Sialolithiasis: mechanism of calculi formation and etiologic factors

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Abstract

Background: Sialolithiasis is a common disease of salivary glands. The etiology of these calculi is little known and their exact mechanism of formation is unknown. Methods: The composition and structure of 21 sialoliths were studied and the composition of the saliva of each corresponding patient was determined (pH, calcium, magnesium, phosphorus, citrate and phytate). Results: Eighteen sialoliths exhibited similar macro and microstructure, being constituted by hydroxyapatite (HAP) and organic matter, normally arranged in a multilayer structure. The three remaining sialoliths were exclusively constituted by organic matter. The salivary Ca of patients with HAP calculi was significantly higher than that found in the saliva of the healthy group. The salivary phytate concentration of patients with HAP calculi was significantly inferior to that found in patients with calculi exclusively formed by organic matter, as well as to that found in saliva of healthy group. Significant differences between the salivary magnesium concentrations of patients with HAP calculi and the control group were also observed. No significant differences between pH and citrate concentrations of the three groups were found. Conclusions: It was concluded that the deficit of crystallization inhibitors such as myo-inositol hexaphosphate (phytate) was also an important etiologic factor implied in the sialolith development.

Keywords: Sialolithiasis; Etiologic factors; Calcium; Phytate; Mechanism

1. Introduction

Sialolithiasis is a common disease of salivary glands characterized by the obstruction of the salivary secretion by a calculus. This is associated with pain and inflammation, and in some occasions with an infection of the affected gland. This disease corresponds to 30% of the salivary pathologies and is more frequent in adults (0.1–1.0% of population) than in children [1–4]. These calculi generally consist of mixtures of different calcium phosphates (mainly hydroxyapatite and carbonate–apatite) together with an organic matrix [2,5–7]. When infection is occasionally present, ammonium and magnesium can be also found.

The etiology of these calculi is little known and their exact mechanism of formation is unknown [3]. The aim of this paper is to study some of the etiologic factors involved with their development, with particular emphasis on those related with saliva composition. Thus, apart from pH and other well-known saliva components (calcium, magnesium, phosphorous, citrate), the presence of myo-inositol hexaphosphate (phytate), a potent crystalli-
zation inhibitor of calcium salts [8,9] was investigated. Phytate is an abundant component of plant seeds and the levels found in blood, urine and mammalian tissues clearly depend on its dietary intake [10,11].

2. Materials and methods

Composition and structure of 21 submaxillar sialoliths belonging to 21 different patients were studied following the methodology indicated below.

The saliva of each patient and controls were obtained in the same standard conditions, early in the morning and without eating, smoking, drinking or washing the mouth for at least 2 h before the sample collection. The saliva sample was obtained without stimulation, during a period of 5 min through spitting. All subjects were on free diet at the time of saliva collection and none of the patients were undergoing pharmacological treatment of any kind. After collection in sterile flasks, the samples were stored at \(-20\) °C until they were assayed. Prior to analysis, the absence of turbidity was checked (if some turbidity was detected, this was eliminated through slight acidification). pH was measured by a Crison pH meter. Calcium, magnesium and phosphorous were determined in saliva by ICP atomic emission spectrometry (Perkin-Elmer P2000), citrate was determined by the UV enzymatic test from Boehringer Mannheim (No. 139076) using previously filtered (0.45 µm pore) saliva and phytate by a procedure detailed below. The maximum time elapsed between calculus removal/expulsion and saliva analysis was 2 months. The presence of infections in saliva of the patient group was excluded due to the total absence of pus and/or inflammation of the salivary glands.

A group of 22 healthy people with the same age and sex distribution as patients was used as a control group to perform the same saliva determinations.

2.1. Sialoliths studies

The 21 sialoliths were studied through the following procedure.

