The effect of phenytoin medication on dentin apposition, root length, and caries progression in rat molars

Markku Larmas and Leo Tjäderhane
Institute of Dentistry, University of Oulu, Oulu, Finland

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To determine the effect of phenytoin (PHT) on dentin apposition and the rate of caries progression in dentin, 40 Wistar rats were treated daily for 35 days with intraperitoneal injections of 5,5-diphenylhydantoin. Twenty-eight controls received saline with pH adjusted to that of PHT. One PHT and one control group were fed a 43% sucrose diet, and the others a non-cariogenic pellet diet. *Streptococcus sobrinus* was inoculated to induce caries. Schiff staining was used to determine caries. The areas of dentin apposition and dentinal caries lesions were quantified after tetracycline staining. The root lengths were measured. PHT reduced slightly the dentin apposition and activated significantly the progression of the dentinal carious lesions. No difference in caries initiation was found. The high-sucrose diet reduced dentin apposition and increased the rate of progression of existing caries greatly. Our study suggests that both the high-sucrose diet and PHT have an effect on secreting odontoblasts, which can be seen as an alteration in dentin apposition and caries progression rates in dentin.
□ *Anticonvulsant; Ca antagonist; dentinal caries; rat*

Leo Tjäderhane, Institute of Dentistry, Aapistie 3, SF-90220 Oulu, Finland

Phenytoin (diphenylhydantoin (PHT)) is a widely used antiepileptic drug that has been successfully used for treatment of grand mal epilepsy. The effect of PHT is believed to be mediated through an influence on calcium transport across plasma membranes and on intracellular calcium movements (1, 2). This limiting action of the drug on calcium fluxes across cell membranes could underlie a common inhibitory mechanism on all secretory cells (1), and thus the function of odontoblasts should be regulated by it during the secretory phases of odontogenesis. There are a few experimental studies supporting this possibility. Tooth size and morphology have been shown to be altered by PHT (3, 4). Girgis et al. (5) demonstrated decreased crown to root ratios and excessive root resorption in histologic examination of mentally retarded children receiving PHT and phenobarbital. However, short dental roots were not necessarily associated with high serum PHT levels.

Larmas et al. (6) have previously suggested that the rate of caries progression in dentin might be cell-mediated, and thus PHT, the well-known regulator of cell secretion, serves as a model for the controlled analysis of the basic cellular mechanisms involved in dentin. The aim of this study was to ascertain the effect of PHT on A) dentin apposition, B) caries prevalence, and simultaneously, C) rate of caries progression in young rats with growing molar teeth.

Materials and methods

Sixty-eight Wistar rats were weaned at the age of 21 days, weighed, marked, and divided into groups. The animals were housed two to four per cage under normal atmospheric conditions at 21°C and subjected to the same regimen of lighting (12 h of light and 12 h of dark) and the same times of feeding, handling, and noise. All the animals

were given an intraperitoneal injection of oxytetracycline hydrochloride (30 mg/kg; Terramycin®, Pfizer Corp., Brussels, Belgium) at the onset of the experiment to mark the areas of dentinal carious lesions and dentin apposition during the test period. All were then inoculated with a fresh suspension of *Streptococcus sorbrinus* (ATCC 27531 K 1 Fitzgerald) on days 2 and 3 of the experiment and then weekly.

The animals were divided into four groups. Groups 1 and 2, 20 animals in both groups, received intraperitoneal injections of 5,5-diphenylhydantoin sodium salt (Sigma, St. Louis, Mo., USA) dissolved in physiologic saline solution, dissolution being promoted with 0.1 mol/l sodium hydroxide. The drug dose injected was 75 mg/kg. Groups 3 and 4, with 14 animals in each, received control injections of physiologic saline with pH calibrated to that of PHT injections (pH 11) with 0.1 mol/l sodium hydroxide. All injections were given daily using Terumo 26 G needles (Terumo Europe N.V., Leuven, Belgium) and syringes with 0.01 ml graduation. Groups 1 and 3 were fed a modified Stephan–Harris diet containing 43% sucrose. Groups 2 and 4 were fed a non-cariogenic powdered pellet diet (Brood Stock Feed for Rats and Mice R3, Ewos AB, Södertälje, Sweden).

After 5 weeks of the experiment the animals were weighed and killed by decapitation under ether anesthesia. The jaws were defleshed and preserved in absolute ethanol.

The lower jaws were sectioned sagittally by the technique of Keyes (7). To measure the size of dentin apposition and dentinal caries lesions, each molar (further referred to as M I, M II, and M III from the first to the third molar, respectively) was examined under an Orthoplan Ploemopak microscope equipped with incident fluorescent light (wavelength, 460 nm), upon which the tetracycline-stained lesions and the stripes surrounding the newly formed dentin could be readily seen (8). The main central transverse fissure of each lower mandibular molar was photographed with Kodak Ektachrome daylight film (400 ASA). The dentinal lesions and tetracycline-marked zones of dentin apposition were measured from the video images by circumscribing their respective areas on a monitor (Salora 445 A RGB, Salo, Finland; Hitachi VKM 96 E camera, Japan) using a serial 'mouse' connected to a PCVision Frame Grabber (Imaging Technology, Inc., Woburn, Mass., USA).

