Effects of continuous glucocorticoid infusion on the progression of dentinal caries in growing rats

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Huumonen S, Larmas M. Effects of continuous glucocorticoid infusion on the progression of dentinal caries in growing rats. Acta Odontol Scand 1998;56:276–280. Oslo. ISSN 0001-6357.

This study was undertaken to test the effects of a low dose of continuous glucocorticoid infusion on the rate of progression of dentinal caries in molars of young rats. Forty-seven rats were inoculated in the mouth with *Streptococcus sobrinus* and fed *ad libitum* a cariogenic diet and 10% sweetened water. After 10 days of caries initiation ten animals were killed to serve as a reference group. In the rest of the animals the cortisone or placebo pellet was implanted subcutaneously in the back of the neck. The daily release of cortisone was 0.42 mg per rat. Sweetened water was changed to pure water, and the diet was the same cariogenic diet. After 6 weeks of medication the areas of dentinal caries were quantified planimetrically. Schiff's staining was used to classify caries. Although cortisone medication slightly increased the number of carious lesions, statistical significance was not reached. However, compared with the placebo group, the rats receiving cortisone medication showed significantly increased dentinal caries progression and severity of lesions. This study suggests that glucocorticoids with a cariogenic diet reduce the intrinsic modulation or response of the odontoblasts to caries attack. □ *Dentin; glucocorticoids; rats*

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Progressing from enamel to dentin, the carious process advances from a non-cellular hard tissue into a living tissue containing an inorganic extracellular component with an organic matrix and connection to living cells, namely, odontoblasts and pulpal cells. The pulpodentinal complex is capable of responding to the caries destruction even in the very initial stages (1–3), and this process includes both the carious destruction and the defensive reactions in dentin and pulp. Disturbances in odontoblast metabolism might predispose teeth to more serious effects of the caries attack. On the other hand, this attack is known to induce the formation of reparative (4) or reactionary dentin (5).

A caries-inducing sucrose diet reduces primary dentin formation (6, 7). One explanation might be that, during primary dentinogenesis, reactions against dental caries are only modulators of the process, whereas during secondary dentinogenesis, the response consists of an increase in the formation of reparative dentin. This concept led to the hypothesis that there may be a cause–effect relation between the rates of caries progression and primary dentin formation.

The widely accepted effects of a high-sucrose diet in the pathobiology of dental caries have established the concept that bacteria easily utilize sucrose for their metabolism. Polysaccharide production by certain strepcococci favors dental plaque formation, and bacterial acids produced by the fermentation of dietary sucrose favor destruction of dentin. We have recently reported a reduction in dentin formation and thereby a possible reduction of dentinal response in young rat molars due to a high concentration (43%) of sucrose in the diet, resulting in an advanced rate of caries progression in dentin (8, 9). Continuous glucocorticoid medication also reduced dentin formation without an effect on caries progression in the absence of a cariogenic diet (10). Apparently, cariogenic bacterial inoculation and a high-sucrose diet are needed to induce rapid dental caries in experimental animals.

We earlier found that a glucocorticoid medication in combination with a high-sucrose diet had little effect on caries initiation or progression, but as the number and severity of the caries lesions were low, no profound conclusions could be reached about the effects of these factors on dentinal caries. Glucocorticoids with a non-cariogenic diet, even with *Streptococcus sobrinus* inoculation, have not been able to induce dental caries (10). The aim of the present study was therefore to investigate specifically the effect of glucocorticoid medication on dentinal caries destruction. We exposed rats to a cariogenic challenge for 10 days after weaning in order to produce enamel lesions before initiating the actual experiment. Thereafter, the progression of dentinal caries was followed in glucocorticoid-treated and placebo-treated groups of rats. The working hypothesis was that, as glucocorticoid medication reduced dentin formation, with a severe cariogenic challenge it would deteriorate the defense of teeth against caries, and larger caries lesions would result.

Materials and methods

All animal procedures were done by persons licensed to perform animal experiments. The protocols were approved by the Animal Experiment Committee of the University of Oulu, Oulu, Finland.

