

Cariogenicity of Different Types of Milk: An Experimental Study Using Animal Model

Regina Célia Rocha PERES
Luciane Cristina COPPI
Eliane Melo FRANCO
Maria Cristina VOLPATO
Francisco Carlos GROPPPO
Pedro Luiz ROSALEN

Department of Physiological Sciences, Area of Pharmacology, School of Dentistry of Piracicaba, UNICAMP,
Piracicaba, SP, Brazil

This study evaluated the cariogenic potential of infant formulas and cow's milk, using a high cariogenic challenge in the animal model. Sixty female Wistar rats infected with *Streptococcus sobrinus* and desalivated were randomly divided into 6 groups, which received *ad libitum*: 1) sterilized deionized distilled water (SDW) with 5% sucrose; 2) cow's milk; 3) Nan 2®; 4) Nestogeno 2®; 5) Ninho® growth supporting; 6) SDW. Groups 1 and 6 also received essential diet NCP#2 by gavage, twice a day. After 21 days, the animals were killed and evaluated according to recovered oral microbiota and caries score by using a modified Keyes method. The analysis of the carbohydrates in the milk samples was performed using HPLC. The results were analyzed by Shapiro-Wilk and Kruskal-Wallis tests. Cow's milk had the lowest cariogenic potential compared to the other test groups, but it was not statistically different from group 6. The percentage of *S. sobrinus* obtained from the oral cavity of the animals was not statistically different among the groups studied, except for the SDW group. It was concluded that cow's milk was not cariogenic and infant formulas showed some cariogenic potential.

Key Words: caries, milk, rat.

INTRODUCTION

Earlier reports have suggested dental caries as an infectious and transmissible disease that continues to be a public health problem in some age groups. Baby bottle tooth decay is closely associated with a sufficient period of time leading to acid production and subsequent demineralization in infant's teeth, when allowed to sleep with a bottle containing sugary solutions (1,2). This kind of lesion appears to be strongly associated with an exceptionally heavy infection by *Streptococcus mutans* on tooth surfaces. Studies have reported that 60% of the microbial population of dental plaque may be composed by *S. mutans* (2).

It is well known that frequency of eating a fermentable substrate is important in the carious process (3). Yet, it is difficult to eliminate sugar from the diet (4), as well as to promote information that does not

stimulate bottle feeding.

Many dietary components such as carbohydrates, lipids and proteins may affect factors associated with dental caries (5). Although lactose solutions can produce rapid drops in pH, milk is a complex solution which contains lactose, calcium, phosphorus, proteins, fat, and vitamins. Cow's milk is generally regarded as being protective against caries, mainly due to the high Ca and P content, but also because of the buffering activity of the milk protein (3,6). Cow's milk contains protein in the form of casein micelles which exhibit properties that allow the formation of very stable complexes of calcium phosphate that are protective against caries (7). It has also been associated with both vitamin D and fluoride, which contribute to the development of teeth and the presence of enzymes with antibacterial potential (8,9).

Some infant formulas have been used as nutri-

tional supplementation for babies and children. Recent studies have shown that infant formulas are acidogenic, support significant bacterial growth and dissolve enamel mineral (10). Infant formulas result from cow's milk, which has its composition altered by industries that add or remove some components such as corn syrup, sucrose, lactose, etc. (11). Although results of many studies have focused on the protective role of bovine milk in caries development (12), further research is needed to fully understand the relationship between infant formulas and baby bottle tooth decay (10).

The purpose of this study was to evaluate and to compare the cariogenic potential of many commonly used infant formulas and cow's milk using a well-defined desalivated rat model. The desalivated rat model was chosen because it provides one of the most severe cariogenic challenges available. This model simulates conditions in the mouth of a sleeping infant using a bottle, where saliva is decreased (13).

MATERIAL AND METHODS

Sixty female Wistar rats spf (specific pathogen free) aged 19 days from CEMIB-UNICAMP were used. This study was approved by the Ethics Committee for Animal Research (CEEA-UNICAMP). The animals were screened for *S. mutans* with usual technique (13,14). The rats were infected with *Streptococcus sobrinus* and received diet 2000, sterile deionized distilled water (SDW) with 10% sucrose *ad libitum* for seven days to establish infection (13).

