Bioavailability and Antioxidant Effects of a Xanthone-Rich Mangosteen (Garcinia mangostana) Product in Humans

MIWAKO KONDO,† LILIANG ZHANG,† HONGPING JI,‡ YAN KOU,‡ AND BOXIN OU*†,

†Brunswick Laboratories, 50 Commerce Way, Norton, Massachusetts 02766, and ‡Brunswick Laboratories (China), 320, A3 Building, 218 Xing Hu Road, Suzhou Industrial Park, Suzhou, Jiangsu, China

Oxidative damage is involved in many chronic diseases including those cited as the major causes of death in Western societies such as cardiovascular disorders and cancer. Antioxidants may prevent these degenerative processes by various mechanisms including scavenging of free radicals. Intake of antioxidant supplements is associated with preventing oxidative damages. This study investigated the absorption and antioxidant effects of a xanthone-rich mangosteen liquid in healthy human volunteers after the acute consumption of 59 mL of the supplement. The liquid contained mangosteen, aloe vera, green tea, and multivitamins. Results indicated that α-mangostin and vitamins B2 and B5 were bioavailable, with observed Cmax at tmax of around 1 h. The antioxidant capacity measured with the oxygen radical absorbance capacity (ORAC) assay was increased with a maximum effect of 18% after 2 h, and the increased antioxidant level lasted at least 4 h. Overall, this study demonstrated the bioavailability of antioxidants from a xanthone-rich mangosteen product and its in vivo antioxidant effects.

KEYWORDS: Aloe vera; antioxidant; bioavailability; green tea; mangosteen; α-mangostin; multivitamin; ORAC

INTRODUCTION

Oxidative damage is involved in many chronic diseases including some of the prominent causes of death in Western societies such as cardiovascular disorders and cancer (1–5). Antioxidants may prevent these degenerative processes by various mechanisms including scavenging of free radicals. There are numerous reports about the reduction of the incidence of degenerative diseases due to the consumption of fruits and vegetables (6–9). These positive bioactivities are considered mainly to be due to the presence of various antioxidants in fruits and vegetables. Most of the antioxidant activity in foods is thought to be due to vitamins C and E, polyphenols, and carotenoids (10, 11).

Mangosteen (Garcinia mangostana) is a tropical evergreen tree originated in Southeast Asia and used for centuries as a folk medicine. The rind has been used for internal and external infections, and poultices can be used to treat skin conditions; an extract of mangosteen pulp has even been used to control fever (12). Recently, the consumption of mangosteen products has increased as a dietary supplement in the United States, because of their potent antioxidant properties (13, 14). Xanthones, a particular class of plant phytochemicals from mangosteen, are highly biologically active, possess anti-inflammatory properties such as COX inhibition, and have cardiovascular protective effects (15–18).

Mangosteen Plus with Essential Minerals, a commercial product, contains a wide variety of natural antioxidant sources including mangosteen, green tea (Camellia sinensis), aloe vera, and multivitamins. Some ingredients in this product are believed to provide preventive effects against diseases associated with aging. However, there have been no human bioavailability studies using commercial mangosteen juice to our knowledge. Due to the health benefits now attributed to mangosteen consumption and the rising popularity of mangosteen botanical supplements, human intervention studies using mangosteen extract supplements are crucial for determining the efficacy of mangosteen extracts in the prevention of chronic diseases and establishing science-based dosing recommendations. In this study, we determined the bioavailability of the free form of α-mangostin and vitamins B2 and B5 found in a xanthone-rich mangosteen product and its efficacy on plasma antioxidant status in the human body.

MATERIALS AND METHODS

Subjects and Study Protocol. A randomized, double-blind, placebo-controlled clinical trial was conducted with generally healthy male and female subjects between 20 and 23 years of age. All subjects were screened by evaluation of a medical history and assessment of diet history and supplement history using a self-developed semiquantitative questionnaire. It was required that subjects had not supplemented their diet with vitamins, minerals, or antioxidants for at least 6 months prior to the study. Written informed consent was obtained from each volunteer participating in this study. All procedures of the protocol were approved by the Institutional Review Board for the Protection of Human Subjects of the Capital Medical University (Beijing, China). Ten men and 10 women participated in this study. Participants were randomly divided into two groups, study or placebo group, with the same number of male and female participants in each group. The average age of both study and placebo groups was 22 years old. The trial duration was 24 h. On the morning before the study started, a blood sample was drawn after a fast of at least 8 h. Each participant was then given a single dose (59 mL) of Mangosteen Plus with Essential Minerals, the Vemna formula, or a matching 59 mL
dose of fructose liquid before breakfast on the trial morning. Table 1 lists the ingredients of each liquid. Blood samples were then collected from each subject at 1, 2, 4, and 6 h after administration of the Vemma formula or the placebo liquid. Plasma was obtained by centrifugation. One milliliter of each plasma sample was freeze-dried and stored at −80 °C until analysis.