Female Sprague–Dawley rats ($n = 47$) were weaned at the age of 21 days, weighed, and marked. To induce caries

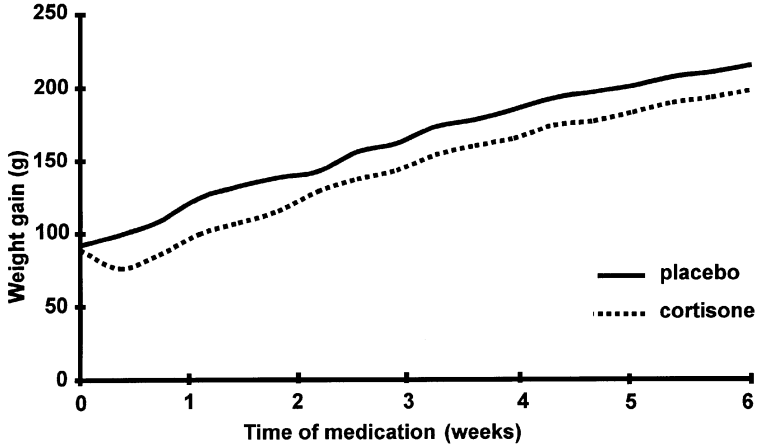

Fig. 1. Growth curves of the rats during the medicated period. The medication started at the age of 31 days and lasted for 6 weeks.

and stabilize the oral flora of the rats, 2–3 drops of newly cultured human *S. sobrinus* (ATCC 27351 K1 Fitzgerald) in a thioglycollate (NIH thioglycollate broth, Difco, Detroit, Mich., USA) and glucose (Merck, Darmstadt, Germany) medium were placed from a pipette into the mouth of all the animals. Infection was assured by also inoculating with a fresh suspension on the 2nd day and weekly thereafter. The animals were fed a modified Stephan–Harris diet, which contained 43% sucrose (plus 22% wheat flour, 32% milk powder, 2% whole liver powder, and 1% corn oil) and sweetened (10% w/v sucrose) water.

The animals were kept two or three to a cage (Makrolon III), on a bed of European aspen shavings, with a 12:12 h light:dark cycle, a room temperature of 21°C, and 40%–60% humidity. Metabolic cages were not used, since group-housed rats are recommended for caries experiments (11). Water and food were given *ad libitum*, and their consumption was evaluated at intervals of 2 or 3 days by measuring the amounts left per cage and calculating the average food consumption for each animal.

After 10 days ten animals were first anesthetized (Dormicum®, Roche, Switzerland: Hypnorm®, Janssen Pharmaceutica, Belgium: sterile water, 1:1:2, 0.2 mL/100 of rat weight, given intraperitoneally) and then killed by decapitation. They served as a reference group at the onset of the medication. At the same time 37 rats were anesthetized, and a cortisone (17 animals) or placebo pellet (20 animals) (Innovative Research of America, Toledo, Ohio, USA) was implanted subcutaneously in the back of the neck. Each cortisone pellet held 25 mg of cortisone, which was sufficient for 60 days' release of 0.42 mg/day. The food was the modified Stephan–Harris diet, and the drink was pure water.

After 6 weeks of medication, the rats were anesthetized and killed by decapitation. The stabilities of the cortisone and placebo pellets were documented by inspecting them through surgical incision. The mandibles were dissected, separated into their two halves, and cleaned of all adherent soft tissue. They were then sectioned using a water-cooled diamond wheel to produce parallel longitudinal sections (12). The hemisection closest to the midline of each molar was selected. All sectioned jaws were coded and randomized before measurements.

In order to measure the area of dentinal caries, the first and second molars were each examined under an Orthoplan Ploemopack microscope equipped with fluorescent light (detector wavelength, 460 nm). The main central transverse fissure of the first molars and the mesial one of the second molars were photographed with Kodak Ektachrome daylight film (400 ASA). The area of caries destruction was determined from the change in dentin fluorescence 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, 14).

The first and second molars were also stained with Schiff's reagent, and the number of intact fissures and the fissures with enamel or dentinal lesions were counted by the method introduced by König et al. (15) and modified by Green & Hartles (16). The teeth were also scored by number and severity of fissure caries, classified as intact fissure, enamel lesion, or dentinal lesion. The maximum potential caries scores per rat were 10 for the number of lesions (all fissures) and 20 for severity (0 for intact, 1 for enamel lesion, and 2 for dentinal lesion).

