Effects of phenytoin on dental caries and plaque in *Streptococcus sobrinus*-infected rats

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Abstract

Effects of phenytoin (PHT) administration on dental caries in rats infected with Streptococcus sobrinus 6715 were investigated. Twenty-day-old specific pathogen-free Fischer male rats were infected with S. sobrinus 6715 and fed diet 2000 containing 56% sucrose with or without PHT for 52 days. Antibody responses against anti-S. sobrinus in serum and saliva failed to show a statistical difference between PHT-treated and nontreated rats. These results indicate that PHT treatment increased plaque deposition and dental caries in the rats infected with S. sobrinus 6715 and fed diet 2000 containing PHT (1–2 mg/g), as compared with those similarly infected and fed diet without PHT ($P < 0.001$). (Pediatr Dent 14:322–25, 1992)

Introduction

Phenytoin (PHT 5, 5-diphenylhydantoin) has been used widely to control epileptic seizures. However, the use of PHT frequently has resulted in several side effects,[1] including gingival overgrowth, which was first reported by Kimball and Horan[2] in 1939. Since then, many reports have been published concerning PHT's effect on gingival tissue in human and experimental animals, or cultured gingival fibroblasts.

It has been emphasized that plaque control is very important for periodontal health in patients taking PHT. However, few reports described the relationship between PHT treatment and dental caries. It was suggested that PHT retarded dental caries development in rats[3] and humans.[4, 5] Other reports indicated that the caries incidence of PHT-treated patients was not significantly higher than that of the epileptic patients taking carbamazepine,[6] or that of normal subjects.[7] Thus, there is a controversy in the information about PHT's effects on dental caries development. This could be attributed to differences in standardizing the environmental conditions that may affect cariogenicity or caries susceptibility in man. To overcome or minimize the environmental variations, this study used an animal caries model system. The purpose of this investigation was to examine the influence of PHT administration on cariogenicity in rats infected with *Streptococcus sobrinus* and fed a caries-inducing diet.

Materials and Methods

Animals and Diets

Specific pathogen-free (SPF) rats (14-day-old, male) were obtained from Charles River Japan Inc. (Osaka, Japan). They were reared in a clean room at 25°C in an animal facility. The animals were fed an ordinary powdered diet (Diet CE-2®, Clea Japan Inc., Osaka, Japan) containing tetracycline (4 mg/ml) and were supplied drinking water containing penicillin G (4000 U/ml) during the 17th and 18th days of age.[8] This procedure suppressed the indigenous oral microflora and facilitated the establishment of *S. sobrinus* 6715 in the rats' oral cavities (Fig 1). They then were fed the caries-inducing diet 2000[9] (Clea Japan Inc., Osaka, Japan), and provided water *ad libitum* for 52 days. Four groups were used in the experiment. Groups A, PHT-infected (PHT-I), and B, infected (I), were infected experimental groups. Groups C, PHT-noninfected (PHT-NI), and D, noninfected (NI), were noninfected controls. Each group comprised 12 rats.

Inoculation and Recovery of *S. sobrinus* 6715

After treatment with antibiotics, the rats were infected orally with 10^{10} colony-forming units (cfu) of *S. sobrinus* 6715 twice a day on the 20th and 21st days of age. To monitor the establishment of inoculated *S. sobrinus*, saliva samples were taken from the rats' mouths with a sterile cotton applicator every two weeks from one week after the infection. They then were dispersed in 1 ml of sterile saline by ultrasonication, and 0.1 ml of these suspensions were plated and cultured on mitis-salivarius (MS) agar (Difco Laboratories, Detroit, MI)

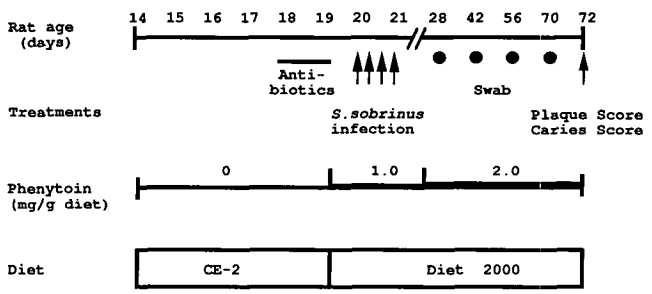

Fig 1. Protocol for rat caries experiment.

containing streptomycin (500 μg/ml). After incubation at 37°C for two days, the numbers of colonies on the plates were counted. Groups A and C were fed diet 2000 containing PHT (Wako Pure Chemical Industry Ltd., Osaka, Japan) ad libitum, while groups B and D were given the diet without PHT. The concentration of PHT in the diet was 1 mg/g for the first seven days after infection, and was increased to 2 mg/g until the end of the experiment.