Material. α-Mangostin was purchased from Chromadex (Irvine, CA), and vitamin B12 was from Acros (Geel, Belgium). Vitamin B5 was obtained as thiamin hydrochloride (as riboflavin USP, mg 1.5 BLD). Niacin was obtained as niacinamide, mg 20 BLD. Riboflavin (as riboflavin USP, mg 1.7 BLD). Thiamin (as thiamin hydrochloride), mg 1.5 BLD. Catechins, epigallocatechin gallate, mg 38 BLD. Plant-source mineral blend, BLD. Tea blend, g 25.2 BLD.

Table 1. Energy, Nutrient, Mineral, and Specific Component Intake with the Mangosteen Product (59 mL) and the Placebo Juice (59 mL)

<table>
<thead>
<tr>
<th>analyte</th>
<th>concentration (ng/mL)</th>
<th>slope</th>
<th>intercept (ng/mL)</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-mangostin</td>
<td>0.4—100</td>
<td>0.000273</td>
<td>0.00</td>
<td>0.996</td>
</tr>
<tr>
<td>vitamin B2</td>
<td>1—200</td>
<td>179</td>
<td>−33.8</td>
<td>0.997</td>
</tr>
<tr>
<td>vitamin B6</td>
<td>8—200</td>
<td>186</td>
<td>−3.38</td>
<td>0.998</td>
</tr>
</tbody>
</table>

with the following conditions: 1 min hold at 100% A, 0.5 min linear gradient from 100 to 60% A, 4.5 min linear gradient from 60 to 20% A, 1.1 min hold at 2% A, 0.1 min linear gradient from 2 to 100% A, and 2.9 min hold at 100% A. The mass detector was equipped with a turbo-ion spray (electrospray ionization, ESI) source and operated in multiple reaction monitoring mode (MRM) under negative ion mode. The heated capillary and voltage were maintained at 550 °C and −4500 V, respectively. The optimized instrument setting is listed in Table 4. Representative chromatograms of MRM scans are shown in Figure 2.

The lower limit of quantification (LLOQ) for α-mangostin was 0.4 ng/mL.

Vitamins B2 and B6. Two hundred microliters of reconstituted human plasma was mixed with 280 μL of methanol and 20 μL of trifluoroacetic acid. The supernatant was collected after vortexing and centrifugation at 14000 rpm for 10 min (4 °C). Five microliters was injected onto the LC-MS/MS for vitamin B analysis. Chromatography was carried out on a 50 × 4.6 mm, 3 μm, Ascentis RP-AMIDE column (Sigma-Aldrich), and the solvent system consisted of a gradient system with water (0.4% formic acid, v/v) (A) and acetonitrile (B). Gradient elution was performed at 0.6 mL/min with the following conditions: 0.9 min hold at 100% A, 0.1 min linear gradient from 100 to 90% A, 3.0 min linear gradient from 90 to 40% A, 1 min linear gradient from 40 to 2% A, 2.0 min hold at 2% A, 0.1 min linear gradient from 2 to 100% A, and 3.0 min hold at 100% A. The mass detector was equipped with a turbo-ion spray (ESI) source and operated in MRM under positive ion mode. The heated capillary and voltage were maintained at 550 °C and 5500 V, respectively. The optimized instrument settings for vitamins B2 and B6 are listed in Table 4. Representative chromatograms of MRM scans are shown in Figure 2. The LLOQ for α-mangostin was 0.4 ng/mL. The LLOQs for vitamins B2 and B6 were 1 and 8 ng/mL, respectively.

