Preliminary Human Study to Determine the Bioavailability of The Right C® and Another Vitamin C In a Blinded Cross-over Study

This study was conducted at Kilden Helse in Oslo, Norway under the direction of Dr. Roald Strand. The protocol was designed by Dr. Anthony J. Verlangieri, Professor of Pharmacology and Toxicology.

PURPOSE

To determine the rate of oral absorption of The Right C® and Another Vitamin C by analysis of Total Vitamin C (ascorbic acid, AA) delivered to plasma at 90 minutes post-ingestion.

STUDY DESIGN

Ten (10) healthy male subjects were randomized into two groups as described below. They were to refrain from taking any vitamin supplements for at least 7 days prior to the start of the study. They were to avoid intake of fruits and vegetables prior to and during the study period. The study required that all subjects fast prior to the administration of the test material.

The study groupings consisted of two groups. Prior to the cross-over, half of the subjects received 1000 mg of Another Vitamin C orally, and the other half received 1000 mg of The Right C®. After a 7 day wash out period, the cross-over treatments began, with the groupings being reversed.

Prior to administration of the test material, a blood sample was collected in a heparin tube (0 time, baseline). Blood samples were then taken at 30 min., 60 min., and 90 min. after administration of the test material.

Plasma AA was analyzed by HPLC as described below. All results are expressed as mg AA/L of plasma.

MATERIALS

Ascorbic acid, dithiothreitol (DTT), tetrasodium EDTA, chloroacetic acid, sodium hydroxide (NaOH), octyl sodium sulfate, and meta-phosphoric acid (MPA) were purchased from Sigma-Aldrich Chemical Co., Milwaukee, WI. High Pressure Liquid Chromatography (HPLC) instrumentation platform included an Alliance 2695 Separations Module (Waters Inc., Milford, MA) with an autosampler, a Waters 2996 Photodiode Array Detector utilizing Empower Pro Software. The column used was a 250 x 4.6 Ultrasphere column (Beckman Instruments, Inc., Fullerton CA) packed with 5-µm octadecylsilane particles. All solvents were degassed with nitrogen prior to use.

METHODS

Isocratic elution at a flow rate of 0.5 mL/min at ambient temperature was used, and the mobile phase consisted of a solution of 14.1 g chloroacetic acid, 4.65 g NaOH, 0.85 g of tetrasodium EDTA, and 200 mg of octyl sodium sulfate in 1L of distilled water. The photodiode array detector was set at a wavelength of 265 nm. Standard curve measurements produced a standard curve with R=0.9931. Ascorbic acid had a retention time of 6.1 min +/- 0.2 min.

Stock solutions of MPA (30 g/L) and DTT (1 g/L) were prepared and stored cold. Ascorbic acid standards were prepared and analyzed as follows. Ascorbic acid (2.0 mg) was weighed and added to a solution containing 0.7 mL of MPA solution (30 g/L) and 0.3 mL of DTT solution (1 g/L). This 2.0 mg/mL stock solution was serial diluted to generate final ascorbic acid standards ranging from 1.25 µg/mL to 20 µg/mL. Human blood samples were collected (3.0 mL), centrifuged, and the plasma (0.5 mL) was supplemented with 0.5 mL of MPA (30 g/L) solution for stability, and frozen at -78°C. After the plasma samples were received (in duplicate), each were treated with 0.2 mL of stock MPA solution, and 0.02 mL of stock DTT solution, vortexed, and centrifuged at 2000xG. Final concentrations of MPA and DTT were 17.2 g/L and 0.2 g/L, respectively. The supernatant (1.0 mL) was transferred to a microfuge tube, stored on ice, and further centrifuged at 13200xG for 1 minute to remove residual suspended particles. The clear supernatant plasma solution was immediately transferred to autosampling vials and analyzed for ascorbic acid. Injection volumes used for standards and plasma samples was 10 µL. A dilution factor of 2.24 was used for measuring final ascorbic acid concentrations based on the method described above. Plasma ascorbic acid concentrations were reported in mg/L.

Results

The data from the vitamin C study clearly shows that The Right C® has significantly better absorption compared to Another Vitamin C.

