Influence of rampant caries in dams on caries activity in their offspring

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Abstract

Available evidence indicates that infants, for the most part, acquire their oral flora, in particular Streptococcus mutans, from their mothers. The purpose of this study was to explore the effect of rampant caries in rat dams on subsequent caries activity in their offspring. Rampant caries was induced by surgical desalivation of four dams, and subsequent infection with Streptococcus sobrinus 6715. Two intact dams served as controls. Desalivated dams, having rampant caries, reared litters that experienced significantly higher caries scores ($P = 0.0001$) on smooth and sulcal surfaces than litters from intact dams. Fluoride given to the dams did not affect caries scores of their pups. The results suggest that the magnitude and virulence of the maternal reservoir of S. sobrinus is a critical factor in the primary infection and caries activity in the offspring. Results support the concept that caries activity in mothers should be controlled in any program designed to prevent caries in infants and children. (Pediatr Dent 13:361–66, 1991)

Introduction

Streptococcus mutans plays a prime etiological role in the initiation of dental caries in humans and in experimental animals (Keyes 1960; Krasse et al. 1967; Gibbons and Van Houte 1975; Loesche 1986). *S. mutans* usually is absent from the oral cavities of human newborn infants (Berkowitz et al. 1980). Nevertheless, the most children eventually become infected with *S. mutans* (Socransky and Manganiello 1971). The magnitude of the maternal reservoir of *S. mutans* is related to the risk of the infant becoming infected by *S. mutans* (Köhler and Brathall 1978; Berkowitz et al. 1980, 1981; Van Houte et al. 1981). High maternal levels of *S. mutans* infection, and frequency of contact of mothers with infants are important in early infection of infants. Nevertheless, the virulence of the organisms requires investigation because it partly determines successful infection in a host for many infectious diseases (Benenson 1980). Childhood acquisition of *S. mutans* and subsequent caries development may be delayed or avoided by reducing the virulence of the transmitted *S. mutans*, or by suppressing the maternal flora.

The dramatic decline in caries prevalence in developed countries in recent years has been attributed to widespread fluoride use. It is possible that in addition to its action at the tooth surface (ten Cate and Duijsters 1983), continual exposure to fluoride has resulted in the selection of a less virulent *S. mutans*, even though we lack direct experimental evidence that this has occurred. Fluoride decreases the acid tolerance of oral streptococci, reducing their potential to survive at low pH values (Bender et al. 1986; Marquis 1990). Caries challenge can be increased by the removing salivary function in humans (Brown et al. 1976) or rats (Schwartz and Shaw 1955; Bowen et al. 1988a). Previous experiments have shown that compared to intact rats, desalivated animals become infected with *Streptococcus sobrinus* rapidly and are colonized by significant levels of this pathogen. Intact animals caged with desalivated animals develop significantly more caries than do intact animals caged together (Bowen et al. 1988a). S. sobrinus reisolated from a desalivated animal is more virulent than *S. sobrinus* isolated from intact animals based on its ability to induce caries (Bowen et al. 1988a; Madison et al. 1990).

Diet and microorganisms clearly play a major role in the pathogenesis of dental caries, but the importance of the virulence of organisms acquired by infants from their mothers is unexplored. The purpose of this investigation was to determine the effect of rampant caries in rat dams on the subsequent bacterial colonization and caries development in their offspring.

This experiment used the desalivated rat model to examine the transfer of *S. sobrinus* from dams with rampant caries to their offspring under controlled conditions of specific infection, diet, and time. Fluoride administration to the dams also was investigated to determine whether environmental selection altered the microorganisms or influenced the virulence of the transferred bacteria. Reducing the virulence of *S. mutans* may be an effective method for controlling dental caries — the principle of reduction has been established for other infectious diseases, such as measles, influenza, and hepatitis (Benenson 1980).

Materials and Methods

Animals

Six specific pathogen-free Sprague Dawley dams were received from Charles River K92 facility (Kingston, NY) for breeding purposes. They were screened as previously described (Bowen et al. 1988b). Upon arrival, the dams were offered laboratory chow (Purina pellet, Ralston-Purina, Richmond, IN, < 10 ppm fluoride ion) and sterile distilled water *ad libitum*. These animals were infected on two consecutive days with an actively growing culture of *S. sobrinus 6715* (rat reisolate P10JK4, which originally had been isolated from a human and recently passaged through two desalivated animals) using oral swabbings. Four dams were desalivated surgically as previously described (Bowen et al. 1988b); the other two dams remained intact.

