Evaluation of the Impact Induced by High Dietary Sucrose Concentration and Glucocorticoid Medication on Dentin Formation of Young Rats

ROXANA OANCEA[1],CRISTIAN FUNIERU[2]*, RUXANDRA SFEATCU[2] , DELIA BERCEANU VADUVA[3], ADELINA CHEVERESAN[3]

[1]Victor Babes University of Medicine and Pharmacy, Faculty of Dental Medicine, Department I, 2 Eftimie Murgu Sq., 300041,Timisoara, Romania

[2]Carol Davila University of Medicine and Pharmacy, Faculty of Dental Medicine, 4-6 Eforie Str., 050037, Bucharest, Romania

[3] Victor Babes University of Medicine and Pharmacy, Faculty of Medicine, 2 Eftimie Murgu Sq., 300041,Timisoara, Romania

The impact of sucrose and systemic cortisone treatment on the response of dentin was examined in an experimental rat model. After 2 months of a modified diet and/or medication period, the areas of dentin formation and dentinal caries were quantified in the mandibular molars of growing animals. The 60% sucrose diet significantly reduced dentin formation and increased dentinal caries progression. The presence of glucocorticoid medication alone reduced dentin formation. Cortisone medication in combination with sucrose diet further decreased dentin apposition. Rats receiving the control diet positively responded to the dentinal caries by increasing dentin formation to prevent pulpal exposure. The rate of dentine formation was lower in rats with high sucrose diet and predentin area was wider compared to the control group fed normal diet. The enlargement of the predentin region in sucrose diet rats reflects changes in the functionality of the odontoblasts, such as reduction of matrix synthesis and alteration of the mineralization process. These results indicate that the functional alterations in the pulpo-dentinal complex might contribute to dentinal caries progression through systemic mechanism irrespective of the causative factors.

Keywords: diet, sucrose, cortisone, dentin

During caries progression the rate of dentin formation is increased to protect the pulp tissue from exposure.

Evidence in literature indicated that sucrose is one of the most important components in the aetiology of caries lesions [1]. Modern diet contains high percentages of carbohydrates. If the topical effect of sucrose is well documented in literature, it is important to test if dietary sucrose may contribute to dentinal caries progression in a dose response through systemic mechanism.

Nowadays in the treatment of different general conditions like polyarticular acute rheumatism, autoimmune diseases synthetic glucocorticoids are widely used [2]. One of the most common side effect of long term use is osteoporosis; also glucocorticoids are well known to affect calcium and collagen metabolism balance so it was chosen a non-sucrose agent of comparison for study of the suppression of dentin formation and possible modulation of caries progression. Children represents a vulnerable population affected by long term treatment with glucocorticoids [3,4]. Reduced bone formation related to decreased osteogenesis and increased osteoblast apoptosis was observed [5]. Since in childhood there is an increasing bone rate, the glucocorticoid treatment might affect bone formation and dentine apposition.

Several effects of cortisone on dental tissues includes: decreasing in height of alveolar bone, osteoporotic aspect with reduced number of osteoblasts, loss of osteoid matrix and fibrous transformation of periodontal space. High doses of corticosteroids have been ascribed to modulate dentin formation. Disorganized odontoblasts in pulp chamber and even fibrosis of the entire dentin could be observed in histological sections [6,7]. Animal models were used to test both sugary diet and glucocorticoid therapy in previous studies [8-10]. Significant decrease in bone strength, trabecular bone mass, changes in bone metabolism and bone architecture was found in glucocorticoid treated rats [11,12]. It was revealed that endogenous glucocorticoids are important for differentiation and proliferation of osteoblast in skeleton development, while long-term therapy inhibits bone formation. It was shown that intravenous administration of high doses of corticosteroids induced dentin formation along the pulp chamber walls in the molars [13].

The aim of this study is to test whether there is a direct relationship between different dietary sucrose concentration and dentin formation in rats model and to compare if the effects of high sucrose concentration and glucocorticoid medication on the formation rate and mineralization of dentin is similar.

Experimental part

Materials and methods

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of animals (Law 6638 from May 8, 1979), and the procedure was approved by the Ethics and Research Committee of Victor Babes University of Medicine and Pharmacy. All experiments underwent full protection according to the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (Strasbourg, France, 1986) and to all European understandings signed by Romanian country (EU Directive 93/35/EEC, Amendment 76/768/EEC, directive 86/609 CEE), as well as the Romanian law (Law 471/2002, Ordinance 37/2002).

