ACTION OF AN ANTIOXIDANT COMPLEX ON THE ANTIOXIDANT POWER OF SALIVA

Action of an antioxidant complex on the antioxidant power of saliva

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Aim. Based on the results of the soluble antioxidants test (SAT), we have produced a combination of oral antioxidants aimed at increasing the antioxidant power of saliva. Several antioxidants are included in this product (Vit E, beta-carotene, Vit A, Vit C, polyphenols, catechins, ellagic acid, antocyanins, coenzyme Q10 and pyridoxin in association with Se, Zn, L-cysteine). The aim of this registry study was to evaluate the efficacy of these antioxidants in saliva, plasma and urines.

Methods. MF Odontovis, an antioxidant complex, was administered to healthy subjects in the evening for one week with a final administration in the morning.

Results. Plasma, urine and saliva showed an increase in antioxidant power following both the evening administration and the final morning administration. The antioxidant action appeared to be present even at night when salival secretion is lower. Plasma SAT levels in the morning following evening treatment were increased by 21% in comparison with controls. Morning administration increased levels up to 34% when measured 4 hours after treatment. Comparable increases were observed in saliva (morning values +44 %; +58% two hours after morning administration and +28 % after 4 hours). In urine the evening administration caused an increase in antioxidant power (+6%); the morning administration did not cause an increase in antioxidants.

Conclusion. This study indicated that antioxidant levels can be increased with specific nutritional supplement. The clinical value of an increased antioxidant power in biological fluids, particularly in saliva, may be relevant for future trials of prevention and treatment.

Key Words: Antioxidants - Saliva - Plasma - Urine.

Saliva, which acts as a barrier for the external environment, includes a number of protective compounds and activities. One important element is the antioxidant system (SAO), which includes different molecules and enzymes, i.e., uric acid (UA) and peroxidases (POXs), both hydrosolubles. Other antioxidants in saliva are liposoluble (Vit E); in association with other not well known antioxidants it produces some 10% of total antioxidant capacity. UA may produce some 70% of the antioxidant capacity, and the role of ascorbic acid may be of secondary importance in comparison with UA. There is a correlation between UA in saliva and UA in plasma because plasma UA is the source of salivary UA. Ascorbic acid levels may be 3 times higher than plasmatic values. Salivary antioxidant power – in relation to lipid oxidation – is lower than the antioxidant power of plasma. POXs are the most important salival enzymes; other secondary enzymes include catalasis, glutathione peroxidase (glu POx), glutatione reductase (Glu red) and glutatione transferase. The last enzymes support the GSH pathway, one of the most important antioxidant systems, which operates in all cells and is present in any body fluid.

Considering weight, POXs represent only 0.01% of the total of salivary proteins, including the actual salivary protein (POX) and myeloperoxidase. This last protein is similar to lactoperoxidase produced by lymphocytes in areas of inflammation of the oral cavity. POXs has two main roles: (a) the control of...
the levels of \( \text{H}_2\text{O}_2 \) produced by bacteria and leukocytes, and (b) a specific anti-bacterial action for some bacteria.\(^{10-13} \)

\( \text{H}_2\text{O}_2 \) also originates with Cl, HOCL in neutrophils; this compound represents an important antibacterial (bactericidal) mechanism in saliva.\(^{14} \) Therefore, antioxidant protection is an important element in oral physiopathology, and its alterations or reduction in power may be associated to pathologies such as caries and periodontopathies.

However, oxidative power may be both beneficial and damaging, and we need to understand and measure these elements to learn how to modulate their actions. The evaluation of antioxidant power in saliva may be obtained using methods comparable to the evaluation of plasmatic antioxidants.\(^{15, 16} \) Salival antioxidant power has been evaluated\(^2 \) measuring, superoxidodismutases, uric acis and TAS (total antioxidant status) and the thiolic groups. To evaluate the antioxidant power of saliva, saliva (SATs), plasma (SATp) and urine (SATu) should be studied.

Based on the results of the soluble antioxidants test (SAT), we have produced a combination of oral antioxidants aimed at increasing the antioxidant power of saliva. Several antioxidants are included in this product (Vit E, beta-carotene, Vit A, Vit C, poliphenoles, cathechins, ellagic acid, antocyanins, coenzime Q10 and piridoxin in association with Se, Zn, L-cisteine). The aim of this registry study was to evaluate the efficacy of these antioxidants in saliva, plasma and urines.

