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ORIGINAL ARTICLE

Tropical fruit camu-camu (*Myrciaria dubia*) has anti-oxidative and anti-inflammatory properties

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Camu-camu;
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Summary
Background: Oxidative stress as well as inflammation plays a pivotal role in the pathogenesis of atherosclerosis. Although, various anti-oxidative dietary supplements have been evaluated for their ability to prevent atherosclerosis, no effective ones have been determined at present. ''Camu-camu'' (*Myrciaria dubia*) is an Amazonian fruit that offers high vitamin C content. However, its anti-oxidative property has not been evaluated in vivo in humans.
Methods: To assess the anti-oxidative and anti-inflammatory properties of camu-camu in humans, 20 male smoking volunteers, considered to have an accelerated oxidative stress state, were recruited and randomly assigned to take daily 70 ml of 100% camu-camu juice, corresponding to 1050 mg of vitamin C (camu-camu group; $n = 10$) or 1050 mg of vitamin C tablets (vitamin C group; $n = 10$) for 7 days.
Results: After 7 days, oxidative stress markers such as the levels of urinary 8-hydroxy-deoxyguanosine ($P < 0.05$) and total reactive oxygen species ($P < 0.01$) and inflammatory markers such as serum levels of high sensitivity C reactive protein ($P < 0.05$), interleukin (IL)-6 ($P < 0.05$), and IL-8 ($P < 0.01$) decreased significantly in the camu-camu group, while there was no change in the vitamin C group.
Conclusions: Our results suggest that camu-camu juice may have powerful anti-oxidative and anti-inflammatory properties, compared to vitamin C tablets containing equivalent vitamin C content. These effects may be due to the existence of unknown anti-oxidant substances besides vitamin C or unknown substances modulating in vivo vitamin C kinetics in camu-camu.
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Introduction

Accumulating evidence has demonstrated that oxidative stress (i.e., dysregulation of the cellular redox state) and inflammation play a pivotal role in the pathogenesis of atherosclerosis interacting with each other. Although, various anti-oxidative dietary supplements have been evaluated for their ability to prevent atherosclerosis, no effective ones have been determined at present. Vitamin C is not only the most important dietary anti-oxidant [1,2], but is also suggested to be a potent anti-inflammatory agent [3—5]. Several studies have found that dietary vitamin C or plasma vitamin C was associated with a protective effect against coronary artery disease [6—9].

''Camu-camu'' is an Amazonian fruit that offers high vitamin C content ranging from 9 to 50 g/kg, which is twofold that of acelora [10]. Additionally, in vitro anti-oxidant activity of 100% camu-camu juice evaluated by DPPH method reaches 50-fold of that of 100% acelora juice (unpublished data), suggesting its potentiality as an effective dietary supplement to prevent atherosclerotic disease. However, the anti-oxidative property has not been evaluated in an in vivo human study. Furthermore, the effects of camu-camu on inflammation have not been elucidated.

This study was designed to establish the anti-oxidative and anti-inflammatory properties of camu-camu in vivo in humans.

Methods

Study design

Study subjects included 20 healthy male volunteers, all of whom were habitual smokers, being considered to have an accelerated oxidative stress state. All of the participants had neither history of atherosclerotic diseases such as coronary artery disease or cerebrovascular disease nor risk factors such as hypertension, diabetes, or hyperlipidemia except smoking habit by the annual physical checkup. The participants were randomly assigned to take daily 1050 mg of vitamin C tablets (vitamin C group; $n = 10$) or 70 ml of 100% camu-camu juice, containing 1050 mg of vitamin C (camu-camu group; $n = 10$) as a dietary supplement for 7 days, and to continued smoking. The dose of 1050 mg/day of vitamin C in both groups was decided, based on previous studies [11—16]. In all subjects, 10 ml of urine and 15 ml of peripheral blood were sampled at baseline before intake of these dietary supplements, 7 days after the intake, and 1 month after ceasing the intake as at a washout stage. The blood samples were immediately centrifuged at $1500 \times g$ for 15 min at room temperature. The urine and serum were frozen and stored at $-80\,^{\circ}$C until analyzed. We measured urine 8-hydroxy-deoxyguanosine (8-OHdG) levels and serum total reactive oxygen species (ROS) levels as oxidative stress markers, and high sensitivity C-reactive protein (hsCRP) and multiple-cytokine levels as inflammatory markers. The local institutional review board approved the study protocol, and written informed consent was obtained from each participant.