The experiments were performed using 80 Sprague-Dawley rats at the age of 21 days. The subjects were kept under the same conditions of lightning (12 h light and 12 h dark) and the same times of feeding, handling and noise. The animals were dived in 8 groups according to their diet and medication received. Each group consisted of 10 subjects.

* email:thycristi@yahoo.com, Phone: +40 723 268 929

In group A, rats were fed with special diet composed of standard protein concentrate for laboratory animals (85%) and sucrose (15%), in group B the same protein concentrate was mixed with sucrose 30%, group C the mixture was done with sucrose 60% and group D was kept as control and it was fed with standard diet without any additional sucrose.

To evaluate whether the glucocorticoid treatment has an influence on dentin formation other 4 groups were kept under control and were divided as follows: group GC was fed with 60% sucrose mixture and received glucocorticoid pellets, group GP was fed with the same mixtures of 60 % sucrose and received placebo pellet (without any cortisone content), group CNG was fed with standard diet and received glucocorticoid pellets and CNP was fed with standard diet and placebo pellet was used. The cortisone pellet contained 25 mg and the release time was 60 days.

50 mg sodium thiopental/kg body weight was injected intraperitoneally to induce anaesthesia. The pellets were implanted through 8 mm incision made by small scissors and pushing it subcutaneously with small forceps. The wound was not sutured to avoid the animal biting and scratching. The stability of the pellet was controlled at the end of the experiment by inspecting it from surgical incisions.

To all the animals an intraperitoneal injection of oxytetracycline hydrochloride (30-40 mg/kg, Terramycin® , Pfizer Corp., Brussels, Belgium) was given for marking the areas of dentin during the test period.

The duration of the experimental period was 2 months (rats were 21-24 days old at the beginning of the experiments and 82-85 days old at the end of experiments).

Rat lower first molars emerge into the oral cavity on postnatal day 19 and reach functional occlusion on day 25. During the experiment only mandibular molars were used for measuring the dentine because mandibles were easier to dissect than maxillas.

They were then sectioned sagittal in halves on the midline of the fissures of the first and second molars under water cooling by the method of Keyes by using a diamond disc.

During the experiment the areas of dentin formed (μm^2) were confirmed by comparing the electron microscope images with the photographs taken under the fluorescent microscope (Nikon Eclipse E 600 with digital imaging system), revealing the tetracycline line. The mandibular molars were stained with Schiff's reagent. Measurements of the distance between two fluorescent labels at 8 geometrically equal intervals were performed. Three measurements of both areas were carried out in each tooth and an average was calculated from these three spots to ensure that the measurements represent the dentinal area as a whole.

Statistical analysis

Means, standard deviations and median values with minimums and maximums were calculated for area of dentin formation. One- way ANOVA with Tukey's and Scheffe's tests were used to identify if there are any differences in dentin formation among different groups. Box plots were used to indicate the shape of the distribution, its central value and its variability related dentin formation in rats. Paired samples T-test was also used, when the effect of cortisone treatment on dentin formation was compared between the first and second molars. For Statistical analyses were performed using the SPSS statistical software package (SPSS Versions 19.0, SPSS, Chigago, IL).

Results and discussions

The main objective was to test whether there was a direct relationship between different dietary sucrose concentration and dentin formation in rats model and to compare if the effects of high sucrose concentration and glucocorticoid medication on the formation rate and mineralization of dentin was similar. Even though many researchers were worked on the dentin formation, the animal model was represented by adult rats. In this study Sprague-Dawley rats at the age of 21 days were used.

Literature data indicated that the required concentration of sucrose to have a significant reduction in dentine formation in young rats varies between 30-40%. In this study it was used 60% sucrose diet considering that nowadays the consumption of carbohydrates is high.

The results indicated that a significantly smaller area of dentin was formed in rats fed with 60 % sucrose diet compared to the groups fed with 15 and 30% sucrose diet (table 1, 2).

The mean value of the dentin apposition is significantly lower in both mandibular molars (fig. 1). When 60% of sucrose was replaced with a standard diet (control diet), the area of dentin formed during the experiment was almost two times greater than in rats fed with 60% sucrose diet. Related to the concentration of sucrose there is no statistical significance between dentinal area observed in 30 % sucrose diet compared to 15% sucrose diet.