The sections were also stained with Schiff reagent, and the number of intact fissures and fissures with enamel, small dentinal, and advanced dentinal lesions was counted by the method of König et al. (9).

To determine the effect of PHT on the length of molar teeth, the upper molars were extracted. The upper jaws were boiled in 1% KOH solution to facilitate the extractions without root fractures. The tooth lengths were measured from the apex of the most prominent root to the adjacent cusp. In M I, in which the mesiobuccal root differs greatly from the others, being much longer, a distobuccal root was also measured. To avoid the effect of attrition, the root length was measured from the apex to the cemento-enamel junction. All fractured roots were excluded. The measurements were made at ×40 magnification using the microscope eyepiece scale with a 0.01-mm graduation.

Any differences in the areas of dentin apposition, root length, and final body weight and in the areas of dentinal caries lesions in the high-sucrose diet group were analyzed with confidence interval analysis. Fisher's exact two-tailed test was used to test the significance of any differences in the number of sound fissures and caries lesions of various depths between the groups. After the teeth with no dentinal caries in non-sucrose groups had been excluded, the differences in the areas of dentinal carious lesions were tested with confidence interval analysis.

Results

PHT reduced dentin apposition slightly when administered to rats fed the pellet diet ($p < 0.05$ in M I and $p < 0.01$ in M II) and had no effect on dentin apposition in the high-sucrose diet groups.

The high-sucrose diet markedly reduced dentin apposition. That reduction was stat-

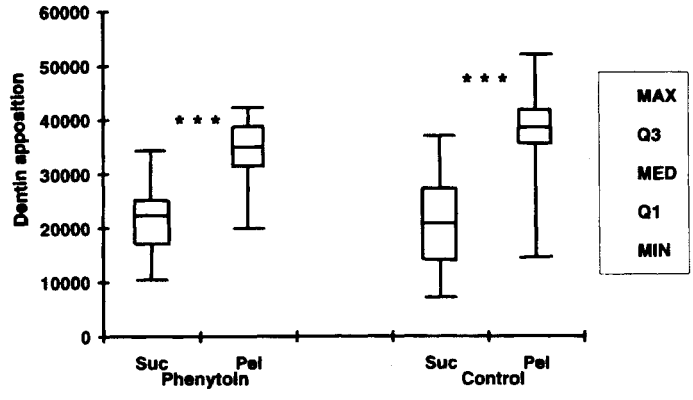

Fig. 1. The box plot comparison of dentin apposition between sucrose and pellet diets in a first molar. Suc = sucrose diet; Pel = pellet diet. Unit on y-axis is square micrometers. (*** = $p < 0.001$: confidence interval analysis). The box plot presents maximum (MAX) and minimum (MIN) values, and median (MED) with upper and lower quartiles (Q3, Q1) in each group.

istically highly significant in every tooth ($p < 0.001$, except $p < 0.01$ in M III in PHT groups) (Fig. 1).

With the pellet diet PHT caused a reduction in the length of the long mesiobuccal root ($p < 0.001$) of M I but not in the distobuccal root. In M II, root length reduction was observed with the high-sucrose diet in both PHT ($p < 0.05$) and control ($p < 0.05$) animals. When the PHT group with the high-sucrose diet was compared with the control group with the pellet diet, root reduction was significant in M I ($p < 0.001$) and M III ($p < 0.05$).

PHT had no significant effect on the number of intact fissures. In PHT groups the number of more advanced lesions was higher than in controls with both the sucrose and pellet diet ($p < 0.001$ and $p < 0.05$, respectively), and in control groups the number of enamel lesions was higher with both diets ($p < 0.001$) (Table 1). The rate of caries progression was significantly increased by PHT with the cariogenic diet ($p < 0.01$ in M I; $p < 0.05$ in M II; $p < 0.001$ in M III) (M I presented in Fig. 2).

PHT reduced weight gains slightly both in high-sucrose and pellet diet groups. These

Table 1. Number of fissures in accordance with the depth of caries penetration (percentage within parentheses)

Group*	Intact fissures	Enamel lesions	Dentinal lesions	Not measured†	No. of fissures
PHT + sucrose	42 (17.5)	73 (30.4)	115 (48.0)	10 (4.2)	240
Ctr + sucrose	27 (16.1)	81 (48.2)	46 (27.4)	14 (8.3)	168
PHT + pellet	159 (66.3)	48 (20.0)	13 (5.4)	20 (8.4)	240
Ctr + pellet	99 (58.9)	63 (37.5)	0 (0)	6 (3.6)	168

† Indicates fissures which proved impossible to classify accurately.
* PHT = diphenylhydantoin; Ctr = control.