At age 25 days, the rats were surgically desalivated using a previously described method (13), and were then randomly placed in individual suspended cages and divided into six experimental groups, which received *ad libitum*: group 1: SDW with 5% sucrose (positive control); group 2: cow's milk (Leite da Granja Tipo A; Campinas, SP, Brazil); group 3: Nan 2[®] (Nestlé Ind. Com. Ltd.; Porto Ferreira, SP, Brazil) reconstituted with SDW; group 4: Nestogeno 2[®] (Nestlé Ind. Com. Ltd.) reconstituted with SDW; group 5: Ninho[®] growth supporting (Nestlé Ind. Com. Ltd.) reconstituted with SDW; group 6: SDW (negative control). The control groups (1 and 6) also received 3 ml of liquid diet (National Caries Program) by stomach tube (gavage) twice a day in order to meet their essential nutritional requirements (1).

The infant formulas and cow's milk were pur-

chased at a local supermarket and belonged to the same lot. The formulas were prepared with SDW according to the manufacturer instructions. The amount that the animals consumed was recorded.

At the end of 21 experimental days, the animals were killed by CO₂ asphyxiation and decapitated. Half of the lower jaw from each animal was dissected aseptically, dipped in 5 ml 0.9% sterile saline and sonicated in 30-s burst in a sonicator (Vibra Cell 400W, Sonics & Materials Inc., Danbury, CT, USA). The resulting suspension was used to inoculate plates, by means of Whitley Automatic Spiral Platter (DW Scientific, Yorkshire, England), blood agar (Difco Co., Detroit, MI, USA), to estimate the total cultivable bacteria, and mitis salivarius agar (Difco Co.) plus streptomycin plates to determine *S. sobrinus* counts (1).

The scored caries were assayed by using the modified Keyes method (15). The total number of lesions and their severity were assessed. Smooth-surface and sulcal-surface caries were expressed as proportions of the maximum possible values of 125 and 56, respectively. The results were analyzed statistically with Shapiro-Wilk and Kruskal-Wallis tests (Bioestat 1.0 for Windows, Fortaleza, CE, Brazil).

The sugar determinations were assessed by high-performance liquid chromatography (16) using Waters HPLC Model 480 E (Milford, MA, USA) with a column operation temperature of 25°C and flow of 1 ml/min. The total fluoride presented in the solutions was determined according to the Taves method (17).

RESULTS

All animals survived the investigations and remained in good health. As expected, weight gain among the groups varied. The animals fed the liquid diet by gavage had the lowest weight gain. The animals fed the infant formulas and cow's milk gained the most weight (Table 1). In general, the control groups consumed less liquid than did the animals in the other groups (Table 1).

There were few differences between groups in the total amount of cultivable bacteria, with the exception of the animals receiving Nestogeno 2[®], which tended to have significantly lower populations and did not show statistical difference in relation to the cow's milk group. No difference was found in the percentage of bacterial populations composed of *S. sobrinus*, except for the animals that received distilled water in

which fewer *S. sobrinus* were detected (Table 2).

Caries data show that some of the infant formulas have significant cariogenic potential. The highest caries scores were observed in the animals receiving sucrose water. Although not as cariogenic as sucrose, Ninho[®] had significant cariogenic potential. The caries severity scores show that the values of Ds (smooth surface) and Ds, Dm (sulcal) observed in the Ninho[®] milk group do not show statistical difference when compared to the sucrose water group. The lowest scores were observed in the cow's milk and water groups (Tables 3, 4).

Cow's milk presented lactose as the single sugar in its composition and in a lower concentration than other milks (Table 5). Infant formulas presented, besides lactose, other sugars such as maltodextrine (Nestogeno 2[®] and Nan 2[®]), sucrose (Ninho[®] and Nestogeno 2[®]), glucose (Ninho[®]) and fructose (Ninho[®]). The Ninho[®] milk had the highest variability of sugar in relation to the others.

The concentration of fluoride in cow's milk and Ninho[®] was lower than the limit of sensitivity of the method used (<0.02), whereas, Nan 2[®] and Nestogeno 2[®] showed 0.5 ppm of fluoride concentration (Table 5).

Table 1. Mean (SD) of weight gains of desalivated animals and amount of fluid consumed daily, after 21 days of the experiment (n=10/group).

	5% sucrose*	Cow's milk	Nan 2 [®]	Nestogeno 2 [®]	Ninho [®]	SDW*
Weight gain (g)	7.0 (0.9)	74.4 (2.3)	55.0 (2.2)	72.3 (1.7)	61.6 (1.5)	0.0 (1.5)
Consumed fluid (ml)	40.5 (1.9)	76.0 (1.1)	77.7 (3.6)	83.9 (1.0)	84.0 (0.8)	18.5 (1.9)

SDW = sterile deionized distilled water.

