by birth weight nor by the number of pups per dam during the lactation period, because there were no differences in the birth weights of the pups between the dams (data not shown) and the pups were divided equally in the experimental litters at the onset of lactation. Thus, alteration of the composition of milk by sucrose or glucose seems to be the only possibility. Theoretically, this is explainable, because the high glucose level in the diet of the dam increases the fat concentration of milk, which makes greater metabolizable energy available to the pups (24). According to Lanoue & Koski (24), the pups of dams that were fed a diet containing no glucose failed to survive more than 24 h postpartum. Sucrose ingestion is known to induce urinary calcium excretion in humans (25–27) and the rat (17). In any event, the calcium balance of lactating rats is negative owing to the high transfer of calcium into milk (28). Increased intestinal absorption (29) and reduced urinary excretion with skeletal calcium transference of lactating rats (28) compensate for the high calcium loss to milk. The sucrose diet, with higher dietary energy levels, may explain the differences in mean weight between the sucrose (13.5 MJ/kg) and control (12.6 MJ/kg) diet pups at the beginning of the experiment period (Table 3). Lack of differences in mean weight and weight gain (Fig. 1) by the end of the experiment period is in good accordance with previous results (7, 30) and supports the concept that the rat regulates food intake to meet energy needs when food is freely available (31).

Generally, these results show that sucrose reduces the rate of primary dentin formation, and the reducing effect is greater in the mandible than in the maxilla of the rat for unknown reasons. Dentinal caries does not affect the rate of dentin apposition during primary dentinogenesis, but the reduction of dentin apposition caused by sucrose increases the rate of caries progression. The rate of dentinal caries progression is less rapid in maxillary than in mandibular molars, possibly as a consequence of different odontoblast responses and morphologic differences between these teeth. In addition, the reduced caries progression in the maxillary molars of young rats offers a chance to observe the response of original odontoblasts to the various dietary challenges during the early stage of dentin formation, without the disturbance caused by large dentinal caries lesions and secondary dentinogenesis thereafter.

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