ACTIVATED AND MICRONIZED ZEOLITE IN THE MODULATION OF CELLULAR OXIDATIVE STRESS IN MEXICAN SMOKERS: A RANDOMIZED CLINICAL TRIAL

ALFONSO ATITLÁN-GIL1,2, MARTÍN M. BRETÓN-DE LA LOZA3, JOSÉ C. JIMÉNEZ-ORTEGA4, HELEN BELEFANT-MILLER5 AND GABRIEL BETANZOS-CABRERA6*

1Innovation Department, Granding International, SA de CV, Jiutepec, Mor., Mexico; 2Research Department, Coordinación de Investigación del Área de Ciencias de la Salud, Centro Universitario Siglo XXI, Pachuca, Hgo., Mexico; 3Department of Translational Investigation, Facultad de Nutrición, Universidad Autónoma del Estado de Morelos, Cuernavaca, Mor., Mexico; 4Department of Molecular Biology, Centro Médico Nacional de Biología Molecular, Pue., Mexico; 5Dale Bumpers National Rice Research Center, Agricultural Research Service, USDA, Stuttgart, USA; 6Department of Nutrition and Clinical Toxicology, Área Académica de Nutrición, Instituto de Ciencias de la Salud, Universidad Autónoma del Estado de Hidalgo, Pachuca, Hgo., Mexico

ABSTRACT

Background: Activated and micronized zeolites are used as detoxifying agents in humans. Detoxification is attributed to their ability to reduce lipid peroxidation by scavenging free radicals. Objective: To evaluate activated and micronized zeolites as modulators of cellular oxidative stress in Mexican smokers without lung diseases. Methods: Randomized clinical trial. Subjects were randomly divided into three groups: activated and micronized zeolites, n = 29; vitamin E, an accepted antioxidant, n = 29; and maltodextrin as control, n = 27. Each group received the corresponding supplementation, dissolved in water, once a day for 30 days as follows: activated and micronized zeolites, 5.4 g activated and micronized zeolite; vitamin E, 400 mg D-alpha tocopheryl acetate; and maltodextrin, 250 mg of maltodextrin. The thiobarbituric acid reactive substances assay was used to screen for lipid peroxidation. Catalase activity, plasma antioxidant capacity, and hydrogen peroxide levels were also measured. Results were analyzed by a one-way ANOVA and post hoc test of Bonferroni. Results: Subjects administered activated and micronized zeolites had equivalent antioxidant activities as subjects administered vitamin E. Conclusions: Activated and micronized zeolites may be useful as a modulator of oxidative stress in smokers. However, inclusion of a comparison group of non-smokers would be useful in future studies to assess the degree to which zeolites reverse the oxidant stress. (REV INVEST CLIN. 2017;69:146-51)

Key words: Activated micronized zeolite. Oxidative stress. Smoking. Catalase. Total antioxidant power.
INTRODUCTION

Cigarette smoking is a major environmental stress factor since smoking enhances reactive oxygen species (ROS) formation, which becomes a significant oxidative stress in vivo1. A smoker is exposed to large amounts of free radicals in each puff, and this exposure is further enhanced when the smoker holds the smoke in the body1,2. ROS cause oxidative damage to cells and are generated by various sources, even under normal conditions such as regular cell metabolism when cells maintain a balance between oxidants and antioxidants1-3. However, external factors can increase ROS production1. Oxidative stress is a condition wherein the cellular production of ROS exceeds the physiological capacity of the antioxidant defense systems to inactivate them3,4. In humans, the most common oxidants are ROS and molecular precursors of ROS such as hydrogen peroxide (H2O2)3. ROS cause oxidation of lipids and lipoproteins, DNA, proteins, and other molecules, altering cellular functions and are probably the cause of a number of chronic diseases2,4-6.

Catalase, an antioxidant enzyme present in most aerobic organisms, catalyzes the dismutation of H2O2 into water and oxygen3,5. The human body has several enzymes that chemically decompose H2O2, including catalase, peroxidases, and peroxiredoxins3. Lipid peroxidation of cell membranes, which produces malondialdehyde (MDA), is considered a good indicator of oxidative stress6. The antioxidant power capacity of a system is measured by the antioxidant capacity of plasma systems to inhibit 50% of the formation of thiobarbituric acid reactive substances (TBARS)3,4,6.