The procedure to analyze and study these calculi required an appropriate combination of observation by means of macroscopic and microscopic conventional techniques (stereoscopic microscope Optomic) with physical techniques such as IR spectrometry (infrared spectroscope Brucker IFS 66) and scanning electron microscopy coupled with X-ray microanalysis (Hitachi S-530 coupled with X-ray microanalysis Oxford Link Isis) [12].

The study of the calculus began through the direct observation of its external aspect, using a stereoscopic microscope. Afterwards, the calculus was sectioned in two parts along a plane as near as possible to its geometric centre, to be able to establish the internal structure. This step indicated which process would be more adequate for further application. This implied:

(a) IR spectrometry analysis of one or several parts of the calculus. If in the fragmented calculus several parts with different aspect appeared, it was necessary to perform an IR analysis of each one. The technique to perform such analysis consisted of the well-known KBr method (it only required 1 mg or even less of sample).

(b) The deep study of the fine inner structure of the calculus and the detection and identification of microcomponents required the use of scanning electron microscopy coupled with X-ray microanalysis.

The methodology applied to carry out the study consisted of locating calculus fragments on a microscope slide fixed with silver. Afterwards, the sample was gold covered (300 Å thickness) by sputtering. The observation of calculus fragments was performed between 30 and 20,000 magnification. The combined use of X-ray microanalysis was of great importance in the identification of some components.

2.2. Phytate determination

Phytate content in saliva samples was determined using a modification of a method previously described [13]. Briefly, 2 ml of saliva were treated with 0.5 ml of HCl, 2 mol/l for 3 h. Samples were centrifuged, and supernatant at pH = 3 was used for phytate determination. Phytate was retained in a chromatographic column containing an anionic res-
in. Then, phytate was hydrolyzed to *myo*-inositol with the enzyme phytase (Sigma). *Scyllo*-inositol (Sigma) was added as internal standard. The solution was frozen to −20 °C and lyophilized. The residue was then derivatized by pyridine and chlorotrimethylsilane to silylated inositol, using a hexamethyldisilazane as catalyst. After reaction, the excess of reagents and organic solvents were blown off in a stream of nitrogen. The solid residue was extracted with hexane and injected in a gas chromatograph with mass spectrometry detection (Shimadzu QP-5000) using fused silica capillary column SPB-20 (Supelco) and He as a carrier. The calibration graph was obtained from peak height corresponding to the silylated compounds of *scyllo*- and *myo*-inositol.

**Fig. 1.** Cross-section of a sialolith constituted by hydroxyapatite and organic matter, arranged in a multilayer structure. The presence of spherulitic hydroxyapatite (arrow) can be observed.

**Fig. 2.** Brushite crystals (arrow) observed in a hydroxyapatite/organic matter sialolith (cross-section).

**Fig. 3.** Fine structure of a sialolith exclusively formed by organic matter (cross-section).

**Fig. 4.** Salivary calcium concentration of healthy control group (control), patients with calculi exclusively formed by organic matter (OM) and patients with hydroxyapatite calculi (HAP). (a) Statistically significant difference ($p<0.05$) vs. control group.
2.3. Statistical analysis

Values in the figures are expressed as mean ± SE. One-way ANOVA was used to calculate significance of differences between groups. Student’s t-test was used to assess the differences of means. SPSS for Windows program was used for statistical computations. A probability of \( p < 0.05 \) was used for assessing statistical significance.
3. Results

From the 21 studied submaxillary sialoliths, 18 exhibited similar macro and microstructures, being constituted by hydroxyapatite and organic matter, normally arranged in a multilayer structure (Fig. 1). As can be seen, hydroxyapatite was found as typical spherulites as well as the aspidinic structure. In one of the salivary calculi, next to hydroxyapatite, a little amount of brushite was detected (Fig. 2). The three remaining sialoliths were exclusively constituted by organic matter, exhibiting a different pattern, as can be seen in Fig. 3. The salivary calcium concentration of patients with hydroxyapatite calculi was significantly higher than that found in the saliva of the healthy group (Fig. 4). The salivary phytate concentration of patients with hydroxyapatite calculi was significantly inferior to that found in patients with calculi exclusively formed by organic matter, as well as to that found in the saliva of the healthy group (Fig. 5). The salivary magnesium concentration of patients with hydroxyapatite calculi was significantly inferior to that found in the healthy group (Fig. 6). No differences between the salivary pH, phosphorus and citrate concentrations of patients with hydroxyapatite calculi and the control group were observed (Figs. 7–9).