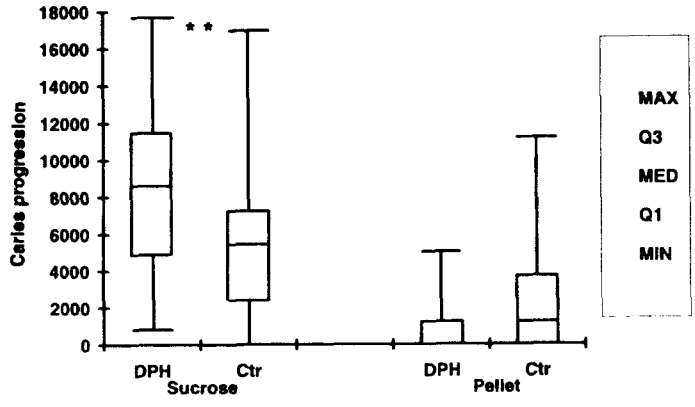

Fig. 2. The box plot comparison of areas of carious lesions in molars with caries (sound fissures excluded) between phenytoin and control groups with different diets in a first molar. Unit on y-axis is square micrometers. (** = $p < 0.01$: confidence interval analysis). DPH = phenytoin group; Ctr = control group; other abbreviations as in Fig. 1.

reductions were, however, not statistically significant.

Discussion

The PHT-induced reduction in dentin apposition was apparent with the animals fed the pellet diet. The same effect, however, was not seen in the animals fed the high-sucrose diet. The high-sucrose diet itself, with or without PHT, effectively reduced dentin apposition during primary odontogenesis, an observation supporting earlier findings (6, 10).

The effect of PHT on dentin apposition is in harmony with the results showing decreased dentin formation in long-term PHT treatment of rabbits (11). This could be an odontoblastic expression of the Ca antagonism that PHT has been found to have in osteoblastic cells (1, 12) and in other cells of different origins (2, 13–15). It has recently been shown that PHT induces an increase in intracellular $^{45}Ca^{2+}$ accumulation in gingival fibroblasts, which is at least partly caused by reduction in Ca efflux (16).

The rat molar is known to produce secondary cementum (in M I from the age of 35 days onwards) (17), which occurs concomitant with the functional stresses imposed on the teeth. Primary dentin apposition in the root area is rather slow, only 2 μm/24 h, which is only one-eighth of the maximal primary dentin apposition found in cuspal tips (17). Because dentin apposition must be faster in the longer than in the shorter root of the same tooth, the findings in the mesiobuccal root of M I further indicate that the metabolically active odontoblasts are more sensitive to PHT. In this study in M III, a significant difference was found only when the PHT group with the high-sucrose diet was compared with the control group with a pellet diet ($p < 0.05$), even though the root was completely formed during the experiment.

Previous studies have demonstrated a weight reduction caused by intraperitoneal injections (3) and/or oral administrations (4) of PHT. The small effect seen in our study may be caused by the relatively small dose used in injections.

It is known that reparative dentin apposition may be mediated by pulpal fibroblasts (18). In this study, however, the dentin apposition on the crown area is mainly involved, representing primary dentinogenesis, and thus it is less probable that the possible effects of PHT on other tissue elements in the dental pulp caused reparative dentin formation. It is also possible that the medication may influence the immune response in the pulp, since many of the events of immune reactions are dependent on calcium homeostasis. This effect, however, could not be studied by this study protocol.

Because there were no differences in the

number of intact fissures in PHT and control animals in cariogenic or non-cariogenic diet groups, the drug had no effect on the initiation of caries. The number of more advanced dentinal caries lesions was higher in PHT-medicated rats than in controls, suggesting that PHT activated the rate of caries progression when caries had reached dentin (Table 1).

It has previously been found that fluoride in drinking water does not diminish the reducing effect of the high-sucrose diet on dentin apposition (19) but does reduce the rate of caries progression in dentin at concentrations of 1 and 19 ppm. PHT worked the opposite way: it corrected the reducing effect of sucrose on dentin and increased the dentinal caries progression. Fluoride is known to be an activator of adenylate cyclase (20, 21), which in turn activates the formation of cAMP from adenosine triphosphate. PHT, a calcium antagonist (1, 2, 12), is a potential adenylate cyclase inhibitor via the calcium/calmodulin-dependent adenylate cyclase pathway (2, 21, 22). The opposite effects of fluoride and PHT on caries progression may thus be transformed by the alterations in cAMP formation in odontoblasts.

Our finding would propose that secreting odontoblasts are the target cells of the drug and the effect of the drug is expressed via accelerated caries progression in dentin. This is also supported by the finding of differences in the sizes of dentinal lesions between the groups. These findings support the hypothesis that caries progression in dentin is partly cell-mediated (6). It remains obscure whether there is a cause-related relationship between diminished dentin apposition and accelerated caries progression.

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