Vitamins E and C (ascorbic acid) are two of many antioxidant molecules that can inactivate ROS3,4. The reduction in vitamin E levels is accelerated in cigarette smokers due to their increased oxidative stress; however, vitamin E levels can be normalized by vitamin C supplementation. There is evidence that ascorbic acid supplementation (500 mg, twice daily for two weeks) would lead to normal rates in smokers' plasma alpha- and gamma-tocopherol disappearance7.

Zeolites are a group of aluminosilicates that exhibit diverse biological activities and have found uses as adjuvant therapy in anticancer therapy, adjuvant for vaccines, treatment of heartburn, and as antiinflammatory agents8-17. Natural zeolites are hydrated porous volcanic microcrystals formed mainly of AlO4 and SiO4, which have defined shapes resulting from tetrahedral building blocks connected through O2 atoms. Their open structure can integrate a wide variety of cations, such as Na+, K+, Ca2+, and Mg2+, based on a size exclusion process derived from the regular pore structure at the molecular level18-20. Clinoptilolite is a natural zeolite with a particular three-dimensional structure that endows it with specific physicochemical properties that include ion-exchange capacity, absorbency, size-exclusion framework, as well as catalytic properties19,20. Activated and micronized zeolites are detoxifying agents because of their selective binding of heavy metals, O2, and ROS. Various studies have shown that these hydrated aluminosilicates have no toxic effects in either humans or animals8-10,14,15,21. Thus, we hypothesized that zeolites could provide a beneficial function of modulating cellular oxidative stress in smokers.

MATERIALS AND METHODS

Materials

The natural zeolite Klinobind® (kindly donated by Granding International, SA de CV, Jutepec, Morelos, Mexico) was activated and micronized by mechanical micronization, and tribochemical and thermal activation. Particle size was D90 1-11 µm and the specific surface area, as measured by the Brunauer-Emmett-Teller method, was 27 m2/g22. Vitamin E, in the water-soluble form of D-alpha tocopheryl acetate (Astroquim, SA de CV, Ecatepec, Mexico), and maltodextrin (Amfher Foods, SA de CV, Mexico City, Mexico) were used as positive and negative controls, respectively.

Study design and subjects

This study was a randomized, double-blind, controlled trial. Smokers without lung diseases were randomized to receive, during 30 days, one of three treatments: activated and micronized zeolite (ZAM), vitamin E, or maltodextrin (Amfher Foods, SA de CV, Mexico City, Mexico). Subjects were included if they had smoked...
for 2–37 years and smoked 8–29 cigarettes per day. Subjects with lung disease or any diseases, including acute infections or chronic illnesses, were eliminated after the initial clinical evaluation performed by physicians. Other exclusion criteria were the use of antioxidant supplementation or any drug containing antioxidants. The study was performed in accordance with the appropriate version of the 1964 Declaration of Helsinki and under the Law of General Health of Mexico. The Ethics Committee of Centro Médico Nacional de Biología Molecular approved the study protocol (register number KB-002-2012). A signed consent form was obtained from all participants.

Treatments

During the 30 days of the study, subjects were required to maintain their usual lifestyle and diet. To ensure that subjects could not distinguish zeolite from vitamin E and the control maltodextrin (polysaccharide that is used as a food additive), a commercial orange flavored powder (ML-G1-001 Doehler Mexico S.A. de C.V) was used to provide color and flavor to the three treatments. Clinical interviews were given by two physicians who were blind to the study’s objective. Likewise, two nutritionists participated in the assignment of treatments to the groups, but did not meet the subjects. The investigator who analyzed the samples also never had contact with the subjects. The number of subjects per group was: ZAM, n = 29; vitamin E, n = 29; and maltodextrin (MALTD), n = 27. The dose of ZAM was calculated based on a previous report by Dogliotti, et al.23. Subjects took the supplements dissolved in water every morning before the intake of any food or beverage during 30 days, as follows: ZAM, 5.4 g of activated and micronized zeolite; vitamin E, 400 mg of D-alpha tocopheryl acetate; and MALTD, 250 mg of maltodextrin.