Pregnancy

The dams were allowed to mate with one of two male Sprague Dawley rats. Pregnancy was confirmed by the presence of a vaginal plug, abdominal enlargement, and lack of estrus behavior. Each dam was housed alone in a plastic cage during pregnancy and offered a diet of pellet chow and sucrose-sweetened water (10% w/v) to aid colonization of *S. sobrinus*.

Following confirmation of pregnancy, the dams were infected with a suspension of *S. sobrinus* P10JK4 in exponential growth phase on two successive days. The six dams delivered a total of 88 pups.

After parturition, all dams were offered Diet 2000 (56% sucrose, Zeigler Brothers, Gardners, PA) in addition to 10% sucrose water *ad libitum*.

Birth Until Weaning

At 7 days of age the gender of the litters was determined, and the animals were culled so that each litter contained 10 or 11 pups. The litters were mixed to provide even representation of litters in each of the experimental groups. Two desalivated dams and one intact dam with their reorganized litters were maintained on Diet 2000 and 10% sucrose water. The remaining three dams received 20 ppm fluoride ion (in the form of sodium fluoride) in sweetened water in addition to Diet 2000. The experimental groups were as follows:

Group 1. Pups reared by desalivated dams fed Diet 2000 and 10% sucrose water until weaning (N = 14).

Group 2. Pups reared by desalivated dams fed Diet 2000 and water containing 10% sucrose and 20 ppm fluoride ion until weaning (N = 16).

Group 3. Pups reared by intact dam fed Diet 2000 and 10% sucrose water until weaning (N = 10).

Group 4. Pups reared by intact dam fed Diet 2000 and water containing 10% sucrose and 20 ppm fluoride ion until weaning (N = 10).

Experimental Period

Pups were weaned at 21 days. Each group was housed together in plastic cages to equilibrate the level of *S. sobrinus* achieved within each group. All groups were fed Diet 2000 and 10% sucrose-sweetened water. No fluoride was administered to any of these animals after weaning. Dams were housed separately and resumed their diet of pellet chow and 10% sucrose-sweetened water. Infection of all animals with *S. sobrinus* P10JK4 was determined weekly on mitis salivarius agar containing 200 μg/ml streptomycin sulfate (MSS). One week after weaning, the pups were paired with a littermate and housed together in suspended cages. Following five weeks of caries challenge, the animals were killed in a CO_2 atmosphere. Their lower left jaws were dissected and prepared for bacteriological examination as previously described (Bowen et al. 1988a). Coronal caries, both sulcal and smooth, was scored using the Keyes method (1958), as modified by Larson (1981).

Statistical Analysis

Caries scores and microbiological data were analyzed using the ANOVA program of STATVIEW II™ Statistical Software package (Abacus Concepts, Berkeley, CA). A *P*-value less than 0.05 was considered statistically significant.

Results

Desalivated dams were colonized readily by *S. sobrinus* after initial infection. Intact dams became infected during pregnancy while being fed 10% sucrose-sweetened water. Eighty-eight pups were born to the six dams; 50 remained in the experiment following culling by gender and weight. All litters became infected with *S. sobrinus*, but the rate of colonization differed (Table 1, see next page). At weaning age (21 days), all the pups of desalivated dams showed signs of *S. sobrinus* infection. Litters reared by intact dams were colonized by *S. sobrinus* more slowly. The litter reared by the intact dam not exposed to fluoride took an additional two weeks to show signs of infection. It took four weeks after weaning for all pups of the intact dam that received fluoride to become colonized by S. *sobrinus*.

Smooth and sulcal caries developed in all litters during the five-week challenge. Pups from desalivated dams developed the most decay, with Keyes scores of 42.1 (± 17.7) on smooth surfaces and 37.4 (± 5.8) on sulcal surfaces (Fig 1, see page 364). In contrast, animals reared by intact dams had significantly lower smooth surface and sulcal caries scores (ANOVA, $P = 0.0001$). Rampant caries developed in the desalivated dams. Caries scores were negligible in the intact dams (Fig 2, see page 364). The protective effect of 20 ppm fluoride in the drinking water was evident in the desalivated dams.

