Hyperglucidic stress in diet - study of the dentine-pulp complex on experimental model

Roxana Oancea 1*, Angela Codruța Podarui 1, Liliana Vasile 2, Valentin Ordodi 3 and Cristian Oancea 4

1 Preventive, Community Dentistry and Oral Health Department, University of Medicine, Timișoara, Splaiul Tădar Vladimirescu No.14, Romania. 2 Department of Cytology and Hystology, University of Medicine, Timișoara, Romania. 3 Department of Molecular Biology, University of Medicine, Timişoara, Romania. 4 Department of Pneumology, University of Medicine, Timişoara, Romania. *e-mail: roancea@umft.ro

Received 8 January 2011, accepted 12 April 2011.

Abstract
High glucose quantity accelerates the initialization and progression of dental caries, but it seems also to affect dentin formation in young rats. Based on the hypothesis that metabolic alterations of odontoblasts might predispose to caries, evidenced through the decrease in dentinal apposition, it was assumed that glucose can contribute to the progression of carious lesion through systemic mechanism. The reactivity of the pulp-dentinal complex on experimental model was investigated in increased carious environment, modifying the diet (high glucose concentration) and the salivary function (ablation of the submandibular salivary gland). In order to analyse the carious mechanism, experimental model was created. Sprague-Dawley rats aged 21 days were used in this experiment. All the animals were injected intraperitoneally with oxytetracycline (30-40 mg/kg) to mark the dentin areas formed during the study. The first molar erupts into the oral cavity on the 19th day after birth and reaches the occlusal plane on day 25, while the second molar erupts on the 22nd day and becomes functional on the 28th day. Control group consisted of feeding rats with standardized protein concentrate with no surgery. Group 2 consisted of feeding rats with the same standardized protein concentrate, with excision of submandibular salivary glands. Group 3 consisted of feeding rats with food made from standard protein concentrate (40%) and glucose (60%), with no surgery. Group 4 consisted of feeding rats with the same food as group 3 and excision of submandibular glands. Samples from 4 groups including 4 rats were collected after ethical approval. The smears were analysed by cytohistological methods. On the experimental model, the effect of the salivary submandibular ablation and the high glucose concentration diet was the increasing carious activity. The high glucose concentration diet reduced mineralised dentinal apposition in young rat. Dentin formation was smaller and the predentin zone wider in rats fed a glucose diet when compared with rats fed the reference diet. Widening of the predentin zone in rats fed a high-glucose diet may reflect changes in odontoblast function, such as reduced matrix synthesis and possibly disturbed mineralization. In the control group the dentinal apposition was significantly higher under the carious lesion. The high dose of glucose in diet decreased dentinal formation in comparison to the control group. The control group showed a defensive pulp-dentinal response while the carious diet group showed a decrease of the pulp-dentinal response. These aspects reveal the importance of diet in modulating the response against progression of dental caries.

Key words: High glucose concentration, diet, experimental model, pulp-dentinal response.

Introduction
The most important factors for dental caries are considered to be infection by the oral microbial flora, a caries susceptible host and dietary factors, glucose being the main cause. Bacterial mediation occurs through the production of organic acids by oral microorganisms, which utilize locally available carbohydrates as substrates. The diet of the host provides the source of carbohydrates. Also the virulence and composition of the bacterial plaque are important factors. The host factors affecting the caries initiation and progression include saliva composition and flow rate, tooth form, physico-chemical nature of tooth surface and host oral hygiene habits.

High amounts of glucose accelerate the initialisation and progression of dental caries, but seem also to affect dentin formation in young rats 1,4,16,19. Pulp-dentinal complex responds to caries destruction from early stages, and this process is obvious not only in the progression of caries destruction, but also in the defensive pulp-dentinal reactions 4.

Based on the hypothesis that morphological metabolic alterations of odontoblasts, evidenced through the decrease of dentinal apposition, can predispose to an increasing carious attack, it was assumed that glucose may contribute to the progression of carious lesion through a systemic mechanism 2, 3, 5. The development of carious lesion determines dentinal apposition in order to prevent pulp exposure.

The hypothesis that under carious lesions the dentinal apposition is increased was tested. The study focused on submandibular salivary glands because in the experimental model further presented, from the physiological and histological points of view, these glands represent over 90 percent of the salivary anti-caries activity. The reactivity of the pulp-dentinal complex on experimental model was investigated in increased carious environment, by modifying the diet (high glucose concentration) and the salivary function (ablation of the submandibular salivary gland).