Figure 1 displays the total plasma AA concentration, correcting for baseline values, over time. A Two-Way Repeated Measures Analysis of Variance was performed. The results from the vitamin C study show that both the main effects of "Time" and "Type of C" were significant (p=0.0001 and p=0.0002, respectively). There was not a significant interaction effect.

Table 1 depicts the mean value for Another Vitamin C and The Right C® at each time point.
Average increase, above baseline, in total plasma AA (mg/L) at each time interval after ingestion of the test material

Time (Min)	Another Vitamin C	The Right C®
30	1.322	4.295
60	4.170	8.331
90	6.222	11.61

Following the Two-Way Repeated Measures Analysis of Variance,
t-test post-hoc analysis was performed comparing Another Vitamin C and The Right C® at each time interval.

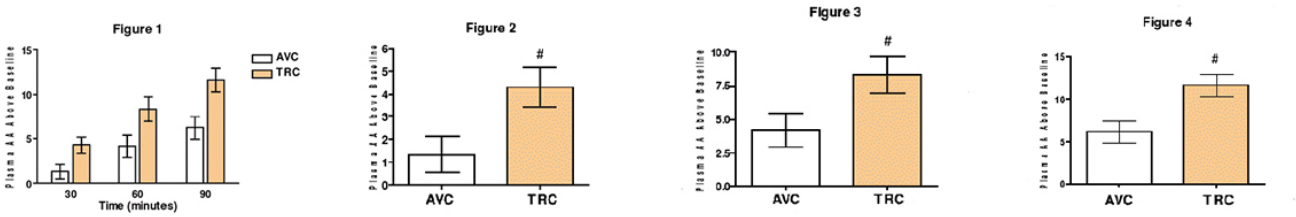

Figure 2 shows the comparison between Another Vitamin C and The Right C® for increase in total plasma AA (mg/L) above baseline at 30 minutes. T-test post-hoc analysis shows that The Right C® had a significantly higher increase in total plasma AA above baseline compared to Another Vitamin C (p=0.0188).

Figure 3 shows the comparison between Another Vitamin C and The Right C® for increase in total plasma AA (mg/L) above baseline at 60 minutes. T-test post-hoc analysis shows that The Right C® had a significantly higher increase in total plasma AA above baseline compared to Another Vitamin C (p=0.0278).

Figure 4 shows the comparison between Another Vitamin C and The Right C® for increase in total plasma AA (mg/L) above baseline at 90 minutes. T-test post-hoc analysis shows that The Right C® had a significantly higher increase in total plasma AA above baseline compared to Another Vitamin C (p=0.0100).

Absorption rate was calculated and is graphically depicted in Figure 5. It was assumed that absorption would be linear over time, and linear regression supports this assumption (R2=0.50575). The absorption rate of The Right C®was 196% higher than the absorption rate of Another Vitamin C. Put another way, the absorption rate of Another Vitamin C was only 50.7% that of The Right C®. Assuming that the absorption rate of either source of ascorbic acid would continue to be linear over time, and that total absorption would be complete after 4 hours, the total amount of ascorbic acid delivered to the plasma from a 1000 mg dose of The Right C® would be 31.883 mg/L, compared to a 16.187 mg/L increase after a 1000 mg dose of Another Vitamin C. Calculating the area under the curve (AUC) of The Right C® and Another Vitamin C shows that the AUC of The Right C® is 205% higher than that of Another Vitamin C (488.6 units versus 238.3 units, respectively).

DISCUSSION

The data indicates that The Right C® formulation is absorbed more rapidly than Another Vitamin C by 196%. Alternatively, Another Vitamin C is absorbed only 50.7% as quickly as The Right C®. The Right C® raises plasma AA levels more rapidly and higher than Another Vitamin C. Higher plasma levels promote more rapid increases in intracellular AA levels. Higher AA levels enable the cell to utilize AA at a higher rate in cell metabolism and provide superior anti-oxidant action in the plasma as well as in the cell. Thus, The Right C® provides superior anti-oxidant protection compared to Another Vitamin C.