Table 1. Rate of infection of litters with *S. sobrinus* from their dams throughout the experiment

	Weaning	*Week 1*	*Week 2*	*Week 3*	*Week 4*
Group 1 (Litters of desalivated dams)	14/14	14/14	14/14	14/14	14/14
Group 2 (Litters of desalivated dams exposed to F)	16/16	16/16	16/16	16/16	16/16
Group 3 (Litter of intact dam)	2/10	8/10	10/10	10/10	10/10
Group 4 (Litter of intact dam exposed to F)	1/10	3/10	7/10	8/10	10/10

F = Sodium fluoride at 20 ppm in drinking water.

The dams that were exposed to fluoride had Keyes scores for smooth caries of 24.5 (± 12.0) and sulcal caries of 10 (± 8.5), compared with 44 (± 4.2) for smooth and 30.5 (± 3.5) for sulcal scores in the dams not exposed to fluoride. Exposing the dams to 20 ppm fluoride in the drinking water did not affect the caries scores in their litters. Buccal, lingual, and proximal surfaces were examined separately to determine whether site-specific patterns of decay were present. No significant differences in caries scores existed among sites, even in those animals whose dams had received fluoride.

Total cultivable flora and recovery of S. *sobrinus* were 10-fold higher in the desalivated dams than in the intact dams. No differences in microbial recovery counts were noted among the dams exposed to fluoride and the dams that were not exposed to fluoride. Total cultivable flora recovered from litters of intact dams (Groups 3 and 4) was significantly lower than that recovered from the pups reared by desalivated dams (ANOVA, $P = 0.0001$). The quantity of cultivable flora recovered from the litters reared by desalivated dams exposed to fluoride was lower than in the animals whose dams did not receive fluoride, but did not reach significance at $P < 0.05$ (Table 2).

S. sobrinus recovery from all pups reared by desalivated dams was significantly higher (ANOVA, $P = 0.02$) than the litters of intact dams (Table 2). Populations of *S. sobrinus* expressed as a percentage of total cultivable flora indicated that fluoride exposure in the dams influenced the contribution of *S. sobrinus* to the composition of the oral flora in their offspring, but apparently did not reduce caries development. Pups of desalivated and intact dams that received fluoride had a smaller percentage of *S. sobrinus* in their total cultivable flora than litters of the dams that were not exposed to fluoride.

Discussion

The high levels of infection by *S. sobrinus* and rampant caries in desalivated dams confirms previous work in our laboratory (Bowen et al. 1985, 1988a, b; Madison et al. 1989, 1990). Our results suggest that desalivation selects a highly cariogenic population of *S. sobrinus*, which is transmitted to and maintained in the offspring. The extent of the caries in the pups of desalivated dams was even greater than that seen in previous results from this laboratory. The virulent organisms were present in the oral cavity during the period of tooth eruption in the present study, whereas previous work on transmission in this laboratory used older animals as recipients. Newly erupted teeth are highly susceptible to demineralization because enamel is hypomineralized for approximately one week following eruption.

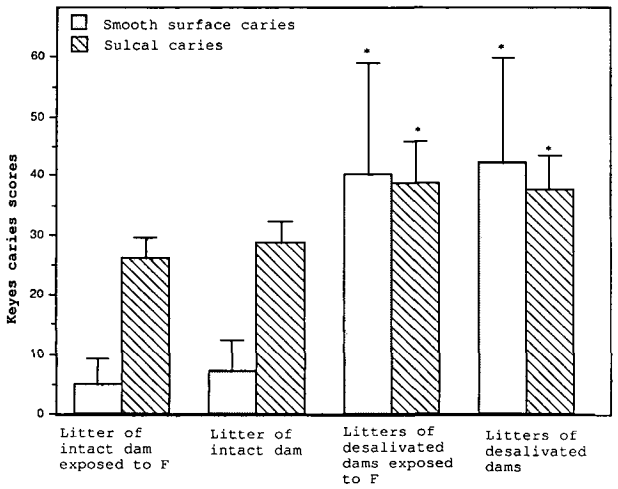
Table 2. Bacterial viable counts and counts of *S. sobrinus* in colony-forming units ($x10^6$) from all litters