Materials and Methods
An experimental model was created which was afterwards exposed to carious risk factors in order to analyse the carious mechanism. Sprague-Dawley rats aged 21 days were used in this experiment. All the animals were injected intraperitoneally with oxytetracycline (30-40 mg/kg, Terramycin®, Pfizer Corp., Brussels, Belgium) to mark the dentin areas formed during the study. The first molar erupts
into the oral cavity on the 19th day after birth and reaches the occlusal plane on day 25, while the second molar erupts on the 22nd day and becomes functional on the 28th day.

An animal model suited to the study of this disease has to be as similar to the human pathology as possible, to allow for the objective measurement of physiological parameters and to show sensitivity and reproducibility. The animals were subjected to normal atmospheric conditions at 21°C, and subjected to the same regimen of lightning (12 h of light and 12 h of dark) and the same times of feeding, handling and noise. Food and drinking water were freely available. Food and water consumption were measured at regular intervals by weighing the amount left in the cage. The rats were weighed at intervals of 2-7 days.

All the experiments were performed by a person licensed to perform animal experiments and the protocols were approved by the Experimental Animal Committee of the Medical Faculty, University of Medicine and Pharmacy Timisoara. Surgical operations were performed under anesthesia. The animals were killed under anesthesia.

The experiment was conducted over three months and performed on four groups of four Sprague-Dawley rats each, weighing 250–300 g. One of the groups was used as control. Group 1 (the control group) rats were fed standard protein concentrate for laboratory animals, no surgical intervention being performed on them. Group 2 rats were fed standard protein concentrate for laboratory animals and they underwent the submandibular glands ablation (excision). Group 3 rats were fed special diet composed of standard protein concentrate for laboratory animals (40%) and glucose (60%), no surgical intervention being performed on them. Group 4 rats were fed special diet composed of standard protein concentrate for laboratory animals (40%) and glucose (60%); rats underwent the submandibular glands ablation (excision).

Glands, dental tissues and maxillas harvesting: Fifty mg sodium thiopental/kg body weight was injected intraperitoneally to induce anaesthesia. Contact anaesthesia of hypopharynx, epiglottis and glottis has been performed using xilene 4%. The trachea was intubated under direct laryngoscopy with a peripheral endovenous catheter, 14 G, and then it was connected to ventilator. Ventilation parameters were: RR = 80–90/min, VT = 10 ml/kg body weight, pressure at the end of inspiration was 1.96 kPa.

PEEP (positive end expiratory pressure) = 0.29 kPa was maintained during the experiment to prevent clogging of pulmonary alveoli as a result of ventilation with pure humidified O2 and absence of negative intrapleural pressure. Ventilation parameters were adjusted later, depending on arterial blood gases values and on arterial acid-base parameters.

Anaesthesia was maintained by a median incision on the anterior part of the neck; the right external jugular vein and the left common carotid artery were approached and catheterised with paediatric peripheral endovenous catheters, 24 G.

The jugular approach allows for the administration of crystalloid solutions to compensate the fluid losses during the anaesthesia and for the maintaining of general anaesthesia with thiopental, 100 mg kg⁻¹ h⁻¹ with an injectomate. The carotid approach allows for the harvesting of blood samples used to assess the acid-base status and the blood gases and for the continuous monitoring of arterial pressure (average values) 10-12. Vital functions maintenance were observed as follows: parameters of acid-base equilibrium (ABE) and of blood gases, which were repeated every 60 min; disequilibria were corrected by changing the ventilation parameters and 8.4% NaHCO₃ solution has been administered depending on the values of blood pH; continuous mean arterial pressure; continuous ECG in DII derivation on a RFT BIOMONITOR-type monitor at a speed of 50 mm/s, as FC in rats = 300 – 600 b/min; continuous monitoring of central temperature measured intrarectal by a thermistor coupled to the RFT BIOMONITOR and normal value 37.3°C, maintained constant by the means of a surgery table with thermostat system. The submandibular glands were excised by surgery, and then the incised site was sutured. At the end of the experiment (after three months), the animals were euthanized using sodium thiopental administered intraperitoneally to induce the anaesthesia, immediately followed by the intracardiac administration of a potassium chloride solution. Death of animals occurs instantly. The procedures observed both biological and ethical rules in force.

Both the teeth and the osseous part (mandibular bone, maxilla, and osseous palate) were fixed in 10% formalin before being decalcified in 5% formic acid. After the decalcification, the jaws were processed with glycemethacrylate (GMA), embedded in blocks and cut into histological sections. The sections were stained with hematoxylin eosin. The smears were analysed through cytohistological methods.

