Aspartame and dental caries in the rat

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Abstract

Aspartame (NutraSweet®™ — The NutraSweet Co., Deerfield, IL) an artificial intense sweetener, was tested for its cariogenicity alone and in the presence of sucrose. Sprague-Dawley™ rat pups (Charles River Laboratories, Bloomington, MA) inoculated with **Streptococcus mutans** *were fed basal diet 2000 with one of the following added: 50% sucrose; 30% sucrose; 30% sucrose + 0.15% aspartame; 0.30% aspartame; 0.15% aspartame and no addition. The animals were sacrificed after eight weeks. Caries was evaluated using Keyes' technique. It was found that the addition of 0.15% aspartame to 30% sucrose diet significantly reduced caries in comparison to rats fed only 30% sucrose diet. In animals fed aspartame only, there was no caries. The* **S. mutans** *counts were high in the animals receiving sucrose diets with and without aspartame. The animals receiving only aspartame had very low* **S. mutans** *counts. (Pediatr Dent 13:217–20, 1991)*

Introduction

Sucrose, a natural sweetener used widely, is a major substrate in caries production, and has a high caloric content. A substitute sweetener is needed for people who must avoid sucrose because of obesity or diseases such as diabetes and dental caries.

Aspartame is the trade name of a dipeptide composed of the amino acids, L-phenylalanine and L-aspartic acid. It is a nonfermentable, intense sweetener proven safe for human consumption. Because of its low caloric contribution in producing the same level of sweetness as sucrose (it is approximately 160–200 times sweeter than sucrose), it is used extensively in "diet" products. The sweet taste of aspartame was discovered accidentally in 1965 by the Searle Research Laboratories in a search for an inhibitor of gastrointestinal secretory hormone, gastrin, as a possible treatment for ulcers. The structure of aspartame is +L aspartyl-L-phenylalanine-methyl ester. Aspartame is an odorless, white crystalline powder with a clean, sweet taste without any aftertaste or cooling effect. It is slightly soluble in water (about 1.0% at 25°C), sparingly soluble in alcohol and insoluble in fats and oils. Being a peptide, it is amphoteric and is metabolized extensively to release its constituent amino acids and methanol (Homler 1984).

Aspartame in dry products is fairly stable even at high temperatures. However, in solution, its stability is a function of time, temperature, pH and available moisture. Aspartame is most stable between pH values of 3 and 5 even with increasing temperature. However, it breaks down and loses its sweetness in normal cooking or baking. Thus its use is limited to table top sweetener (Equal®™ — The NutraSweet Co., Deerfield, IL) and as NutraSweet in dry foods, soft drinks, and frozen foods like ice cream.

There are few studies on the cariogenicity of aspartame. Most investigations have been limited to in vitro experiments and most have shown aspartame to have anticariogenic capabilities (Olson 1975, 1977; Linke and Chang 1976; Mishiro and Kaneko 1977; Grenby and Saldanha 1983; Linke 1983; Frank and Berry 1984; Fu and Bibby 1984; Thompson et al. 1986; Smidt et al. 1987). There are a few in vivo investigations related to the caries preventive potential of aspartame (Reussner and Galimidi 1982; Tanzer and Slee 1983; Lout and Messer 1987; Siebert et al. 1987). None of these have studied the effects of different levels of aspartame fed to rats.

The following report presents the results of an in vivo study to determine whether aspartame (at two different levels) in the presence or absence of sucrose, can influence the incidence of caries.

Methods and Materials

Pregnant Sprague-Dawley™ rats from Charles River Laboratories were inoculated with *S. mutans* 6715–13 (resistant to 200 μg/ml streptomycin) according to Larson et al.'s technique (1977) so that the pups could be infected via coprophagy (Ooshima et al. 1988). They were fed cariogenic diet 2000 (Teklad) and deionized, double distilled water. Basic experimental procedures for rats, such as caging and rotating of the pups among dams, were observed (Navia 1981). The pups were weaned at day 17. All continued on the same diet and received fresh cultures of *S. mutans* on days 17, 18, and 19 in the drinking water. They were checked on day 21 for *S. mutans* colonization of their oral cavities. Sixty pups with *S. mutans* colonization were divided randomly into six groups of 10 animals each. This investigation met all conditions for a double blind study. They

were fed (ad lib) basal diet 2000 (Teklad cariogenic diet 2000 without sucrose) with 60% cornstarch by weight. Cornstarch was replaced with various proportions of sucrose and aspartame as follows: Group 1 — 50% sucrose; Group 2 — 30% sucrose; Group 3 — 30% sucrose + 0.15% aspartame; Group 4 — 0.30% aspartame; Group 5 — 0.15% aspartame; Group 6 — no replacement. They received double distilled deionized water. After sacrifice at the end of six weeks they were decapitated. The heads were defleshed and both upper and lower jaws were used to evaluate caries in all the molars by Keyes' technique (1958).