	Total cultivable flora ($x10^6$)	*Total S. sobrinus recovery ($x10^6$)*	*% S. sobrinus*
Group 1 (Litters of desalivated dams)	44.25 ± 15.9*	18.04 ± 9.3*	40.99
Group 2 (Litters of desalivated dams exposed to F)	40.09 12.2*	11.88 7.5*	28.66
Group 3 (Litter of intact dam)	19.73 15.5	9.64 9.2	41.66
Group 4 (Litter of intact dam exposed to F)	25.6 ± 4.16	8.45 ± 2.7	32.88

Mean ± SD

* Significant at $P < 0.0001$ from group 3 and 4 (ANOVA)

F = Sodium fluoride at 20 ppm in drinking water



KEY

Mean ± SD

* Significantly different to intact groups (p= 0.0001,ANOVA).

F = Sodium fluoride at 20 ppm in drinking water.

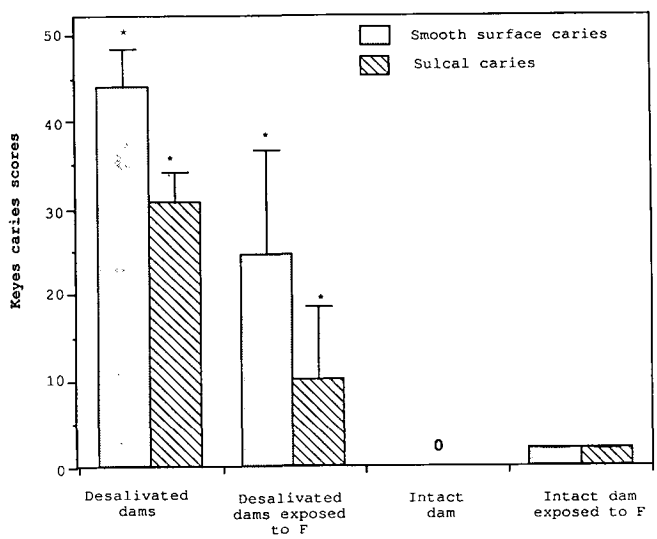
Fig 1. Smooth surface and sulcal scores of the experimental litters.

In addition to being more acidogenic and aciduric, strains of *S. sobrinus* with increased virulence may be more effective colonizers of the oral cavity than unaltered organisms. The rate at which the offspring acquired infection from the dams differed among the groups (Table 1). The selection of highly aciduric *S. sobrinus* at elevated levels by desalivation of the dams resulted in rapid, complete colonization of their offspring. Successful colonization by *S. sobrinus* took up to four weeks after weaning in all pups of the intact dams. The dams, who were the only source of *S. sobrinus* infection were removed at weaning. Transmission between the intact dams and their pups must have occurred within this time, but the levels of infection were undetectable by our methods during this period.

Implantation experiments in rodents indicate that a minimum infectious dose of streptococcal cells is not necessary for successful infection if the frequency of inoculation is increased (Ooshima et al. 1988a, b; Van Houte et al. 1976, 1977). The inherent ability to colonize, an aspect of virulence, may be of greater significance than the size of inoculum in establishing infection. Previously, we have investigated the issue of the cariogenicity of micro-organisms vs. the level of infection in rats. The population of *S. sobrinus* in desalivated animals was suppressed by daily application of chlorhexidine (Bowen et al. 1988a). The reduced level of infection in chlorhexidine-treated rats did not alter caries experience in their cagemates when compared with controls, suggesting that the virulence, and not simply the level of infection, influences caries development. However, the relative importance of virulence, size, and frequency of inoculum cannot be established from this study, since the pups of the desalivated dams that developed rampant caries also became infected rapidly and maintained the highest levels of infection with *S. sobrinus*.