In previous studies performed on elderly rats the association between depression of odontoblasts function and diet was not indicated which proved that the alteration of odontoblast function is age dependent [14].

During the experiment molars 1 and 2 of the rat were erupted, so the dentin formed alongside the study was secondary dentine or reactive dentine as a result of the carious process.

Literature data indicated that the required concentration of sucrose to have a significant reduction in dentine formation in young rats varies between 30-43% [15]. Dentinal apoptosis was present in young rats fed with enriched diet in sucrose. Predentin area is wider and dentin formation is reduced compared with rats fed a normal diet. The change in the function of the odontoblasts in rats fed a rich diet in sucrose was indicated by the enlargement of the predentin area by the decrease in matrix synthesis or alteration of mineralization; so the progression of the caries

Concentration ↓	Sample Volume	Mean	Std. Deviation	Minimum	Maximum
15% sucrose	20	139950	8947,07	123000	150000
30% sucrose	20	141370	23509,49	109000	170000
60% sucrose	20	101200	28559,45	60000	156000
Control diet	20	156300	25441,57	105000	198000
Total	80	134705	30405,45	60000	198000

Table 1
THE MEAN, STANDARD DEVIATION, MAXIMUM AND MINIMUM VALUES OF DENTIN FORMATION (μm^2) IN GROUPS FED WITH DIFFERENT CONCENTRATION OF SUCROSE (15, 30, 60%) IN DIET

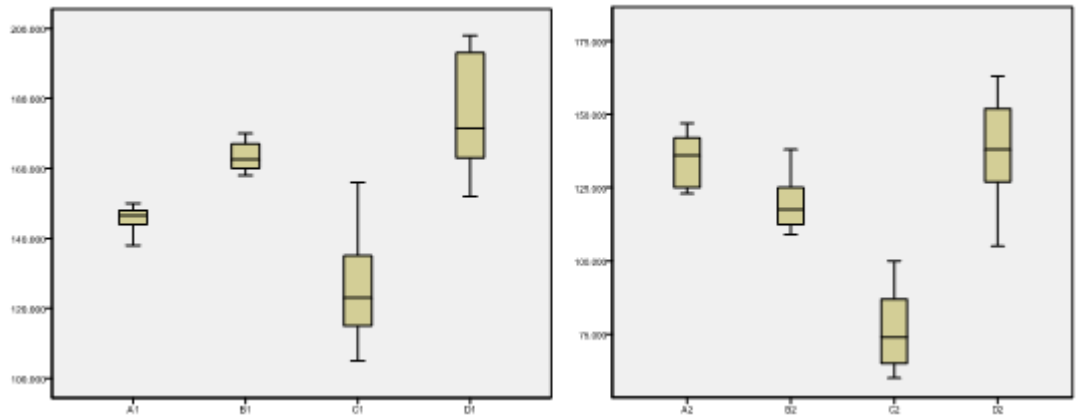

Fig. 1. The area of dentin formed (in squareμm) in groups (A-D) in the first and second mandibular molars. A1, B1,C1=area of dentin formation in the first molar in rats fed with 15% ,30%,60% concentration of sucrose in diets. D1= area of dentin formation in the first molar in rats fed with standard diet. In a box and whisker plot: the ends of the box are the upper and lower quartiles , so the box spans the interquartile range. The median is marked by a vertical line inside the box. A2, B2,C2= area of dentin formation in the first molar in rats fed with 15% ,30%,60% concentration of sucrose in diets. D2= area of dentin formation in the first molar in rats fed with standard diet.

(I) group	(J) group	p value for Tukey HSD	p value for Scheffe
15%sucrose	30%sucrose	.997	.998
	60%sucrose	.000	.000
	control diet	.117	.174
30%sucrose	15%sucrose	.997	.998
	60%sucrose	.000	.000
	control diet	.175	.244
60%sucrose	15%sucrose	.000	.000
	30%sucrose	.000	.000
	control diet	.000	.000
control diet	15%sucrose	.117	.174
	30%sucrose	.175	244
	60%sucrose	000	000

Table 2

COMPARATIVE ANALYSIS OF DENTIN FORMATION (TUKEY'S HSD AND SCHEFFE'S TESTS) IN GROUPS FED WITH DIFFERENT CONCENTRATION OF SUCROSE. WE APPLIED AN ANOVA TEST IN ORDER TO SEE IF WE HAVE SIGNIFICANT DIFFERENCES WITHIN THE STUDIED GROUPS. WE CONSIDERED α=0.05 AS CONFIDENCE LEVEL.

can be modulated by the functionality of the odontoblasts [16]. Comparative analysis of dentin formation (Tukey's HSD and Scheffe's tests) in groups fed with different concentration of sucrose was shown in table 2.