In order to measure the dentine formed during the experiment only mandibular molars were used in these experiments, because mandibles were easier to dissect than maxillas. They were then sectioned sagittally in halves on the midline of the fissures of the first and second molars under water cooling by the method of Keyes 20 by using a diamond disc. Only the outer side of the disc was diamond surfaced to minimize the loss of specimen during the cutting. The disc was mounted on a technical handpiece.

To measure the area of dentin formed during the experiment, a method based on the fluorescence reaction of tetracycline labeling was used as described by Larmas and Kortelainen 21. The areas of dentinal caries lesions were measured from the spontaneous fluorescence seen under ultraviolet light.

Means, standard deviations and median values with minimums and maximums were calculated for area of dentin formation. One-way ANOVA with Tukey’s, Duncan’s and Scheffe’s tests (group 1-4) were used to identify differences in dentin formation among different groups. Statistical analyses were performed using the SPSS statistical software package (SPSS Versions 17.0, SPSS, Chigago, IL).

Results and Discussion

In this study we have studied the behaviour of oral mucosa and dental tissues with variable histological aspects depending on the diet of experimental animals with or without the salivary glands.

In the case of hyperglucidic diet, the caries frequency in experimental model was increased, so that one of the animals died of starvation after multiple tooth fractures and inflammatory lesions of periapical osteitis type under advanced carious lesions conditions (Fig. 1-2).

Under conditions of hyperglucidic diet, by developing carious environment conditions there were severe damages in inflammatory and lithium tissue context of cover and support parodontium (gum, alveolar crest, support alveolar bone) (Figs 3-5). Significant structural changes were also present in the pulp-
Hypocalcified carious dentin, with porous aspect and dilated dentinal tubules, sclerotic dentin areas including odontoblasts, lesions of reactive pulp with fibroblasts and odontoblasts hyperplasia in the experimental model with gland and hyperglucidic diet, hematoxylin eosin stain, magnification x 200.

Periapical osteitis in the case of an advanced caries in the experimental model with gland and hyperglucidic diet, hematoxylin eosin stain, magnification x 200.

Area of dentinal carious cavity with enamel remnants at the periphery of carious crater, deposits of amorphous material from plate and suppurative-necrotic parodontitis in the experimental model with hyperglucidic diet, hematoxylin eosin stain, magnification x 100.

Aggressive suppurative-necrotic osteitis and parodontitis in the experimental model with hyperglucidic diet, hematoxylin eosin stain, magnification x 100.

Chondroid bone of alveolar crest adjacent to a suppurative osteitis in a complicated carious lesion in the experimental model with hyperglucidic diet, hematoxylin eosin stain, magnification x 200.

dentin complex as regards the dentinal matrix, which presents hypomineralization areas, as well as basophile cement lines situated at the predentin-dentin junction, partly joining the globular dentin areas. We noticed the increase of dentinal permeability by widening of dentinal tubules concomitant with congestive serous pulpitis reactions, which explains the risk of rapid development of the carious process once the amelo-dentinal line was over passed (Figs 6-7).

Differences in dentin formation revealed by statistical analysis are shown in Tables 1 to 4. The mean values, with standard deviation of the groups in question would give some reason to presume that differences might exist. Because the data did not meet the assumption of homogeneity of variances Tukey’s HSD, Duncan’s and Scheffe’s tests were used to test the differences between groups. By using the Scheffe’s test, group 3 was different from group 1 (p = 0.01) and different from group 2 (p = 0.08), but no other significant differences were found between groups 1 and 2 and also between groups 3 and 4.
Figure 6. Transversal section through carious tooth in the experimental model associated with hyperglucidic diet, with large areas of sclerotic dentin and reactive osteodentin presenting cell inclusions, moderate hypercementosis adjacent to dentinal lesion, incipient serous pulpitis in pulp, edema, discrete atrophy of odontoblasts layer and decrease of the thickness of predentin deposition, hematoxylin eosin stain, magnification x 200.

Figure 7. Osteodentin in carious context, adjacent hypercementosis, decalcified tooth; transversal section also including the chondroid bone of the alveolar crest in the experimental model with hyperglucidic diet, hematoxylin eosin stain, x 100.