Plaque Index and Caries Scores

Blood and pilocarpine-stimulated saliva samples were collected from each rat under Nembutal® (Dainippon Pharmaceutical Co., Ltd. Osaka, Japan) anesthesia at the end of the experiment, and then the rats were killed. Rat jaws were stained with a plaque-disclosing Red-Cote® solution (John O. Butler Co., Chicago, IL), and buccal, occlusal, and lingual plaque accumulation on each molar tooth was recorded under a dissection microscope using an index from grade 0 to 4, according to the method of Regolati and Hotz.[10] Rat jaws then were autoclaved at 115°C for 5 min, and defleshed. The degree of caries development of molar teeth in both the maxilla and mandible was evaluated under a dissecting microscope on the unsectioned jaw layout by the method of Keyes,[11] as modified by Hamada et al.[8] Student's *t*-test was used for statistical analysis of plaque and caries scores.

Measurement of Antibody Levels

Serum and salivary antibodies against *S. sobrinus* 6715 whole cells were titrated by an enzyme-linked immunosorbent assay (ELISA) as described previously.[12] Briefly, 100 μl samples of rat serum (x25 dilution) or saliva (x5) were added to the wells of flat-bottom microtiter plates (Sumitomo Bakelite Co. Ltd., Tokyo, Japan) onto which *S. sobrinus* whole cells had been coated in quadruplicate. Alkaline phosphatase conjugated sheep anti-rat IgG and IgA antisera were purchased from Serotec (Oxford, England) and appropriate dilutions were made. As a substrate of alkaline phosphatase, p-nitrophenylphosphate (Sigma 104, Sigma, St. Louis, MO) was used. Antibody responses are expressed as the arithmetic mean of ELISA readings at 405 nm from each group.

Results

At the end of the experiment, there was difference in the body weight gain between groups A (136.0 ± 7.3) and B (162.9 ± 8.9), and between groups C (134.9 ± 7.9) and D (157.8 ± 11.7 g, $P < 0.01$ by the Student's *t*-test). However, no statistical difference was found in weight gains between groups A and C or groups B and D.

Establishment of the inoculated *S. sobrinus* 6715 was confirmed one week after the challenge in groups A and B. The level of *S. sobrinus* colonization was not significantly different between these two groups throughout the experimental period. No *S. sobrinus* was detected in groups C and D. Average plaque scores of rat jaws from each group are shown in Fig 2. More plaque accumulation was found on mandibular than maxillary molars. PHT in diet 2000 markedly enhanced plaque deposition induced by *S. sobrinus*. Statistically significant differences in the average plaque scores were noted between group A and other groups.

Total caries scores for maxillae and mandibles in each experimental group are shown in Fig 3 (page 324). Group A rats developed significantly more caries in the upper and lower molars than group B rats ($P < 0.001$, Table, page 324). Phenytoin-treated and *S. sobrinus*-infected rats showed approximately twice the mean caries score of the rats infected alone. The first molars in the mandible showed the most severe caries, followed by the second and third molars. Most group A rats showed complete loss of the crowns of their mandibular first molars with the pulp chambers being exposed in some cases. The buccal surfaces of the mandibular molars were destroyed more severely than the lingual. Incipient caries lesions were observed in the rats of groups C and D, which had not been infected with *S. sobrinus* 6715.

Serum anti-*S. sobrinus* 6715 IgG and IgA antibody responses in rats from each experimental group are also shown in Table 1. The highest serum anti-*S. sobrinus* IgG antibody responses were found in group B rats. PHT-treated group A rats showed 22% less average IgG

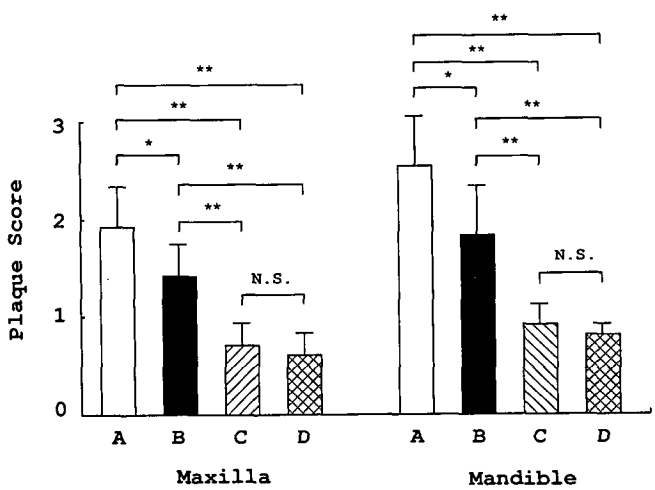

Fig 2. Effects of phenytoin treatment on plaque deposition on molar surfaces in Fischer rats, N = 12 rats for each group. Bars are the mean plaque score ± SE from groups A, PHT-I, (□); B, I, (■); C, PHT-NI, (▨); and D, NI, (▩) rats. Statistical analyses were made (N.S., not significant; *; $P<0.01$; ** $P<0.001$) by Student's *t*-test.