Baseline characteristics

Prior to starting the intake of camu-camu or vitamin C, each participant was interviewed with regard to brand of the cigarette and daily number of cigarettes consumed and daily intake of tar and nicotine was calculated as the content of tar or nicotine for each cigarette brand $\times$ daily number of cigarettes consumed. Blood pressure was measured to the nearest 2 mmHg in the same arm at baseline, using a mercury sphygmomanometer with an appropriately sized cuff.

Measurements

Urinary 8-OHdG levels were determined by competitive enzyme-linked immunosorbent assay (ELISA) using the commercially available kit (8-OHdG Check, Japan Institute for the Council of Aging, Fukuroi, Japan) [17]. Serum levels of total ROS were measured using a DEPPD reaction method by Hayashi et al. [18] applying the Fenton reaction on a multi-well plate. The hsCRP levels were measured by particle-enhanced technology on the Behring BN II nephrometer (Dade Behring Inc., Newark, DE, USA) [19]. This assay used monoclonal anti-CRP antibodies and a calibrator that was also traceable to World Health Organization reference materials. The Luminex micro-beads array system was used for a multiplex assay of simultaneous quantification of the following 10 cytokines: interleukin (IL)-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, tumor necrosis factor-α, granulocyte-macrophage colony stimulating factor, and γ-interferon. The assay was conducted as per the manufacturer's instructions (Luminex Corp., Austin, TX, USA), using a commercially available kit (BioSource International, Inc., Camarillo, CA, USA) [20].

Statistical analysis

Values are expressed as the mean $\pm$ S.D. for parametric data, and median values and interquar-

Table 1 Baseline characteristics

	Vitamin C ($n = 10$)	Camu-camu ($n = 10$)
Age (year)	36 ± 4	37 ± 8
Height (cm)	171 ± 4	170 ± 5
Weight (kg)	72 ± 10	69 ± 15
Body mass index (kg/m^2)	25 ± 3	24 ± 5
Number of cigarette (/day)	24 ± 8	21 ± 9
Tar intake (mg/day)	117 ± 93	143 ± 110
Nicotine intake (mg/day)	10 ± 8	13 ± 10
Systolic blood pressure (mmHg)	124 ± 8	123 ± 9
Diastolic blood pressure (mmHg)	79 ± 11	77 ± 5

tile ranges for non-parametric data. Intergroup comparisons were performed using unpaired *t*-tests for parametric data and Mann—Whitney's U tests for non-parametric data. Intragroup comparisons for non-parametric data were assessed using Wilcoxon Rank Sum tests. Correlations between two variables were assessed using Spearman rank correlation coefficient. $P < 0.05$ was considered to be significant.

Results

Baseline characteristics such as the number of cigarettes consumed, daily intake of tar and nicotine, and systolic and diastolic blood pressure were similar in the two groups (Table 1). Overall, at baseline before taking the dietary supplements, urinary 8-OHdG levels ($R = 0.47$, $P < 0.05$), but not serum levels of total ROS ($R = 0.15$, NS) were correlated with the number of cigarettes (Fig. 1). Among 10 cytokines measured, the levels of 8 cytokines (except IL-6 and IL-8) were under the detection limit in almost all subjects, and therefore, were excluded from the analysis. The baseline levels of hsCRP ($R = 0.32$, NS), IL-6 ($R = 0.07$, NS), and IL-8 ($R = 0.12$, NS) were not correlated with the number of daily cigarettes consumed.

In the camu-camu group, 8-OHdG levels (9.0 [4.0, 21.2] to 7.0 [4.6, 14.6] ng/mg ·Cr, $P < 0.05$) and total ROS levels (128 [116,192] to 123 [110,162] Unit, $P < 0.01$) significantly decreased 7 days after taking the dietary supplements, and were restored at the washout stage (to 11.2 [4.9, 20.9] ng/mg Cr in 8-OHdG, $P < 0.05$ and to 131 [121, 188] Unit in total ROS, $P < 0.01$). In the vitamin C group, however, both levels did not change (8.5 [4.1, 22.3], 8.7 [3.0, 20.2], 10.5 [7.5, 14.3] ng/mg Cr in 8-OHdG and 135 [88, 162], 134 [106, 156], 136 [113, 167] Unit in total ROS) (Fig. 2).