Statistical analyses were performed by using SPSS for Microsoft Windows Release 6.1. To evaluate scientifically meaningful differences, 95% confidence intervals for means (17) were used to determine statistical differences in the body weight gain, the areas of dentinal caries lesions, and the number and severity of the sound fissures and the carious lesions according to Schiff's staining. The Mann–Whitney U-test was used to test the statistical significance between the cortisone and placebo groups.

Table 1. Caries lesions in different rat groups analyzed.* Means, standard deviations (*s*), and 95% confidence intervals (CI)

	Group	Mean	*s*	95% CI
Intact fissures	Cortisone	1.53	1.23	0.90;2.16
	Placebo	2.75	1.91	1.85;3.64
	Reference	4.30	2.91	2.22;6.38
Enamel lesions	Cortisone	3.11	1.17	2.52;3.71
	Placebo	4.45	2.32	3.36;5.54
	Reference	5.50	2.63	3.61;7.39
Small dentinal lesions	Cortisone	3.71	1.65	2.86;4.55
	Placebo	2.40	2.03	1.44;3.35
	Reference	0.20	0.63	−0.25;0.65
Advanced dentinal lesions	Cortisone	1.52†	1.46	0.78;2.28
	Placebo	0.40	0.99	−0.07;0.87
	Reference	0.0		0.0
Dentinal lesions	Cortisone	5.24†	1.15	4.65;5.83
	Placebo	2.80	2.46	1.65;3.95
	Reference	0.2	0.63	−0.25;0.65

* First and second mandibular molars were analyzed together.
† Significantly different from placebo group.

Table 2. Number and severity of caries lesions in different groups analyzed. Means, standard deviations (*s*), and 95% confidence intervals (CI)

Group	Mean	*s*	95% CI	Fissures*
Number of lesions				
Cortisone	8.35	1.32	7.67;9.03	68
Placebo	7.25	1.91	6.35;8.15	80
Reference	5.50	2.63	3.61;7.39	40
Severity index†				
Cortisone	13.59‡	2.18	12.47;14.71	68
Placebo	10.05	3.75	8.30;11.80	80
Reference	5.50	2.64	3.61;7.38	40

* Maximum number of carious fissures per rat is 10.
† Severity index was calculated from the penetration depth of carious lesions per rat (maximum, 20).
‡ Significantly different from placebo group.

The non-parametric test was chosen because the data did not pass the normality test. The level of statistical significance was set at $P < 0.05$.

Results

In the cortisone group the animals lost weight at the beginning of medication, but after a few days began to gain weight again. However, they never quite reached the weight of the animals in the placebo group (Fig. 1). There were no differences in food or water consumption between the groups (data not shown). The location of the cortisone and placebo pellets was stable in all of the rats during the experiment. All of the animals appeared healthy throughout the study.

The development of dental caries in terms of number and severity of carious lesions on the 10th day of caries challenge was about half that on the 52nd day of caries challenge (Tables 1 and 2). Although the number of carious lesions did not significantly differ between the medicated groups, the severity of dentinal lesions was significantly greater in the cortisone group (Tables 1 and 2). The number of dentinal lesions in the glucocorticoid-medicated group (Table 1) was double that in the placebo group.

Enamel lesions developed within 10 days of cariogenic challenge in the reference group, whereas only two dentinal fissure lesions occurred. Also, the areas of dentinal caries lesions in both the first and second molars were significantly larger in the cortisone group than in the placebo group (Table 3).

Discussion

The initiation of medication after a short period of cariogenic challenge to a rat offers a model especially suited for investigating dentinal caries progression. As the initial cariogenic periods were similar in the two groups, the differences in caries destruction were due to alterations by the medication.

The onset of the weight gain that normally occurs was delayed in the cortisone group, and in fact they became lighter after insertion of the pellets. They never reached the level of weight gain of the other group (Fig. 1). Our nutritional and housing conditions met normal recommendations (11, 18). Glucocorticoid medication is known to retard the weight gain in growing animals (10, 19, 20). In our previous work the reduction of weight gain was seen in only sucrose-fed and glucocorticoid-medicated rats. Glucocorticoids raise the blood glucose level and may retard the growth of animals fed a high-sucrose diet in the same way as in experimental diabetes animals (21). At the beginning of the experiment the relative dose per animal was greater, and after a short period the animals modified their own hormonal balance. This reduced the total amount of glucocorticoids available, and thereafter the rats gained weight as fast as those in the placebo group.