Before sacrifice, plaque was collected from the molars (buccal and lingual surfaces) with a sterile toothpick. The toothpick then was dropped into a tube with 1 ml of sterile, reduced, anaerobic transport medium. The medium consisted of Trypticase® Soy Broth (TSB, BioQuest, Cockeysville, MD) with 0.1% agar (Bacto®-Agar, Difco, Detroit, MI) and 0.025% resazurin (Allied Chemicals, NJ). The tubes were mildly sonicated for 10 sec. Dilutions in TSB covering the range 10^1 to 10^6 were placed onto Mitis Salivarious Agar (Difco) containing Bacto® Chapman Tellurite (Difco, Detroit, MI) and 200 μg/ml streptomycin and were anaerobically incubated for one week in GasPak jars (BioQuest). The colony forming units (cfu) of *S. mutans* present in each sample were determined, and reported as high (> 300 cfu), medium (100–300 cfu) and low (< 100 cfu).

Group 1 was used as a test group for checking the efficiency of the caries production system. Group 6 was a control group to check the cariogenicity of the basal diet alone. Our purpose was to test the effect of adding aspartame to a 30% sucrose diet, and the effect of aspartame itself at different levels of concentration. The comparisons were between Groups 2 and 3, and Groups 4 and 5. Pairwise two-tailed analyses were performed using the Mann-Whitney-U Method to compare Groups 2 vs. 3, and Groups 4 vs. 5.

Results

All rats appeared to be in good health throughout the experiment. The food intake was similar in the six groups. There were no significant differences in weight gain in the six groups. The caries incidence (sulcal and smooth surface) in Group 3 (which received aspartame in addition to 30% sucrose) was significantly lower ($P < 0.001$) than in the group receiving only 30% sucrose. The animals which received only aspartame (Groups 4 and 5) had no caries (Table 1).

All animals had *S. mutans* colonization in their oral cavities at the beginning, but by the end of the experiment, animals receiving only aspartame had a very low count (< 100 cfu) (Table 2). In the three groups which received sucrose (with or without aspartame) the *S. mutans* population was high (> 300 cfu) compared to the groups receiving no sucrose. The difference was significant. There was no significant difference in *S. mutans* population between Groups 1, 2, and 3, (fed sucrose) or between Groups 4, 5, and 6 (fed no sucrose).

Table 1. Mean enamel caries units and weight gain of the six groups

Group	*Smooth Surface Caries Units ± SE*	*Sulcal Caries Units ± SE*	*Weight Gain in Grams ± SE*
1 – 50% S	56.57 ±23.33	62.83 ±15.46	172.60 ±31.33
2 – 30% S	11.63 ±2.56	12.0 ±3.34	174.80 ±13.86
3 – 30% S + 0.15% A	5.50 ±2.69	3.40 ±2.01	142.00 ±75.78
4 – 0.30% A	0	0	189.50 ±22.09
5 – 0.15% A	0	0	157.90 ±88.62
6 – None	0	0	155.10 ±92.08

SE – Standard error of the mean

Only groups 2 and 3 with similar sucrose content compared for caries. Caries units in 2 vs. 3 significantly different ($P < 0.001$). No significant difference in weight gain in the six groups.

Table 2. Number of animals showing *S. mutans* colony forming units (cfu)

Group	*High*	*Medium*	*Low*
1 – 50% S	10		
2 – 30% S	9	1	
3 – 30% S + 0.15% A	8	2	
4 – 0.30% A			10
5 – 0.15% A		1	9
6 – None			10

S – Sucrose, A – Aspartame

High (> 300 cfu), Medium (100 – 300 cfu), Low (< 100)

Difference between S groups (1, 2, 3) and without S groups (4, 5, 6) was significant ($P < 0.005$).

Discussion

The addition of aspartame to 30% sucrose reduced decay in the rats when compared to 30% sucrose only. Aspartame alone also did not promote caries. Thus, our

in vivo data supports several in vitro studies which have shown the caries preventive potential of aspartame (Olson 1975, 1977; Thompson et al. 1986; Smidt et al. 1987).