Rats fed with 60% sucrose diet and treated with cortisone pellet had smaller area of dentin in both mandibular molars compared to the respective control groups. When the groups with contisone medication were compared, the sucrose diet decreased the area of dentin formation (Table III). Cortisone medication reduced the area of dentin apposition in the group fed with sucrose but also in the group fed with standard diet. There is a significant difference between all the groups (with or without sucrose diet) that received cortisone treatment ($p<0.05$) (Table 4). Glucocorticoid treatment may have been related to the more advanced developmental stage of the first molar rather than the type of dentin. Cortisone medication in association with increased sucrose diet revealed the lowest dentin formation (fig. 2).

Previous studies indicated reduction of the trabecular bone volume, bone loss in femoral cortical bone, along with a reduction of cortical bone volume after glucocorticoid treatment. Increased apoptosis of osteoblasts in trabecular bone and apoptosis of osteocytes in the metaphyseal cortical bone in the mice treated with glucocorticoid was reported [17]. Decreased production of osteoclasts and osteoblasts may also contribute to the diminished bone formation and bone turnover caused by glucocorticoides [18]. Though there are similar work, dentin apposition and the activity of osteoblasts was not so much evaluated, but in the present work mean values of dentin formation in groups fed with different concentration of sucrose were measured.

Concentration ↓	Sample	Mean	Std. Deviation	Minimum	Maximum
60%sucrose+cortisone	20	67850	14557,59	49000	94000
60%sucrose+placebo	20	87400	23956,98	49000	167000
control diet +cortisone	20	151900	22936,76	90000	210000
control diet +placebo	20	179000	10920,77	153000	192000
Total	80	121537	49378,91	49000	210000

Table 3

THE MEAN VALUE OF DENTIN FORMATION (μm^2) IN GROUPS TREATED WITH CORTISONE AND FED WITH 60%SUCROSE OR CONTROL DIET. WE CALCULATED THE MEAN, STANDARD DEVIATION, MAXIMUM AND MINIMUM VALUES FOR THE TESTED SAMPLES.

(I) medicated groups	(J) medicated groups	p value for Tukey HSD	p value for Scheffe
60%sucrose+cortisone	60%sucrose+placebo	.009	.018
	control diet+cortisone	.000	.000
	control diet+placebo	.000	.000
60%sucrose+placebo	60%sucrose+cortisone	.009	.018
	control diet+cortisone	.000	.000
	control diet+placebo	.000	.000
control diet+cortisone	60%sucrose+cortisone	.000	.000
	60%sucrose+placebo	.000	.000
	control diet+placebo	.000	.000
control diet+placebo	60%sucrose+cortisone	.000	.000
	60%sucrose+placebo	.000	.000
	control diet+cortisone	.000	.000

Table 4
COMPARATIVE ANALYSIS OF DENTIN FORMATION (TUKEY'S HSD AND SCHEFFE'S TESTS) IN GROUPS TREATED WITH CORSTISONE AND FED WITH 60% CONCENTRATION OF SUCROSE OR CONTROL DIET. WE APPLIED AN ANOVA TEST IN ORDER TO SEE IF WE HAVE SIGNIFICANT DIFFERENCES WITHIN THE STUDIED GROUPS. WE CONSIDERED α=0.05 AS CONFIDENCE LEVEL