Table 1. Descriptive statistics of dentin formation in the 4 groups. Group 1-control group. Group 2-standard protein diet + submandibular glands ablation. Group 3-glucose enriched diet (60% glucose). Group 4-glucose enriched diet (60% glucose)+ submandibular glands ablation.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Standard error</th>
<th>95% Confidence interval for mean</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>group 1</td>
<td>5</td>
<td>10.0000</td>
<td>1.58114</td>
<td>0.70711</td>
<td>8.0368</td>
<td>11.9632</td>
<td>8.00</td>
</tr>
<tr>
<td>group 2</td>
<td>5</td>
<td>9.0000</td>
<td>1.58114</td>
<td>0.70711</td>
<td>7.0368</td>
<td>10.9632</td>
<td>7.00</td>
</tr>
<tr>
<td>group 3</td>
<td>5</td>
<td>4.8000</td>
<td>1.92354</td>
<td>0.86023</td>
<td>2.4116</td>
<td>7.1884</td>
<td>2.00</td>
</tr>
<tr>
<td>group 4</td>
<td>5</td>
<td>4.2000</td>
<td>1.30384</td>
<td>0.58310</td>
<td>2.5811</td>
<td>5.8189</td>
<td>3.00</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>7.0000</td>
<td>2.99122</td>
<td>0.66886</td>
<td>5.6001</td>
<td>8.3999</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Table 2. Analysis of variance in dentin formation

<table>
<thead>
<tr>
<th>Levene Statistic</th>
<th>df1</th>
<th>df2</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.190</td>
<td>3</td>
<td>16</td>
<td>0.901</td>
</tr>
</tbody>
</table>

The effect of the salivary submandibular ablation and the high glucose concentration on the experimental model was the increase in carious activity, the study revealing the existence of complicated carious lesions and changes in the oral ecosystem under two carious risk factors association conditions. The high glucose concentration diet reduced mineralized dentinal apposition in young rats.

Dentin formation was smaller and the predentin area wider in rats fed glucose diet when compared with rats fed the reference diet. Widening of the predentin area in rats fed high-glucose diet may reflect changes in odontoblast function, such as reduced matrix synthesis and possibly disturbed mineralization. Dental caries progression may thus be modulated by odontoblast function, not as much by matrix formation, but rather by mineralization. The dentinal apposition under the carious lesion was significantly higher in the control group.

Only young rats undergoing active secondary and/or reparative dentin formation were involved in the present experiments. As earlier reports did not show any diet-related differences in old rats, this kind of depression of the odontoblast function may, in fact, depend on age 4, 6, 8, 9.

Both in the first and the second molar having emerged into the oral cavity before or at the onset of the experiment, the dentin formed during the experiment represented secondary dentin or, in the case of a caries lesion, reparative dentin.

Many reports have shown that a 43% sucrose diet markedly reduces dentin formation in the molars of young rats. The sucrose concentration needed to markedly reduce dentin formation was between 30 and 43% 1, 2, 5, 13, 17-19. In relation with dentin formation other diets have been tested dietary carbamyl phosphate and calcium deficient diet indicating no relationship between this diets and dentin apposition 7, 15.

The present study indicated that 60% glucose diet reduced formation of dentin in young rats. An increase in the number and severity of carious lesions was also seen. At a 60% glucose concentration in diet, the pulp-dentin complex is incapable of protection through the increase of dentin apposition ratio. In the control groups, the pulp-dentin complex responds to the carious lesion by dentin apposition. Dentinal apposition under the carious lesions was significantly higher in the control group, while the formed dentinal area does not differ between fissure and intact lesions in the carious diet group. The effect of increased glucose quantity was the decrease in the dentin formation, as compared with the control group. A defensive pulp-dentin response...
occurrence of dental caries. Dentinogenesis during the experimental period and enhanced the dentinogenesis, but also predisposed to a reduction of function of the pulp dentine complex leading to reduced prevention of dental caries.

It was concluded that the glucose diet given, changed the function of the pulp dentine complex leading to reduced dentinogenesis, but also predisposed to a reduction of the pulpo-dentinal complex to dental caries in young rats. Acta Odontol. Scand. Apr. 59(2):83–87.

The effect of high dose of glucose was the decrease of dental formation compared with the control group. The control group showed a defensive pulp-dental response whereas the carious diet showed a decrease of the pulp-dental response. The present findings showed that glucose load reduces dentinogenesis by impairing the synthesis of dentin matrix, but also point out the crucial importance of the local glucose challenge in the initiation of dental caries.

It was concluded that the glucose diet given, changed the function of the pulp dentine complex leading to reduced dentinogenesis, but also predisposed to a reduction of dentinogenesis during the experimental period and enhanced the occurrence of dental caries.

References