Table 2. Concentration Range and Linearity of Calibration Curve

The measured values were used to summarize the characteristics of the subjects. The measured values were used for the maximum plasma concentration, Cmax, and the time to reach the maximum plasma concentration, tmax. A paired t test was performed to determine the significance of the test using Microsoft Excel software (Office 2003, Microsoft Corp., Redmond, WA).
Table 3. Recovery of Tested Analytes in Human Plasma

<table>
<thead>
<tr>
<th>analyte</th>
<th>spiked concentration (ng/mL)</th>
<th>mean measured, ng/mL (n = 5)</th>
<th>CV (%)</th>
<th>recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-mangostin</td>
<td>0.4</td>
<td>0.37</td>
<td>11.9</td>
<td>92.2</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>2.05</td>
<td>8.67</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>9.78</td>
<td>3.55</td>
<td>97.8</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>44.1</td>
<td>3.58</td>
<td>88.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>106</td>
<td>2.49</td>
<td>106</td>
</tr>
<tr>
<td>vitamin B2</td>
<td>1</td>
<td>0.89</td>
<td>9.22</td>
<td>88.8</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>7.13</td>
<td>4.94</td>
<td>89.2</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>85.3</td>
<td>2.91</td>
<td>85.3</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>172</td>
<td>2.68</td>
<td>85.9</td>
</tr>
<tr>
<td>vitamin B5</td>
<td>8</td>
<td>7.66</td>
<td>4.70</td>
<td>95.8</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>82.8</td>
<td>4.43</td>
<td>82.8</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>177</td>
<td>2.49</td>
<td>86.7</td>
</tr>
</tbody>
</table>

Table 4. Molecular Weights (MW) and Optimized Instrument Settings

<table>
<thead>
<tr>
<th>analyte</th>
<th>MW</th>
<th>parent ion</th>
<th>production</th>
<th>DP&lt;sup&gt;a&lt;/sup&gt; (V)</th>
<th>CE&lt;sup&gt;b&lt;/sup&gt; (V)</th>
<th>CXP&lt;sup&gt;c&lt;/sup&gt; (V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-mangostin</td>
<td>410.5</td>
<td>409.0</td>
<td>351.1</td>
<td>−90</td>
<td>−35</td>
<td>−10</td>
</tr>
<tr>
<td>vitamin B2</td>
<td>376.4</td>
<td>377.3</td>
<td>243.2</td>
<td>85</td>
<td>35</td>
<td>8</td>
</tr>
<tr>
<td>vitamin B5</td>
<td>219.2</td>
<td>220.4</td>
<td>90.2</td>
<td>50</td>
<td>20</td>
<td>12</td>
</tr>
</tbody>
</table>

<sup>a</sup> Dedustering potential.  <sup>b</sup> Collision energy.  <sup>c</sup> Collision cell exit potential.

RESULTS AND DISCUSSION

LC-MS/MS Analysis of Plasma Samples. One xanthone and two vitamins in human plasmas were measured by LC-MS/MS methods. α-Mangostin, one of the most abundant xanthones in mangosteen, is known as an antioxidant. The plasma level of α-mangostin was analyzed in this study. The bioavailability of vitamins B2 and B5 was also investigated. Although they are not antioxidants, they are essential nutrients and important for cell metabolism. Therefore, they are interesting as a part of one’s health promotion. A complete data set was not available for several subjects due to concentrations being below LLOQ. For that reason the missing values were replaced by 0 to obtain the mean for all subjects. Figure 3 illustrates the mean plasma concentration–time curves of tested analytes.

A significant increase of α-mangostin was detected in the study group, whereas no α-mangostin was found in the placebo group within the detection range. The α-mangostin plasma concentration reached its maximum (C<sub>max</sub>) of 3.12 ± 1.47 ng/mL at t<sub>max</sub> of 1 h after the Vemma formula consumption. After that time point, the concentration of α-mangostin decreased to one-third of C<sub>max</sub> by the fourth hour, and the level remained through the sixth hour. After mangosteen product consumption, at 2 h, C<sub>max</sub> values of vitamins B2 and B5 were 7.52 ± 2.72 and 48.9 ± 11.7 ng/mL, respectively. Significant increases of vitamins B2 and B5 were observed in the treatment group (P = 0.022 and 0.041, respectively), whereas no significant changes were seen in the placebo group (data not shown). As seen in Figure 3, the concentrations of vitamins B2 and B5 roughly doubled within 1 h and gradually decreased to the level at time 0.

Antioxidant Capacity (ORAC Assay). The human plasma antioxidant capacity increased by >16% at 1 h following consumption of the Vemma formula (Figure 4). The ORAC value increased as much as 18% after 2 h and stayed elevated through 6 h, the last blood draw in the trial. In the placebo group, no change was observed (data not shown).