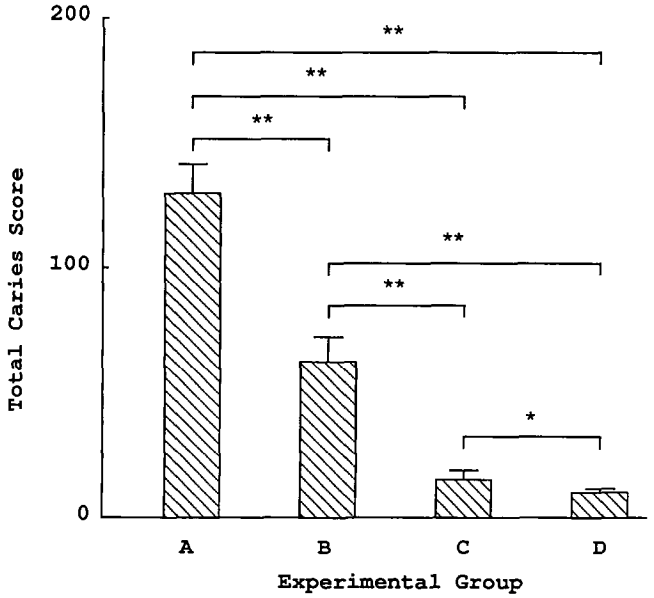

Fig. 3. Effects of phenytoin on rat caries. Bars are the average total caries scores, the sum of caries scores from maxillary and mandibular molars from groups A, PHT-I; B, I; C, PHT-NI; and D, NI, of each experimental group; N = 12 for each group. Statistical analyses were made (*$P < 0.05$; **$P < 0.001$) by Student's *t*-test.

antibody titer than untreated group B rats. However, those IgG as well as IgA antibody responses were not significantly different in any combination of experimental group irrespective of PHT-treatment and *S. sobrinus* infection. Although salivary anti-*S. sobrinus* antibodies were measured in the same manner as those of serum samples, no detectable IgG or IgA antibody responses were obtained (data not shown).

Discussion

More than 500 studies concerning PHT-induced gingival overgrowth were reviewed by Hassell.[1] Further investigations have been conducted; however, few studies attempted to correlate PHT treatment and dental caries development in man or animals. Our study clearly indicates that the rats fed diet 2000 containing PHT and infected with *S. sobrinus* 6715 showed significantly higher caries incidence, as well as higher plaque scores than those infected without PHT treatment. Enhanced plaque accumulation and caries incidence in rats treated with PHT could be attributed to overgrown gingival tissue.[13]

In our experiment, no statistical difference was detected in humoral immune response to infected *S. sobrinus* whole cells. Furthermore, although it was reported that PHT treatment decreased salivary IgA responses in humans,[14] no detectable levels of salivary IgA antibody to *S. sobrinus* 6715 could be found in this study, irrespective of PHT administration.

Under experimental conditions, the serum concentration of PHT in the rats given PHT was shown to be 13–14 μg/ml.[13] This level is similar to that found in patients prescribed PHT. In this regard, significantly lower body weight gains were noted in groups A and C treated with PHT, compared with groups B and D rats. However, PHT-treated group A rats had significantly greater caries scores than group B, the untreated controls. König et al.[15] showed that a higher incidence of caries occurred in rats in taking cariogenic diets more frequently. Furthermore, Huxley[16] reported a positive correlation between weight gain and the incidence of carious lesions under the same diet conditions except for eating frequency. Because an automatic feeding machine was not available in our laboratory, these results could not be compared directly with the results of our study.

There are several possible explanations for the results of our study. 1) The severe dental caries found in PHT-treated rats may be attributed to the accumulation of dental plaque following PHT-induced gingival overgrowth.[13] 2) PHT decreased the production of IgG antibodies in the rats, lowering their immunodefense mechanism.[17] 3) PHT treatment changed the salivary components or flow rate. 4) PHT or its metabolites might have acted directly on *S. sobrinus* and enhanced its cariogenicity. 5) PHT might have altered the formation of the dental hard tissues such as dentin formation[18] and tooth size.[19] Further experiments will be required to clarify the effects of PHT on dental diseases such as dental caries and periodontal disease, and formation of the dental hard tissue. We hope that these studies will

Table. Effects of phenytoin on caries and antibody responses in Fischer rats*

Group	*Caries Scores*		*Anti-S. sobrinus response*†	
	Maxilla	*Mandible*	*IgG*	*IgA*
A	37.1 ± 5.0	93.2 ± 5.9	0.31 ± 0.06	0.050 ± 0.01
B	14.9 1.7	47.5 7.9	0.40 0.08	0.074 0.01
C	1.2 0.2	14.1 2.8	0.25 0.08	0.056 0.01
D	0.7 ± 0.1	9.0 ± 0.9	0.26 ± 0.04	0.063 ± 0.01

* Arithmetic mean ± SE; † IgG and IgA antibodies were measured by ELISA (A 450 nm).
Group A = PHT-I; Group B = I; Group C = PHT-NI; Group D = NI. N = 12 rats for each group.

clarify the relationship between PHT treatment in epileptic patients and dental caries development.

Conclusions

1. Phenytoin treatment enhanced plaque deposition and dental caries in *S. sobrinus*-infected rats.
2. No statistical difference of antibody responses to *S. sobrinus* was found between PHT-treated rats and untreated rats.

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