The cariostatic effect of fluoride is attributed to its inhibition of demineralization and enhancement of remineralization at the tooth surface. However, fluoride also may affect bacterial metabolism by decreasing the efficiency of proton extrusion via ATPases from the bacterial cell (Marquis 1990; Bender et al. 1986). The outcome is a reduction in the acid production by the organism, and an attenuated pH drop in the oral environment (Hamilton 1990). The extent of a cariogenic challenge depends on the magnitude and duration of the drop in pH at the tooth surface; any reduction in acid tolerance of the bacteria will render it a less cariogenic organism. Our hypothesis proposed that animals exposed to fluoride would maintain less cariogenic populations of *S. sobrinus*, independent of any effect of fluoride on enamel, and that these organisms would be transferred to and established in the offspring.

Caries was reduced in the dams exposed to fluoridated drinking water but no effect on the caries development was seen in any of the pups. The contribution of *S. sobrinus* to total cultivable flora in litters reared by desalivated dams was greater (41%) than in those of



KEY

Mean ± SD

0-Intact dam experienced zero caries.

*Statistically significant from intact dams at p< 0.05(ANOVA)

F-Sodium fluoride at 20 ppm in drinking water

Fig 2. Caries experience in dams throughout entire experiment.

dams exposed to 20 ppm fluoride (29%). Our results are not consistent with the hypothesis that fluoride can select for less virulent organisms.

However, further investigation is indicated to explore whether alternate fluoride regimens can select for populations of *S. sobrinus* with reduced virulence, and whether mutations or phenotypic adaptations are induced by bacteria to overcome unfavorable environmental conditions.

The importance of virulence and level of infection in the successful transfer of cariogenic organisms and the evidence that transmission of bacteria is primarily from mother to infant have significant clinical implications. Rampant caries in mothers increases the risk of high caries activity in their children (Klein 1938, 1946). Caries prevention for children may be enhanced by manipulating the oral flora in the mother. Evidence exists that suppression of cariogenic bacteria can be achieved in mothers by diet counselling, chlorhexidine treatment, and restorative care (Köhler et al. 1982, 1983, 1984). People who harbor cariogenic bacteria with increased virulence may potentially increase the risk of caries activity among those living in their immediate vicinity. Patients with salivary gland hypofunction, those receiving radiation therapy for malignancies, and those with rampant caries may harbor high levels and potentially virulent strains of *S. mutans*. Caries prevention programs should target these individuals, and those who are in close contact with them.

Conclusions

In conclusion, the results from this study indicate that transmission of *S. sobrinus* from dams to their litters influences the subsequent caries development in the pups. Fluoride did not select a less virulent population of *S. sobrinus* as measured by caries development. The possibility of such a selection cannot be ignored until the effects of fluoride on the organisms are clarified further. The infection of offspring can be transmitted from and influenced by the level and type of *S. sobrinus* infection in dams. The clinical implications of these findings suggest that as practitioners, we should strive to control caries activity in expectant parents and caretakers of young children to delay or prevent infection of the children with cariogenic organisms and reduce virulence of the transmitted bacteria.

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Cocaine use by men may harm fetuses

Cocaine use by men may be a factor in abnormal fetal development, according to a study published in the October 9, 1991 issue of the *Journal of the American Medical Association.*

"These results support the hypothesis that sperm may act as vectors to transport cocaine into an ovum," according to Ricardo A. Yazigi, MD, from the Department of Obstetrics and Gynecology, Washington University School of Medicine, St. Louis, and colleagues. "This novel mechanism could be involved in the abnormal development of offspring of cocaine-exposed males."

The authors incubated prepared sperm specimens with cocaine solutions for 10, 20, 60, and 90 min at 0, 23, and 37°C (32, 73.4 and 98.6°F, respectively). They found that concentrations such as those used in the study may be achieved easily with doses of cocaine commonly used by human addicts.

The authors noted that their study clearly demonstrated the binding of cocaine to human spermatozoa, with optimal binding occurring at 23°C. Cocaine binding to sperm was optimal after 20 min of incubation, with a decline following longer incubation periods.

Additionally, they reported that cocaine had no effect on sperm motility or viability.

The authors recommend further study of the effects of drug use on fetal development, particularly in light of the epidemiologic data that demonstrate developmental abnormalities in offspring of cocaine users, and the findings of their study.