*liquid diet by stomach tube (gavage).

Table 2. Mean and (Score mean) of total cultivable bacteria, *S. sobrinus* and percentage of *S. sobrinus* (n=10/group).

	5% Sucrose	Cow's milk	Nan 2 [®]	Nestogeno 2 [®]	Ninho [®]	SDW
Total (x10 ⁶)	14.4 (40.9) ^a	11.0 (26.6) ^{ab}	14.2 (29.7) ^a	8.0 (14.3) ^b	12.7 (37.0) ^a	13.9 (31.0) ^a
<i>S. sobrinus</i> (x10 ⁶)	7.4 (41.2) ^a	4.2 (34.0) ^a	3.9 (27.3) ^a	3.7 (30.3) ^a	7.4 (41.9) ^a	0.05 (5.6) ^b
<i>S. sobrinus</i> (%)	47.3 (36.9) ^a	38.5 (32.7) ^a	32.5 (27.2) ^a	50.0 (37.4) ^a	54.5 (40.3) ^a	0.5 (5.7) ^b

Different letters in the horizontal rows indicate statistically significant differences (Kruskal-Wallis, p<0.05).

Table 3. Mean and (Score mean) of caries score* of total smooth surface lesions and severity in desalivated rats (n=10/group).

	5% sucrose	Cow's milk	Nan 2 [®]	Nestogeno 2 [®]	Ninho [®]	SDW
Total smooth surface lesions	74.0 (53.5) ^a	4.0 (10.7) ^b	33.8 (36.7) ^c	30.4 (33.9) ^c	30.6 (33.8) ^c	2.3 (9.3) ^b
Ds	50.9 (54.3) ^a	1.5 (19.0) ^b	3.6 (26.2) ^{bc}	7.5 (26.9) ^{bc}	11.8 (40.0) ^{ac}	0.7 (11.7) ^b
Dm	16.9 (50.9) ^a	0.5 (21.7) ^{bc}	2.4 (30.4) ^{bc}	1.4 (26.0) ^{bc}	3.3 (34.5) ^b	0.2 (15.5) ^c

*Scored caries using Keyes method modified by Larson (15).

Different letters in the horizontal rows indicate significant differences (Kruskal-Wallis, p<0.05).

Ds = lesion extended into dentin.

Dm = dentin exposed.

Table 4. Mean and (Score mean) of caries score* of total sulcal lesions and severity in desalivated rats (n=10/group).

	5% sucrose	Cow's milk	Nan 2®	Nestogeno 2®	Ninho®	SDW
Total sulcal lesions	57.9 (53.7) ^a	24.0 (19.1) ^{bd}	35.7 (31.7) ^{bc}	34.1 (31.7) ^{bc}	38.3 (35.7) ^c	11.0 (7.0) ^d
Ds	35.1 (51.6) ^a	3.4 (12.3) ^b	15.4 (29.9) ^c	15.3 (29.4) ^c	32.5 (46.7) ^a	2.1 (8.2) ^b
Dm	11.2 (50.5) ^a	1.1 (16.9) ^{bd}	2.8 (28.6) ^{bcd}	3.0 (31.7) ^{bc}	3.8 (35.8) ^{ac}	3.1 (15.0) ^d

*Scored caries using Keyes method modified by Larson (15).

Different letters in the horizontal rows indicate significant differences (Kruskal-Wallis, $p < 0.05$).

Ds = lesion extended into dentin.

Dm = dentin exposed.

Table 5. Percentage (w/v) of carbohydrate and total fluoride (ppm) present in milk tested (n=10/group).

Milk	Maltodextrine mean % (SD)	Lactose mean % (SD)	Sucrose mean % (SD)	Glucose mean % (SD)	Fructose mean % (SD)	Total Fluoride ppm F ⁻ mean (SD)
Cow	-	4.9 (0.04)	-	-	-	0.02 (0.01)
Ninho®	-	29.0 (0.25)	9.3 (0.13)	1.5 (0.01)	2.1 (0.02)	0.02 (0.02)
Nestogeno 2®	8.0 (0.07)	29.0 (0.25)	14.0 (0.19)	-	-	0.51 (0.19)
Nan 2®	13.0 (0.12)	44.0 (0.37)	-	-	-	0.53 (0.15)

Carbohydrate determinations by HPLC method (16).