### Materials and methods

We used the SAT to evaluate antioxidant power in saliva, plasma and urines.\(^{17} \) This method utilizes the capacity of tociyanate (SCN) to react with iron (\( \text{Fe}^{3+} \)) causing formation of \( \text{Fe}[(\text{SCN})_6]^{3-} \). This compound is characterized by a brown-red color. The possible reducing elements within the test fluid transform \( \text{Fe}^{3+} \) into \( \text{Fe}^{2+} \), avoiding the reaction with tociyanate. The color is revealed at 505 nm. The base reactant including F is \( \text{Fe(NO}_3)_3 \). However \( \text{Fe}^{3+} \) may react with phosphates present in saliva. Therefore it has been modified adding to the fluids under zirconium salts, which inactivate the phosphates without causing precipitation. This passage avoids centrifugation. This test allows the evaluation of the antioxidant power of saliva and plasma in relation to hydrosoluble antioxidants capable of reducing \( \text{Fe}^{3+} \).

### Subjects

We studied 26 subjects (13 males); the age range was between 28 and 34 years. Inclusion criteria were the absence of clinical problems and no treatment with drugs or food supplements. All subjects were non-smokers.

### Administration

Before their night rest all subjects were asked to drink MF Odontovis\(^{“} \) (“MFO“), which contains a combination of antioxidants, or a comparable solution without antioxidants for a period of one week. MFO was administered using comparable packaging for the active and non-active formulations (10 ml vials separately including a powder and the relative fluid that were mixed just before drinking). The last administration was made in the morning just before evaluation in the study center. A total of 8 vials (7 in the evening and the 8th in the morning before evaluation) were administered. The formulation of MF Odontovis, is shown in Table I. Both treatments (active and non active) were given in comparable boxes (10 vials for the two phases). Compliance was evaluated controlling unused vials.

### SAT evaluation

The test was made for plasma, saliva, and urine at inclusion and after the last administration.

Plasma SAT (SATp) was made at 8:00 am (basal value), at 11:00 and 12:00 am (the subjects were fasting since the previous evening meal). A drop of blood was collected from the finger in heparinated cuvettes. These were centrifuged to obtain plasma. The total blood used was less than 0.15 mL. SAT measurement was made on plasma samples of 10 µL.

### Table I.—Composition of MF Odontovis (MFO).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Selenium</td>
<td>48 mcg</td>
</tr>
<tr>
<td>Vit C</td>
<td>30 mg</td>
</tr>
<tr>
<td>Red Fruits, Reach Berry</td>
<td>90 mg</td>
</tr>
<tr>
<td>Zn</td>
<td>5 mg</td>
</tr>
<tr>
<td>L-cisteine</td>
<td>12 mg</td>
</tr>
<tr>
<td>Coenzime Q10</td>
<td>10 mg</td>
</tr>
<tr>
<td>Vit E</td>
<td>15 mg</td>
</tr>
<tr>
<td>Piridoxin</td>
<td>1 mg</td>
</tr>
<tr>
<td>Vit A</td>
<td>1 mg</td>
</tr>
<tr>
<td>Beta-carotene</td>
<td>0.5 mg</td>
</tr>
</tbody>
</table>

\(^{“} \)
Salival SAT (SATs) was made every hour from 8:00 a.m. (at least 30 minutes after teeth brushing) and at 12. Saliva was collected with a defined procedure: subjects were asked to chew a standard cube of medical cotton (150±20 mg) for one minute. The cotton ball was collected in a plastic cup of known weight to obtain the weight of the saliva included in the cotton ball. If salival weight was <0.7 mL/min or >1.5 mL/min, the test was repeated. All measurements were obtained with volume/weights of saliva bewteen 1.1 e 1.3 mL/min.

Urine SAT (SATu). Urine was collected the evening before testing using the night urine (between 11:00 pm and 8:00 am). Morning urine was also collected in another container between 8:00 and 12:00. Therefore SATu was the expression of night urine and morning urine (after the last administration).