Biochemical analyses

On days 0 and 30 of the intervention, blood samples were obtained by venipuncture after an overnight fast. The blood was immediately centrifuged at 4,000 rpm for 10 minutes and the serum samples were separated into aliquots and frozen at −70 °C until tested.

Four different tests for antioxidant activity were utilized. All of them were assayed in microtiter plates using commercial enzymatic colorimetric kits (Bioassay Systems, Hayward, CA): Plasma antioxidant capacity (QuantiChrom™ DTAC-100), catalase activity (EnzyChrom™ ECAT-100), H₂O₂ (QuantiChrom™ DIOX-250), and the thiobarbituric acid reactive substances (TBARS) assay (QuantiChrom™ DTBA-100). Changes in H₂O₂ levels were directly measured, and lipid peroxidation was determined by measuring the inhibition of the formation of TBARS. Absorbance was measured in a spectrophotometer (ATI-Unicam 300, Cambridge, UK). If necessary, samples were diluted in a buffer and results were multiplied by the dilution factor. Each experiment was performed in triplicate.

Statistical analysis

Results were expressed as mean ± SD of the value at 30 days minus the value at 0 time in each subject. The Kolmogorov-Smirnov test was performed to ensure that the data distribution was equivalent among the test groups. The Bonferroni test was used to compare the means after rejecting the null hypothesis of equality of means by a one-way ANOVA. Differences were considered significant at p < 0.05. Statistical analysis was performed with SPSS v.12.0 (IBM).

RESULTS

A total of 85 smokers, 49 male and 36 female, participated in the trial. The baseline characteristics are described in table 1. All groups had similar values and there were no statistically significant differences between the test groups.

As expected, no changes were apparent in the subjects treated with the control compound MALTD in their plasma antioxidant capacity (Fig. 1), catalase levels (Fig. 2), hydrogen peroxide levels (Fig. 3), or lipid peroxidation activity (Fig. 4). The administration of either the standard antioxidant vitamin E or the test detoxifying agent ZAM resulted in large increases in the amount of antioxidants present in the plasma (Figs. 1 and 2) and decreases in ROS (Fig. 3) and oxidative stress (Fig. 4).

The ZAM was even slightly more effective than vitamin E in raising the level of antioxidant capacity of the plasma (Figs. 1 and 2). ZAM also decreased H₂O₂ levels (Fig. 3) and lipid peroxidation activity (Fig. 4) to nearly those of the vitamin E treatment.
DISCUSSION

Free radicals are produced in excess during mitochondrial respiration and can react with biomolecules, altering their functions\textsuperscript{2,6}. This molecular damage can be minor in healthy subjects because the free radicals can be trapped or neutralized by the subject’s natural antioxidant systems\textsuperscript{1,3,4}. However, the antioxidant system can be overwhelmed by increases in certain factors, including age, sedentary lifestyle, weight, and smoking\textsuperscript{1,3}. Oxidative stress is exacerbated in smokers\textsuperscript{3}, and smoking is a proven risk factor for cancer, respiratory disease, and circulatory disease because the free radicals in smoke enhance oxidative stress\textsuperscript{1,2}.

Although the prevalence of coronary heart disease is staggering, many of its risk factors are ultimately preventable through a healthy diet, exercise, and other positive lifestyle habits\textsuperscript{24}. Of these, cigarette smoking is considered to be the most significant. As the number-one cause of preventable death, it is estimated that smoking is responsible for over 440,000 heart-related deaths every year in the USA\textsuperscript{25}. In Mexico, over 38 million people have smoked at least once in their lives (two men to every woman). In 2011, 17.3 million

Table 1. Baseline data of treatment groups

<table>
<thead>
<tr>
<th></th>
<th>ZAM (n = 29)</th>
<th>Vit E (n = 29)</th>
<th>MALTD (n = 27)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>12</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Male</td>
<td>17</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Age, mean y ± SD</td>
<td>35 ± 13</td>
<td>28 ± 11</td>
<td>29 ± 12</td>
</tr>
<tr>
<td># Cigarettes/day ± SD</td>
<td>20 ± 7</td>
<td>19 ± 8</td>
<td>18 ± 8</td>
</tr>
<tr>
<td>Smoking time, y ± SD</td>
<td>14 ± 12</td>
<td>10 ± 11</td>
<td>10 ± 10</td>
</tr>
</tbody>
</table>