In the camu-camu group, the levels of hsCRP (0.05 [0.01, 0.62] to 0.02 [0.01, 0.32] mg/dl, $P < 0.05$), IL-6 (6.0 [4.9, 6.9] to 5.1 [4.2, 6.4] pg/ml, $P < 0.05$), and IL-8 (24.8 [22.8, 30.6] to 22.4 [21.6, 24.5] pg/ml, $P < 0.01$) also significantly decreased 7 days after taking the dietary supplements. The levels were restored at the washout stage for hsCRP (to 0.05 [0.01, 0.58] mg/dl, $P < 0.05$) and IL-8 (to 25.4 [23.8, 27.9] pg/ml, $P < 0.01$), but did not for IL-6 (to 5.3 [4.9, 10.8] pg/ml). In the vitamin C group, however, each level did not change (0.03 [0.01, 0.22], 0.06 [0.01, 0.68], 0.05 [0.01, 0.13] mg/dl for hsCRP, 5.3 [4.8, 7.3], 5.1 [4.4, 7.6], 5.5 [4.5, 7.8] pg/ml for IL-6 and 27.3 [24.9, 32.8], 25.1 [23.0, 31.6], 26.4 [24.8, 33.4] pg/ml for IL-8) (Fig. 3).

Discussion

The major finding of this study is that both oxidative stress markers, urinary 8-OHdG and serum total ROS levels, and inflammatory markers, hsCRP, IL-6 and IL-8, decreased 7 days after taking daily 70 ml of the

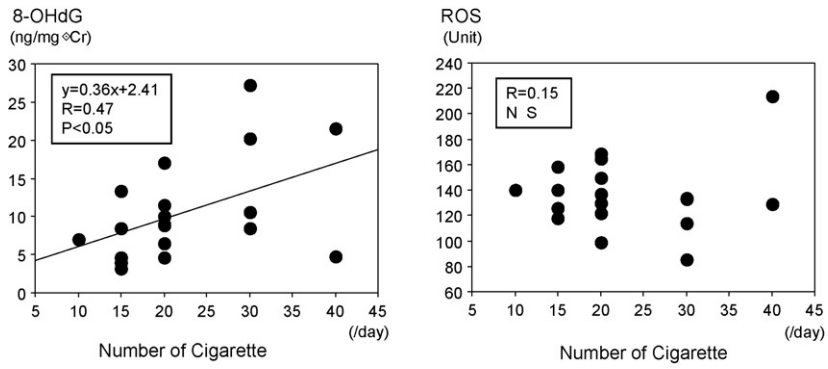

Figure 1 Relationship between baseline levels of oxidative stress markers and the number of cigarettes consumed. Urinary 8-OHdG levels were correlated with the number of cigarettes (left), but total ROS levels were not (right).