Statistical analysis

Mean and standard deviation (SD) were evaluated for all measurements. To evaluate the differences in the effects of the administration of MFO we used both parametric test (Student T-test) and non parametric evaluation (ANOVA, Mann-Whitney) considering independent data for variations before and after treatment. Considering previous SAT evaluations (unpublished data on file) for \( \alpha \) value of 0.05 and 1-\( \beta \) 0.90 the difference of one SD in homogeneous groups including 20 subjects should be able to discriminate differences due to treatment. Considering possible dropouts (at a dimension of 30% of included subjects) the dimension of the groups was defined with a total of 26 subjects.

Results

All subjects completed the study. Compliance was very good (>98 %), and no tolerabilty problems were reported.

After administration of MFO (Table II), plasma antioxidant capacity increased at 4 hours after treatment (P<0.05) with minimal effects at 2 hours. Basal SATp following evening administration of MF Odontovis" for seven days was significantly higher (P<0.05) in comparison with controls. This indicates that there is a persistent residual effect of MFO up to the following morning. Non-significant effects were observed with the non-active compound. Saliva was correctly collected in all subjects (all volumes were between 1.1 e 1.3 mL/min).

Evening treatment with MFO increases the antioxidant power of saliva. To evaluate the acute effects of MFO, the treatment was administered in the morning after the last evening administration. An increase of the antioxidant power was shown within one hour after the morning administration, which remained higher than the baseline value for at least 4 hours.

In Table IV the values of urinary excretion of antioxidants have been divided into night excretion after the evening administration and morning excretion after the morning administration. SATu values after MFO treatment were significantly higher (P<0.05) in comparison with controls both considering night excre-
tion and the daily excretion within 5 hours after administration. There is no significant difference in urine volumes between the two groups.

**Discussion**

The value of these observations is somewhat limited due to the limited time of administration. The primary aim of the study was to evaluate saliva for the kinetic value of an antioxidant compound. It is possible to increase the antioxidant effects of saliva using different types of antioxidants.

The types of antioxidants include all 4 categories: circulating antioxidants, membrane antioxidants, intracellular antioxidants (pyridoxine and coenzyme Q10), and those defined as system antioxidants. All these antioxidants may generate a protective shield. However, it is important to underline the importance of circulating antioxidants that may, more easily, reach saliva.

The presence of antocyanins, catechols, polyphenols and ellagic acid in the red-fruit extract has an important ant-bacterial action (i.e. against *Streptococcus mutans* and other pathogenic bacterial elements of the oral cavity). Whether the action is effective against bacterial adhesion (either bacteriostatic or bactericidal) will have to be determined in specific studies involving caries or paradontosis or other oral infective processes.

MF Odontovis™ transports into saliva an antioxidant complex rather than a single product/compound. As shown by the antioxidant action in other clinical and preventive applications, the combination of these elements appears to be synergic. Dosages aimed at producing antioxidant action are kept low on purpose, and compounds including RDA have been kept at 100% RDA to avoid an unwanted pro-oxidant action that might result from using doses much higher than normal physiological intake.

The concept of having different synergic antioxidant actions is produced using different antioxidant products. Whether this combined antioxidant and, possibly, anti-inflammatory action may be enough to produce an antibacterial, anti-infective action should be evaluated in longer prospective studies.

Morning SATp levels following the evening treatment increased on average 21% (P<0.05) in comparison with controls. Morning administration increased levels up to 34% (4 hours after administration; P<0.05); the increase was clear one hour after administration (showing a fast absorption).

Comparable increases were observed in saliva (SATs); morning values were increased up to 44%; after 2 hours they increased 58%; after 4 hours the increase was 28%. Therefore, hydrosoluble antioxidants are quickly transferred into saliva.

In urine (SATu) evening administration caused an increase in night secretion of +6%; morning administration did not show any change in urinary antioxidants. This may indicate that the excretion of metabolites without antioxidant power is prevalent in urine.

With a daily production of about 1.5L per day, saliva is basically recycled as antioxidants flow again into the intestinal tract and follow reabsorption and metabolism. This causes an antioxidant overload in the evening, which is shown in urine. The clinical value of an increased antioxidant power in biological fluids, particularly in saliva, may be relevant for future trials including both prevention and treatment.

The SAT is very important to measure in a simple way the levels of antioxidants. These tests may indicate how antioxidant levels can be increased with the use of specific nutritional supplements within physiological modulation.

**References**