Caries lesions were revealed in the animals both by staining and by measuring the areas of fluorescence in the dentin. Any totally demineralized areas (carious areas) will take up the stain, and these areas are scored as carious (22). The fluorescence method reveals the mineral loss at the earlier stage and is therefore a more sensitive method (14). As the two methods were done together, the results are more reliable and support each other.

The number of enamel lesions in the reference group demonstrates the high cariogenic potential of the combination of a high-sucrose diet and sweetened water. Since the number of caries lesions differed only slightly, but not significantly, between the cortisone and placebo groups, we assume that the medication has only some modifying effects on extrinsic factors such as plaque and saliva. On the other hand, the progression of dentinal caries was faster in the cortisone group; the medication at least partly reduced the defense mechanisms of the pulpodentinal complex, possibly through a direct effect on odontoblastic

Table 3. The area of dentinal caries lesions ($\mu m^2 \times 10^3$). Mean areas, standard deviations (*s*), 95% confidence intervals (CI), and numbers of rats

Group	Mean area	*s*	95% CI	No. of rats
First mandibular molar				
Cortisone	60.70*	73.85	34.93;86.46	17
Placebo	23.64	33.74	12.85;34.43	20
Reference	0.86	3.06	−0.66;2.38	10
Second mandibular molar				
Cortisone	74.20*	82.32	45.48;102.92	17
Placebo	24.06	23.88	16.43;31.70	20
Reference	1.84	4.34	−0.32;4.00	10

* Significantly different from placebo group.

function or by indirectly altering the availability of both organic and inorganic elements needed for predentin and dentin formation. In our previous experiment (10) glucocorticoids enhanced slightly, but not significantly, the effect of sucrose in inhibiting dentin formation, and this further supports a decrease in the defense mechanisms of the pulpodentinal complex by glucocorticoids, even at the concentration used in this experiment.

The effects of glucocorticoids on salivary function are partly conflicting. In an earlier study (23) the relative submandibular and parotid gland weights remained the same as those of the controls. In another study (24), on dexamethazone-treated rats, there were no significant effects on submandibular gland ultrastructure or on the elemental composition of the acinar cells, and flow rate was not affected; however, the concentrations of protein, calcium, and potassium were significantly increased. A reduction in the parotid salivary flow rate after simultaneous cholinergic and adrenergic stimulation has been shown, but at the same time the protein concentration was increased threefold (25). It seems that there is no evidence showing that the cariogenic effect of glucocorticoids is due to salivary function.

Our results show that there was an increase in the severity of the dental caries lesions as a result of glucocorticoid medication after the teeth had been predisposed to a high level of cariogenic challenge, consisting of both a high concentration of sucrose in the diet and cariogenic bacterial inoculation. Because dental caries as a biologic phenomenon is a combination of tissue destruction and defensive measures (26), there is the possibility that the depression of dentinal defensive reactions precedes the rapid progression of dentinal caries development. In other words, odontoblast function may first be depressed and the degree of defensive reactions therefore diminished, allowing rapid caries progression to follow. This kind of odontoblast alteration was seen in our previous study, in which either a high-sucrose diet or glucocorticoid medication alone reduced dentin formation (7, 10). This suggestion is supported by the findings that resection of molar root tips increases dentinal caries scores and that there is a positive correlation between pulpal necrosis and dentinal caries in hamsters (27). In carious teeth dietary sucrose increased dentinal caries progression in a dose-response fashion (6).

The results of this study suggest that carious lesions progress faster and become more severe in rats medicated with glucocorticoids than in placebo-treated rats.

Acknowledgements.—This work was partly supported by a grant from the Finnish Dental Society.