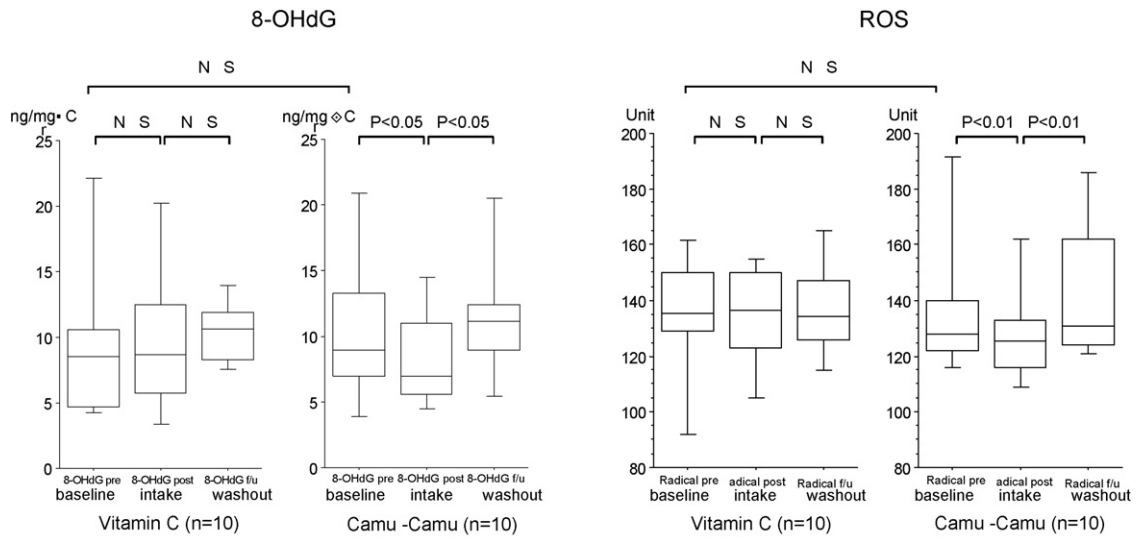

Figure 2 Serial changes in the levels of oxidative stress markers. In the camu-camu group, both levels of urinary 8-OHdG and serum total ROS significantly decreased 7 days after taking the supplement, and were restored at the washout stage of 1 month after cease of intake. In the vitamin C group, however, both levels did not change. Solid line represents median value, box represents interquartile range, and error bar represents 95% confidence interval.

100% camu-camu juice, including 1050 mg of the vitamin C. The levels of these markers except IL-6 were restored at the washout stage of 1 month after ceasing camu-camu intake. However, these changes were not seen after intake of daily 1050 mg of vitamin C tablets in similar subjects. These results suggest that camu-camu juice has more powerful anti-oxidative and anti-inflammatory activities, compared to vitamin C tablets, although the contents of vitamin C are equivalent. In this study, we enrolled male volunteers who were smokers but with neither atherosclerotic disease nor other risk factors so that we aimed to evaluate the anti-oxidant and anti-inflammatory effects of camu-camu using subjects with accelerated oxidative stress independently of other risk factors. Our result of correlation between baseline urinary 8-OHdG levels and the number of daily cigarettes consumed may suggest the validity of subject selection.

Atherosclerosis is characterized by the response of the vessel wall to chronic multifactorial injury leading to the formation of atheromatous or fibrous plaques. Following vascular endothelial dysfunction representative of an initial stage, smooth muscle dysfunction, metabolic abnormalities such as breakdown of neurohormonal balance occur in var-

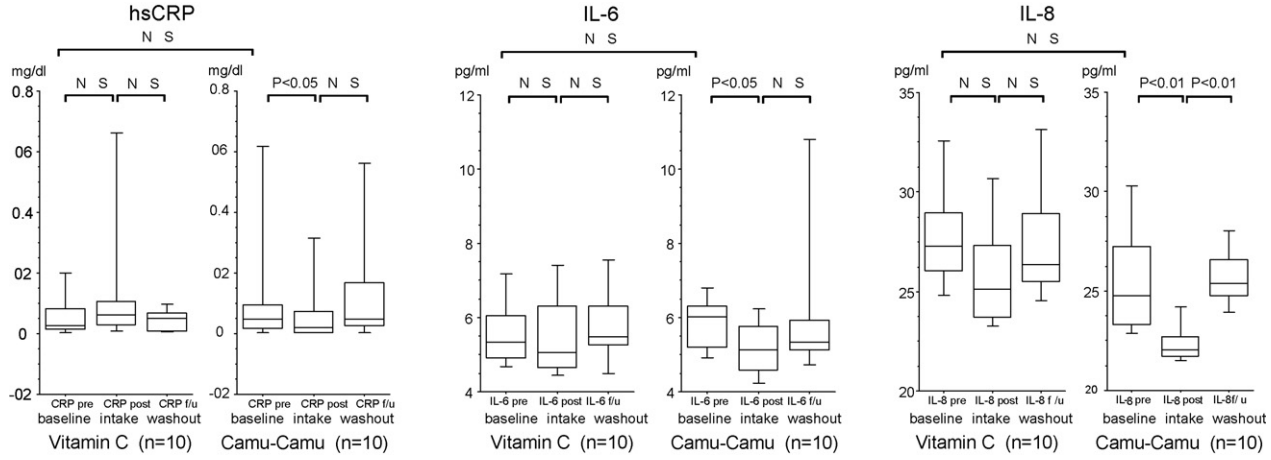

Figure 3 Serial changes in the levels of inflammatory markers. In the camu-camu group, the levels of hsCRP, IL-6, and IL-8 significantly decreased 7 days after taking the supplement, and were restored at the washout stage of 1 month after cease of intake in hsCRP and IL-8. In the vitamin C group, however, each level did not change. Solid line represents median value, box represents interquartile range and error bar represents 95% confidence interval.