The bioavailability of vitamins B2 and B5 is low after the ingestion, and they are not antioxidants themselves. For that reason, they are not expected to contribute to ORAC value. α-Mangostin has antioxidant activity both in vitro (13) and in vivo, but the increase in ORAC after the consumption of the xanthone-rich product could not be explained solely by the increase in plasma α-mangostin. The possible reasons are due to the t<sub>max</sub> and plasma level of α-mangostin. The t<sub>max</sub> of α-mangostin and that of plasma ORAC value do not match: 1 and 2 h, respectively. Plasma α-mangostin concentration was increased by 3.12 ng/mL, which accounts for only a small percentage of the significant increases in plasma ORAC. Therefore, we can conclude that α-mangostin is not the main contributor to the increase in plasma ORAC.

Even though the increase of antioxidant capacity may be related to α-mangostin, other antioxidants in the Vemma formula are likely to contribute to the increase. Vitamins C and E are well-known antioxidants contained in this xanthone-rich product. However, significant increases of vitamins C and E were not observed (data not shown). The increase of vitamins C and E in plasma would not be sufficient to give such a large impact on plasma ORAC (21), and there is no potentiation observed on the antioxidant effects by vitamins C and E (22) due to the high base concentrations. Although one serving (59 mL) of the Vemma formula contains 300 mg of vitamin C and 60 IU of vitamin E, more than double the recommended daily values, they would not be main contributors to the increase in ORAC value in this study.

This xanthone-rich product also contains green tea, which is full of catechins. Green tea catechins belong to the flavonoid family and are known to have fairly strong antioxidant activity with ORAC values of 81,000–220,000 μmol of Trolox equiv/g (23). As possible contributors to ORAC, the bioavailability of catechins was tested. The assay was carried out as described by Henning et al. (24). Because the majority of catechins are present as glucoronides and sulfates, plasma samples were hydrolyzed using β-d-glucuronidase type X-A from Escherichia coli and sulfatase type VIII from abalone entails. Analyzed catechins are, namely, catechin, epicatechin catechin gallate, epicatechin gallate, gallo catechin gallate, and epigallocatechin gallate. However, we did not detect any green tea catechins in the plasma samples (data not shown). This may be due to the low concentration of catechins in one serving. Data presented by Henning et al. showed that the bioavailability of four catechins including epicatechin, epicatechin gallate, epigallocatechin, and epigallocatechin gallate. The intake of the four catechins was approximately 680 mg, and plasma level at t<sub>max</sub> was 395 ng/mL in total. Whereas the total catechin concentration in Vemma formula was 119 mg, we would expect the lower concentration in plasma. As we expected, none of eight catechins were detected by HPLC-ECID system. Therefore, we can conclude that green tea catechins do not make a major contribution to the observed ORAC increase.

There are other possible contributors to the ORAC increase in this product including flavonoids, some other phenolics, and plant-sourced minerals. Particularly, some flavonoids are known to have several times higher antioxidant activities than those of vitamins C and E (19, 25, 26), which may contribute greatly to the increasing ORAC. However, this study did not focus on the bioavailability and structural elucidation of flavonoids except green tea catechins. Moreover, this study did not reveal if the ORAC increase was due to some strong antioxidants or synergistic effects of many components. Therefore, a detailed study is required to confirm all possible contributors and the mechanism of the antioxidant capacity increase.
In summary, we determined the physiological availability of xanthones and vitamins found in a xanthone-rich product and their effects on the degree of antioxidant potency in the human body. Noteworthy bioavailability was seen in α-mangostin and vitamins B2 and B5 along with the increase of antioxidant capacity. In the experiment group within 2 h after consumption of the xanthone-rich product, recognizable increases of α-mangostin, vitamins B2 and B5, and ORAC (antioxidant levels) were observed in these subjects. In the placebo group, no significant change was observed in vitamins B2 and B5, and no α-mangostin was detected. Our results suggest that Mangosteen Plus with Essential Minerals is an excellent source to increase the blood

Figure 2. LC-MS/MS MRM chromatograms of blank human plasma (A), spiked human plasma at limit of quantitation (B), and representative sample (C) for each standard compound.

Figure 3. Concentration of each analyte in human plasma versus time curve after a single oral administration of a xanthone-rich mangosteen product (59 mL): (A) α-mangostin; (B) vitamin B2; (C) vitamin B5. Values were expressed as means ± SEM.
level of antioxidants, thus possibly offering protection against chronic diseases caused by the aging process. However, further studies need to be done to characterize the antioxidant properties of the xanthone-rich product as a dietary supplement.

LITERATURE CITED

(1) U.S. National Center for Health Statistics, Health, United States, 2007, with Chartbook on Trends in the Health of Americans, Hyattsville, MD.

Received March 26, 2009. Revised manuscript received May 27, 2009. Accepted July 31, 2009.