Total fluoride determination by Taves method (17).

DISCUSSION

The present study confirms previous investigations using the same model which reported significant cariogenic potential in infant formula milk (1).

The animals from the test groups received no additional nutrition during the experimental period. Therefore, any carious lesions that were developed could be attributed to milk ingestion. However, previous exposure to carbohydrate from diet 2000, used in order to establish infection by *S. sobrinus*, may have caused a minimal level of caries before the experiment. The animals from the control groups regurgitated after receiving the liquid diet by gavage. Thus, the diet 2000 and the liquid diet by gavage could have contributed to the small amount of caries observed on the teeth of the SDW group (negative control).

It was observed that all animals consumed increasing amounts of fluid. This observation was important because it allowed comparisons among solutions. Some studies reported that there seems to be little relationship between the amount of fluid consumed and

caries scores (18). When more or equal amount of test fluid is consumed in relation to the positive control (sucrose), comparisons can be made (6). In the present study, the test milk groups consumed more liquid than the positive control group; therefore, programmed feeding was not necessary to achieve significant data.

Previous reports as well as this study showed that the formulas sustained the infection of *S. sobrinus* (Table 2). This confirms that sucrose is not the only sugar that can maintain infection (1). The highest levels of infection were observed in animals receiving sucrose solution, and the lowest levels were observed in the negative group. These data emphasize the importance of the interaction between bacteria and diet in the pathogenesis of caries, because the levels of infection observed in the water that fed the animals were meaningless (1,19).

Cow's milk is widely consumed in the world especially by children. It is a natural and healthy drink and contains macro- and micro-components which benefit dental health. As expected, our results confirm previous ones, that cow's milk is not cariogenic. Its

caries score was the lowest and did not show statistical difference compared to the negative control (SDW). This may be attributed to the composition of cow's milk, which has some components (calcium, phosphorus, peptides) that protect the teeth (20).

The reduced salivary flow and the suckle-sleep-suckle cycle may lead to stagnation of the formula. It has been shown that this stagnation may cause an enzymatic breakdown of protective proteins, such as casein (10). The data of the present study show that Ninho® and Nestogeno 2® milks have more sucrose (9.3 and 14%, respectively) than the positive control (5% sucrose). However, the positive control was the most cariogenic of the groups tested. Thus, there are some components in these infant formulas that modify the cariogenicity of sugars in order to reduce it. Although we did not measure enzymatic activity, components of these milks, such as protein, calcium, phosphorus, iron, and peptides were likely to protect the teeth during all experiment (20).

Table 5 shows that some milks presented fluoride, which has well known cariostatic effects. Therefore, it is possible to conclude that the presence of this element in Nestogeno 2® and Nan 2® reduced the cariogenic potential of these milks when compared to Ninho®, which presented the highest variability of carbohydrates and the lowest quantity of fluoride (0.02 ppm F). The composition of Ninho® could explain why this group did not present a statistical difference in relation to the positive control (5% sucrose) for the severity caries scores such as Ds (smooth-surface caries) and Ds, Dm (sulcal caries).

The quantity of fluoride in milk can be of concern due to the risk of fluorosis. Children consuming fluoridated drinking water are at risk for the development of fluorosis. Independent of the presence of fluoride in some powder milks, the prolonged and frequent daytime, naptime, and nighttime bottle feedings with milk reconstituted with fluoridated water can also be considered a risk factor for fluorosis in children.

The cariogenic potential of any product depends on the manner and pattern of its use. Parents should be made aware of the cariogenicity of milk, so that an infant does not sleep with a bottle containing any cariogenic substance. Dental practitioners and other health care professionals should actively discourage the use of cariogenic liquids so that this practice, which has a deleterious effect on teeth, does not progress to adulthood.

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RESUMO

Peres RCR, Coppi LC, Franco EM, Volpato MC, Groppo FC, Rosalen PL. Cariogenicidade de diferentes tipos de leite - Estudo experimental usando modelo animal. *Braz Dent J* 2002;13(1):27-32.