Our data also supports several in vivo studies. Soparker et al. (1978) found that aspartame-sweetened gum raised the pH that was lowered by a sucrose rinse. Lout and Messer (1987) have reported that frequent rinsing with 0.05% aspartame in the presence of a cariogenic diet significantly lowered dental caries in rats. Reussner and Galimidi (1982) found a dose-related nonsignificant decrease in rat caries with aspartame. However, Tanzer and Slee (1983) and Siebert et al. (1987) found that aspartame had no effect on caries in rats in the presence of sucrose. This may be due to the fact that Tanzer and Slee used 50% sucrose with 0.5% aspartame, and Siebert et al. used 30% sucrose with 3% aspartame, whereas we used 30% sucrose with 0.3% aspartame. A lower level of aspartame may be more effective in reducing cariogencity in the presence of a lower level of sucrose. This should be investigated further with different levels of aspartame and sucrose.

Aspartame alone (in the diet) did not support the growth of *S. mutans* as indicated by the dwindling population of *S. mutans* in Groups 4 and 5. This may be because aspartame is utilized poorly as a nitrogen source by acidogenic microorganisms (Grenby and Saldanha 1983), or because of adverse changes in the bacterial structure caused by aspartame (Smidt et al. 1987). Smidt et al. (1987) found that when *S. mutans* was incubated with aspartame, electron microscopic examination revealed that the bacterial cells were damaged (they do not specify the type of damage) and their population dwindled. This supports Frank and Berry (1984) who found the *S. mutans* population reduced in the presence of aspartame. Our findings support both Frank and Berry (1984) and Smidt et al. (1987).

Bowen (1984) reported a cariostatic effect on smooth surface caries in rats. The present study found that aspartame, when added to a sucrose diet, was cariostatic when compared to a diet containing sucrose without added aspartame. This was true for smooth surface as well as sulcal caries, almost to the same extent (Table 1). However, this reduction in caries in animals fed the aspartame-supplemented sucrose diet was not associated with reduction in microbial flora (*S. mutans* cfu) when compared with animals on the sucrose only diet. Similarly, incidence of either type of caries (sulcal/smooth surface) was unrelated to *S. mutans* cfu.

Aspartame, in other words, did not reduce the *S. mutans* population when added to diets with sucrose. This supports the report by Mishiro and Kaneko (1977) wherein they found that adding aspartame to a sucrose-containing medium did not reduce the *S. mutans* population. The caries reduction in these groups must be due to some additional mechanism(s). Olson (1977) reported that the addition of aspartame to a sucrose-containing medium resulted in a significant reduction in the formation of adherent plaque by *S. mutans* as compared to a sucrose-only containing medium. Reussner et al. (1982) found that aspartame is effective in reducing acid-induced enamel demineralization in rats. Further, it has been reported that in spite of the lactic acid production, the pH increased when aspartame was incubated with dental plaque due to neutralization of the acids by the breakdown products of aspartame (Mishiro and Kaneko 1977; Fu and Bibby 1984; Bibby and Fu 1985). The damage to *S. mutans* cell structure, in the presence of aspartame in sucrose medium, reported by Smidt et al. (1987) also may make the cells less virulent. These in vitro studies may explain the reduction of caries when aspartame is added to a cariogenic diet.

Conclusions

1. Aspartame is noncariogenic in rats.
2. Added to a 30% sucrose diet, it reduces caries in rats.

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Medical waste technology may benefit dentistry

Dentists may benefit from technology developed in response to the federal medical waste tracking experiment, according to an Environmental Protection Agency (EPA) report to Congress. The 1988 Medical Waste Tracking Act encouraged development of innovative waste treatment and destruction technologies, and most of the newer treatment methods are disposal of sharps.

The EPA report suggests the agency may recommend extending parts of the program, but seek exemptions for dentists and other small generators. American Dental Association officials continue to meet with EPA officials to urge exemption or minimal regulation for dentists. EPA will send a final report to Congress in September, 1991.

Almost all (98%) of 2,684 dentists covered by the two-year experiment generate less than 50 pounds of medical waste a month. Some 95% of physicians and veterinarians also are "small quantity generators."

Hospitals, which are less than 4% of regulated facilities, produce the vast majority of medical waste. Nearly 80% of the waste generators in the experimental program — dentists and other small-quantity groups — produce only 3% of the waste.