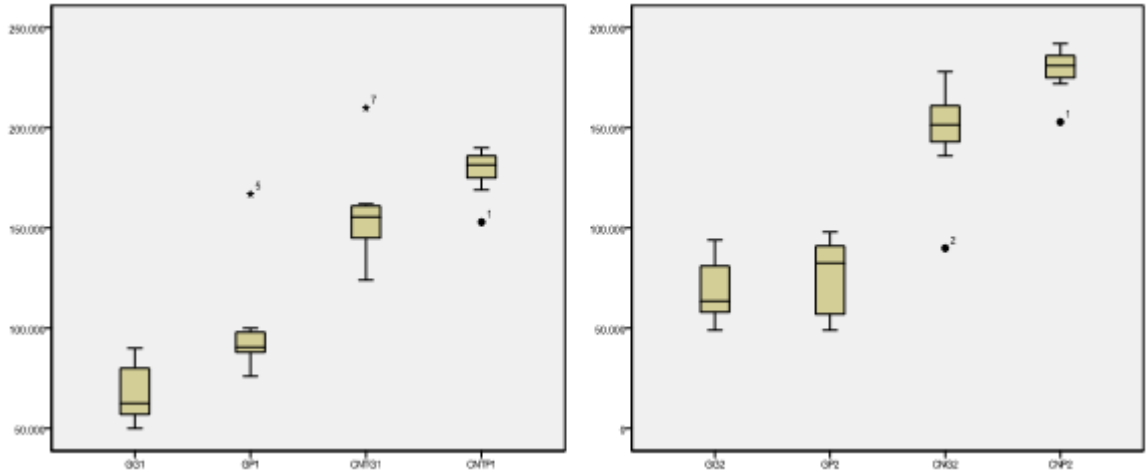

Fig. 2. The area of the dentin formed in groups treated with cortisone in first molar : GC1= 60% sucrose diet + cortisone pellets, GP1 =60 % sucrose diet + placebo pellet, CNTG1 =standard diet + cortisone pellets CNPP 1= standard diet + placebo pellet was used The box reveals the 1st and 3rd quartiles with the median value inside, and the whiskers show the minimum and maximum. Dots and asterisk represent the values bigger than the maximal or smaller than minimal values statistically calculated in the *box and whiskers.*
The area of the dentin formed in groups treated with cortisone in second molar : GC2= 60% sucrose diet + cortisone pellets, GP2 =60 % sucrose diet + placebo pellet, CNTG2 =standard diet + cortisone pellets CNPP 2= standard diet + placebo pellet was used The box reveals the 1st and 3rd quartiles with the median value inside, and the whiskers show the minimum and maximum. Dots and asterisk represent the values bigger than the maximal or smaller than minimal values statistically calculated in the *box and whiskers.*

After 2 months of cortisone treatment, despite of diet there was lower dentine formation. When modified sucrose diet was added the action of cortisone indicated lower dentin apposition.

The area of the dentin formed in groups treated with cortisone in second molar : GC2= 60% sucrose diet + cortisone pellets, GP2 =60 % sucrose diet + placebo pellet, CNTG2 =standard diet + cortisone pellets CNPP 2= standard diet + placebo pellet was used The box reveals the 1st and 3rd quartiles with the median value inside, and the whiskers show the minimum and maximum. Dots and asterisk represent the values bigger than the maximal or smaller than minimal values statistically calculated in the *box and whiskers.*

Several studies have shown that dentin is a potential source of several growth factors including insulin-like growth factor, skeletal growth factor/insulin-like growth factor and transforming growth factor beta [19]. Bone morphogenetic proteins have been found to be involved already in the early tooth morphogenesis [20].

These growth factors appear to be trapped in the mineralized dentin matrix. In pathological situations such as caries, growth factors could be liberated from the dentin during the demineralization process and thus stimulate subjacent odontoblasts to lay down reparative dentin. The release of these growth factors might permit their diffusion into the pulp, where they can activate appropriate genes to initiate repair processes either by odontoblasts or in the case of odontoblast destruction, to induce differentiation of pulpal cells into hard-tissue forming cells. On the other hand there is evidence that high glucose concentration impairs the responsiveness of osteoblasts to insulin-like growth factor stimulation in vitro [19]. Compared to other studies, [1] we found that cortisone medication in combination with sucrose diet, can further decrease dentin apposition. The reduction of matrix synthesis and alteration

of the mineralization process can lead to the enlargement of the predentin region.

Conclusions

In conclusion, the presence of modified diet decreased the dentin area formation when the sucrose was added, the apposition depending on the concentration of sucrose. These results demonstrate that dietary sucrose with over 30% concentration reduces dentin formation in the molars of growing rats. After 2 months of cortisone treatment, despite of diet there is lower dentine formation. When modified sucrose diet was added the action of cortisone indicated lower dentin apposition.

With the 60% sucrose diet the pulpo-dentinal complex was not able to defend against

dentinal caries by increasing the rate of dentin formation. In the control diet groups the pulpo-dentinal complex responded to dentinal caries by increasing dentin formation.

This adds further evidence of the importance of the systemic contribution to the regulation of dentinal caries.

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