Este estudo avaliou o potencial cariogênico de fórmulas infantis e do leite de vaca, usando um modelo de alto desafio cariogênico em animais. Sessenta ratas Wistar infectadas com *Streptococcus sobrinus* e dessalivadas foram aleatoriamente divididas em 6 grupos os quais receberam *ad libitum*: (1) água destilada e esterelizada (ADE) com 5% de sacarose; (2) leite bovino tipo A; (3) Nan 2®; (4) Nestogeno 2®; (5) Ninho® coadjuvante de crescimento; (6) ADE. Os grupos 1 e 6 também receberam dieta essencial NCP#2, através de gavage, 2 vezes/dia. Após 21 dias, os animais foram sacrificados e avaliados quanto a microbiota oral recuperada e o índice de cárie de Keyes modificado. A análise dos carboidratos nos leites foi feita através de CLAE. Os resultados foram submetidos aos testes de Shapiro-Wilk e Kruskal-Wallis. O leite bovino apresentou o menor potencial cariogênico quando comparado aos outros grupos testes, porém não diferiu estatisticamente do grupo 6. As porcentagens de *S. sobrinus* dos grupos 1 a 5 não apresentaram diferenças estatisticamente significantes entre si, exceto em relação ao grupo 6. Concluímos que o leite bovino não foi cariogênico e as fórmulas infantis mostraram potencial cariogênico.

Unitermos: cárie, leite, ratos.

REFERENCES

1. Bowen WH, Pearson SK, Rosalen PL, Miguel JC, Shih AY. Assessing the cariogenic potential of some infant formulas, milk and sugar solutions. *J Am Dent Assoc* 1997;128:865-871.
2. Van Houte J, Gibbs G, Butera C. Oral flora of children with "nursing bottle caries." *J Dent Res* 1982;61:382-385.
3. Rugg-Gunn AJ, Roberts GJ, Wrigth WG. Effect of human milk on plaque in situ and enamel dissolution in vitro compared with bovine milk. Lactose and sucrose. *Caries Res* 1985;19:327-334.
4. Parajas IL. Sugar content of commonly eaten snack foods of school children in relation to their dental health status. *J Philip Dent Assoc* 1999;51:4-21.
5. Vacca-Smith AM, Bowen WH. The effect of milk and kappa casein on streptococcal glucosyltransferase. *Caries Res* 1995;29:498-506.
6. Bowen W, Pearson SK. Effect of milk on cariogenesis. *Caries Res* 1993;27:461-466.
7. Rollema HS. Casein association and micell formation. In: *Advanced Dairy Chemistry: Proteins*. Fox PF, ed. Vol 1. London: Elsevier Applied Science, 1992. p 111-140.

8. Reynolds EC. The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra oral model. *J Dent Res* 1987;66:1120-1127.
9. Westover KM, Diloreto MK, Shearer TR. The relationship of breast feeding to oral development and dental concerns. *ASDC J Dent Child* 1989;56:140-143.
10. Erickson PR, McClintock KL, Green N, LaFleur J. Estimation of the caries-related risk associated with infant formulas. *Pediatr Dent* 1998;20:395-403.
11. Sheikh C, Erickson PR. Evaluation of plaque pH changes following oral rinse with eight infant formulas. *Pediatr Dent* 1996;18:200-204.
13. Bowen W, Madison KM, Young DA. The effect of desalivation on coronal and root surface caries in rats. *J Dent Res* 1988;67:21-23.
14. Gold OG, Jordan HV, Van Houte JA. A selective medium for *Streptococcus mutans*. *Arch Oral Biol* 1973;18:1357-1364.
15. Larson RW. Merits and modifications of scoring rat dental caries by Keyes method. In: *Symposium and Workshop on Animal Models in Cariology*. Sturbridge, Washington 1980. Washington: Information Retrieval, 1981. p 195-203.
16. Alpenfels WF. The rapid determination of neutral sugars in biological samples by high-performance liquid-chromatography. *J Liq Chromatog* 1982;9:1711-1723.
17. Taves DR. Separation of fluoride by rapid diffusion using hexamethyldisiloxane. *Talanta* 1968;15:969-974.
18. Bowen WH, Pearson SK, VanWuyckhuysse BC, Tabak LA. Influence of milk, lactose-reduced milk, and lactose on caries in desalivated rats. *Caries Res* 1991;25:283-286.
19. Tanzer JM. On designing animal model experiments on caries. In: *Symposium and Workshop on Animal Models in Cariology*. Sturbridge, Washington 1980. Washington: Information Retrieval, 1981. p 143-149.
20. Bowen WH, Amsbough SM, Monell-Torrens S, Brunelle J, Kuzmiak-Jones H, Cole MF. A method to assess cariogenic potential of foodstuffs. *J Am Dent Assoc* 1980;100:677-681.

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