4. Discussion

The macro and microstructure of the hydroxyapatite salivary calculi described in Results is practically identical to those found in the hydroxyapatite renal calculi (non-infective phosphate renal calculi) [14]. Thus, the inner fine structure of both types of hydroxyapatite calculi (salivary and renal) are characterized by an ample occurrence of layers of amorphous material, called “aspidinic” hydroxyapatite layers. Aspidinic layers are unstructured from a macroscopic viewpoint (Fig. 1), but detailed inspection of broken surfaces reveals them to be composed of small spheres of amorphous material cemented together. These stones contain a substantial amount of organic matter both on their outer surface and inside the stone. Hydroxyapatite spheres are largely accumulated in stone cavities as either individual entities or agglomerates (Fig. 1). The structural similarities between both types of calculi must also involve a similar mechanism of formation. Thus, similar to the hydroxyapatite renal calculi, small particles of organic matter are the initial substrate on which the calculus development starts. This organic matter is gradually calcified by hydroxyapatite, due to the particular saliva composition and it can be assumed to be later transformed through ageing into an aspidinic structure. The hydroxyapatite crystallization in saliva must be favoured by an appropriate crystallization driving force (thermodynamic factor), i.e. a higher calcium phosphate supersaturation and by a low level of crystallization inhibitors (kinetic factor). Thus, effectively, in the present study, the salivary calcium concentration of hydroxyapatite calculi patients was higher than the salivary calcium concentration of the healthy control group. With respect to crystallization inhibitors, it is very interesting to observe how phytate, a potent inhibitor of hydroxyapatite crystallization [8,9], exhibited significantly lower concentrations in saliva of hydroxyapatite stone formers when compared with saliva of the healthy control group and also when compared with the saliva of uncalcified (organic matter) calculi patients. Also the salivary magnesium concentration (another crystallization inhibitor of hydroxyapatite) of hydroxyapatite stone formers was lower than the salivary magnesium concentration of the healthy control group. Nevertheless, no differences were found in endogenous saliva citrate con-
centrations between the three studied groups. Citrate has been also described as a hydroxyapatite crystallization inhibitor [16], but it must be also considered that citrate can easily increase in saliva due to exogenous contribution through toothpaste, beverages and citrate-rich foods. Obviously, this increase is only produced into the mouth but not into the salivary ducts. Thus, it can be concluded that the etiologic factors implied in the sialolith formation can be classified in two large groups: (a) saliva retention due to morphoanatomic factors (salivary duct stenosis, salivary duct diverticuli, etc.) [4,15], and (b) saliva composition factors (high supersaturation, crystallization inhibitors deficit, etc.) [16–18].

Obviously, the existence of a bacterial infection can favour the development of sialoliths through the increase of salivary pH (this produces an increment of calcium phosphate supersaturation) and due to the increase of organic matter that can obstruct the salivary ducts, favouring the nucleation and retention of hydroxyapatite [19].

As can be seen, an interesting parallelism between the formation of these calculi and the renal calculi exists. So, in both cases, the presence of retained organic matter, a high hydroxyapatite supersaturation and the deficit of crystallization inhibitors, would permit the development of the first spherulites of hydroxyapatite in the organic matrix, which upon causing a still more effective obstruction of the salivary duct, favours the calculus growth through repetition of the mentioned process. As a conclusion, it can be stated that the deficit of crystallization inhibitors as phytate must play an important role in the sialolith development.

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References