References

1. Brännström M, Garberoglio R. The dentinal tubules and the odontoblast processes. A scanning electron microscopic study. Acta Odontol Scand 1972;30:291–311.
2. Läikkö I, Larmas M. Adenosine triphosphate in normal and carious human dentine. Arch Oral Biol 1979;24:15–20.
3. Björndal L, Darvann T, Thylstrup A. A quantitative light microscopic study of the odontoblast and subodontoblastic reactions to active and arrested enamel caries without cavitation. Caries Res 1998;32:59–69.
4. Magloire H, Bouvier V, Joffre A. Odontoblast response under carious lesions. Proc Finn Dent Soc 1992;88 Suppl 1:257–74.
5. Smith AJ, Cassidy N, Perry H, Begue-Kirn C, Lesot H. Reactionary dentinogenesis. Int J Dev Biol 1995;39:273–80.
6. Huumonen S, Tjäderhane L, Larmas M. Greater concentration of dietary sucrose decreases dentin formation and increases the area of dentinal caries in growing rats. J Nutr 1997;127:2226–30.
7. Tjäderhane L, Hietala E-L, Larmas M. Reduction in dentine apposition in rat molars by a high-sucrose diet. Arch Oral Biol 1994;39:491–5.
8. Kortelainen S, Larmas M. Effects of low and high fluoride levels on rat dental caries and simultaneous dentine apposition. Arch Oral Biol 1990;35:229–34.
9. Larmas M, Tjäderhane L. The effect of phenytoin medication on dentin apposition, root length, and caries progression in rat molars. Acta Odontol Scand 1992;50:345–50.
10. Huumonen S, Pajari U, Bäckman T, Tjäderhane L, Larmas M. Effect of low dose of glucocorticoid treatment on dentin formation and dentinal caries in rats. Acta Odontol Scand 1996;54: 282–6.
11. Navia JM, Narkates AJ. Dental research. In: Baker HJ, Linsay JR, Weisbroth SH, editors. The laboratory rat. Vol II. New York: Academic Press; 1980p. 59–74.
12. Keyes PH. Dental caries in the molar teeth of rats. J Dent Res 1958;37:1088–99.
13. Larmas M, Kortelainen S. Quantification of the areas of dentinal lesions and secondary dentin in fissures of rat molars. Caries Res 1989;23:32–5.
14. Hietala E-L, Tjäderhane L, Larmas M. Dentin caries recording with Schiff's reagent, fluorescence and back-scattered electron image. J Dent Res 1993;72:1588–92.
15. König KG, Marthaler TM, Mühlemann HR. Methodik der kurzfristic erzeugten Rattenkaries. Dtsch Zahn, Mund-Kieferheilkd 1958;29:99–127.
16. Green RM, Hartles RL. A modification to an established technique for the assessment of fissure caries in the rat. Arch Oral Biol 1967;12:427–8.
17. Potter RH. Significance level and confidence interval. J Dent Res 1994;73:494–6.
18. National Research Council, Subcommittee on Laboratory Animal Nutrition. Nutrient requirements of the laboratory rat. In: Nutrient requirements of laboratory animals. 2nd ed. Washington (DC): National Academy of Sciences; 1972p. 56–93.
19. Sissons GA, Hadfield GJ. Influence of cortisone on structure of bone. J Anat 1955;89:69–78.
20. Winter CA, Silber RH, Stoerk HC. Production of reversible

hyperadrenocortinism in rats by prolonged administration of cortisone. Endocrinol 1950;47:60–72.
21. Rebuffat P, Malendowitcz LK, Mazzocchi G, Gottardo G, Nussdorfer GG. Streptozotocin-induced experimental diabetes causes a time-dependent inhibition of growth and steroidogenic capacity of rat adrenal zona glomerulosa. Res Exp Med 1990; 190:1–12.
22. Dreizen S. Mechanism of dental caries. In: Lazarri EP, editor. Dental biochemistry. Philadelphia: Lea & Febiger Press; 1979. p. 274–88.
23. Liu FT, Lin HS. Influence of corticosterone on the integrity of salivary glands and the incidence of dental caries in the rat. J Dent Res 1969;48:467–72.
24. Sagulin GB, Roomans GM. Effects of thyroxine and dexamethasone on rat submandibular glands. J Dent Res 1989;68: 1247–51.
25. Johnson DA, Alvares DA, Etzel KR, Kalu DN. Regulation of salivary proteins. J Dent Res 1987;66:576–82.
26. Soames JV, Southam JC. Dental caries. In: Oral pathology. 2nd ed. New York: Oxford University Press; 1993p. 19–33.
27. Mascrés C, The Luu H, Magnan R. Influence of pulpal necrosis on experimental caries in the golden Syrian hamster. Actual Odontostomatol 1982;36:565–76.

Received for publication 14 April 1998
Accepted 9 July 1998