**Plasma antioxidant**

<table>
<thead>
<tr>
<th></th>
<th>ZAM (mM) ± SD</th>
<th>Vit E (mM) ± SD</th>
<th>MALTD (mM) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capacity Trolox</td>
<td>1,232.5 ± 250.1</td>
<td>1,227.1 ± 313.0</td>
<td>1,152.4 ± 283.5</td>
</tr>
<tr>
<td>Catalase Activity</td>
<td>24.9 ± 7.9</td>
<td>26.2 ± 8.8</td>
<td>21.4 ± 5.8</td>
</tr>
<tr>
<td>H\textsubscript{2}O\textsubscript{2} (mg/dl)</td>
<td>44.9 ± 4.4</td>
<td>45.1 ± 6.6</td>
<td>43.7 ± 5.3</td>
</tr>
<tr>
<td>Malondialdehyde (mM)</td>
<td>5.3 ± 0.8</td>
<td>5.2 ± 1.0</td>
<td>5.4 ± 0.7</td>
</tr>
</tbody>
</table>

Baseline measurements were not significantly different (\(p > 0.05\)) between the treatment groups for either the characteristics of the group or biochemical data.

MALTD: maltodextrin; Vit E: vitamin E; ZAM: zeolite.

Figure 1. The effect of zeolite on levels of plasma antioxidant capacity in plasma of human cigarette smokers.
The letters a and b indicate significant differences (\(p < 0.05\)) between zeolite and maltodextrin, between vitamin E and maltodextrin, respectively.
MALTD: maltodextrin; Vit E: vitamin E; ZAM: zeolite.

Figure 2. The effect of zeolite on catalase activity in plasma of human cigarette smokers.
The letters a and b indicate significant differences (\(p < 0.05\)) between zeolite and maltodextrin, between vitamin E and maltodextrin, respectively.
MALTD: maltodextrin; Vit E: vitamin E; ZAM: zeolite.
Mexicans between 12 and 65 years of age were active smokers (12 million men and 5.2 million women). Mexico City had the highest numbers of smokers in the country, with 30.8%, followed by the western region of Mexico, with 24.7%. The lowest percentage of smokers is in the south, between 14 and 18%. There is also evidence to suggest a strong relationship between that of passive smoke exposure, or second-hand smoke, and the development of coronary heart disease (CHD). It is now widely considered that exposure to tobacco smoke increases the risk of CHD by 25-30%, making it imperative to find alternative prevention methods for both active as well as passive smokers. The ability of zeolites to absorb molecules of particular sizes has been used in medicine due to their anti-carcinogen and antioxidant properties, among others. As shown in previous reports, zeolites have high levels of antioxidant activity based on their capacity to scavenge free radicals and to inhibit lipoperoxidation.

To our knowledge, this is the first clinical study that measures the effects of oral supplementation with zeolite on the oxidative stress levels of smokers. We observed that zeolite was as effective as vitamin E in reducing oxidative stress and this was achieved without any adverse effects on the study subjects. Thus, zeolites may be considered as an alternative way to attenuate oxidative stress, not only in smokers, but also in any disease that involves the generation of oxidative stress. Because of the great heterogeneity in the intensity and time span of smoking (smoking time: 2-37 years; number of cigarettes per day: 8-29), it is difficult to make an analysis of the effectiveness of zeolites for different durations of this addiction. The limitations of the study are the number of subjects included and the number of ZAM determinations, making it worthwhile to expand this research to larger populations and with more testing to confirm the promising results observed from this pilot study. Furthermore, including a control group of non-smokers would be useful to assess the degree to which zeolites reverse the oxidative stress of smoking so that its effectiveness can be fully evaluated. Eventually, it would be valuable to clarify the mechanisms of action of the zeolite compounds.

In conclusion, the activated and micronized zeolite tested here (ZAM), acted as a modulator of oxidative stress in smokers when it was orally administered daily for 30 days. ZAM significantly improved the plasma antioxidant capacity and the enzymatic antioxidant system and decreased the H$_2$O$_2$ production and lipid peroxidation levels in smokers.

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