ious stages of the atherosclerotic process. In this sequence of the atherosclerotiuc process, oxidative stress and inflammation play an essential role interacting with each other [21,22]. It is well accepted that vitamin C has anti-oxidant effects with respect to scavenging both ROS and reactive nitrogen species [1,2]. The Dietary Reference Intake Panel of the US Institute of Medicine recommends a vitamin C dietary allowance of 90 mg/day for men and 75 mg/day for women [23]. This recommendation was based on many lines of evidence, including the amount required to maintain near-maximum neutrophil concentrations with minimal urinary excretion of ascorbate. A higher intake of fruit and vegetables [24,25] as well as vitamin C supplements [26] has been demonstrated to prevent cardiovascular disease. In particular, higher intake of vitamin C is shown to be associated with a decreased risk of coronary artery disease in a population with a high prevalence of smoking [27]. In contrast, Lee et al. [28] reported that supplemental vitamin C increased cardiovascular disease risk in postmenopausal women with diabetes. They considered that this contradictory result might be caused by the fact that vitamin C can also act as a pro-oxidant under certain circumstances [2,29,30]. It may also possibly explain our result that neither urinary 8-OHdG levels nor serum total ROS levels decreased after intake of 1050 mg vitamin C tablets. On the other hand, the anti-inflammatory effects of vitamin C are controversial. Vitamin C has been shown to inhibit the LPS-induced number of monocytes producing IL-6 and TNF-α [4]. Another in vitro study using the human endothelial cell line ECV 304 and HUVECS demonstrated that vitamin C inhibited nuclear factor κB, a central mediator of altered gene expression during inflammation [5]. In humans, daily intake of 1500 mg vitamin C supplement in runners attenuated an increase in IL-1 receptor antagonist, IL-6, IL-10, and IL-8 following 90 km ultramarathon [11]. In a cross-sectional analysis of the third US National Health and Nutrition Examination Survey data, Ford et al. [31] reported that plasma CRP levels were inversely and significantly associated with concentration of vitamin C after adjustment for age, sex, race-ethnity, education, body mass index, leisure-time physical activity, and aspirin use. In addition, Wannamethee et al. [32] reported a significant inverse association of dietary and plasma vitamin C and fruit and vegetable intakes with biomarkers of inflammation in a cross-sectional study of 3258 men aged 60—69 years who had no history of cardiovascular disease or diabetes. However, a review of the prospective studies to date found that 4 of the 5 studies with doses ranging from daily 250 to 3000 mg vitamin C in persons with diabetes, hypercholesterolemia, hemodialysis, or coronary artery disease reported no anti-inflammatory effects [12—16], corresponding to our results that 1050 mg vitamin C tablets did not decrease the inflammatory markers.

Because of its particularly high vitamin C content, camu-camu is considered to have high nutritional value. Our study provides the first in vivo human data demonstrating the anti-oxidative and anti-inflammatory effects of camu-camu juice. Moreover, the effects of camu-camu were more powerful, compared with those of vitamin C tablets, despite the equivalent vitamin C contents. In this respect, there are two possible mechanisms. First, camu-camu possibly contains other anti-oxidative substances besides vitamin C. In addition to high contents of vitamin C, camu-camu contains carotenoids [33] and anthocyanines [34] as potential anti-oxidants. Otherwise, there may be other unknown contents potentially acting as anti-oxidative and/or anti-inflammatory substances in camu-camu. Second, camu-camu may have substances that increase in vivo availability of vitamin C such as by absorption or excretion. Camu-camu also contains high levels of potassium [35], which is considered to accelerate intestinal absorption of vitamin C [36]. To elucidate these mechanistic issues, the efforts such as search for anti-oxidative or anti-inflammatory substance, or comparison of in vivo vitamin C kinetics between camu-camu juice and vitamin C supplement would be required.

Although the mechanisms are not clear, camu-camu juice has more powerful anti-oxidative and anti-inflammatory properties, compared to vitamin C tablets containing equivalent vitamin C contents, and thus may be expected as a dietary supplement to prevent atherosclerosis.

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