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A Convenient Method for Measuring Blood Ascorbate Concentrations in Patients Receiving High-Dose Intravenous Ascorbate

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Abstract

Objective—A simple method of using fingerstick blood glucose monitors (FSBG) to estimate blood ascorbate values after high-dose intravenous (IV) ascorbate infusion is evaluated as a substitution for HPLC measurement.

Methods—In 33 participants, readings from FSBG were taken before and after IV ascorbate infusions at various time points, with the post-infusion FSBG readings subtracted by the baseline glucose readings. The results of the subtractions (AA_{FSBG}) were correlated with ascorbate concentrations detected by HPLC (AA_{HPLC}).

Results—A linear regression was found between ascorbate concentrations detected by the fingerstick method (AA_{FSBG}) and by HPLC (AA_{HPLC}). The linear correlations were identical in healthy subjects, diabetic subjects and cancer patients. ANOVA analysis obtained an AA_{FSBG}/AA_{HPLC} ratio of 0.90, with 90% confidence interval of (0.69, 1.20). The corrections of AA_{FSBG} improved similarity to AA_{HPLC}, but did not significantly differ from the un-corrected values.

Conclusion—The FSBG method can be used as an approximate estimation of high blood ascorbate concentration after IV ascorbate (>50 mg/dL, or 2.8 mM) without correction. However this measurement is not accurate in detecting lower or baseline blood ascorbate. It is also

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important to highlight that in regard to glucose monitoring, FSBG readings will be erroneously elevated following intravenous ascorbate use and insulin should not be administered to patients based on these readings.

Keywords

Intravenous vitamin C; Ascorbate (Vitamin C ascorbate acid); blood glucose monitor; HPLC; complementary; alternative; integrative medicine

INTRODUCTION

For many years, high-dose intravenous (IV) ascorbate (Vitamin C or ascorbic acid) has been used by complementary, alternate and integrative medicine (CAIM) practitioners to treat various conditions including infections, cancer, autoimmune diseases and illnesses of uncertain origin [1–6]. Clinical use of IV ascorbate is growing as recent studies revealed the scientific basis for this use [4, 7–10]. Although the actual effective clinical dose of IV ascorbate has not yet been established by rigorous clinical studies, a target plasma concentration of 350 ~ 400 mg/dL (20~23 mM) is recommended based on clinical experience for effectiveness and safety considerations.

As the clinical use grows, monitoring of plasma ascorbate concentrations in patients receiving IV ascorbate remains of prime importance. High performance liquid-chromatography (HPLC) with coulometric electrochemical detection or UV detection has been the gold standard for detection of ascorbate in biological samples [11,12]. The HPLC method is highly sensitive and accurate, with great advantages in detecting low physiological ascorbate concentrations. However, because of the instability of ascorbate in biological fluids [13–15], samples must be immediately and properly processed to prevent ascorbate degradation [11,12]. Accurate measurement by HPLC requires strict sample processing/extraction methods, low-temperature (–80°C) storage and low-temperature sample handling (4 ~ –20°C) [11,12]. In addition, the sophisticated and expensive HPLC instruments are not often accessible on a daily basis on site where IV ascorbate infusions are performed. Blood samples need to be drawn and immediately prepared, frozen and shipped to laboratories equipped with HPLC instruments for further analysis. This process is complicated and costly and might ultimately lead to ascorbate degradation and faulty laboratory readings if samples are mishandled at any step along the chain.

In this study, we investigated a fast and easy method to assess blood ascorbate concentrations in patients receiving IV ascorbate. Commercially available fingerstick blood glucose (FSBG) monitoring is a fast way of testing blood glucose as commonly applied in diabetic patient care. In the clinical setting, we observed that glucose readings with glucometer FSBG testing resulted in very high readings following IV ascorbate infusions. By concurrent laboratory verification of serum glucose levels, these high FSBG readings have been found to be erroneously elevated. This suggests that ascorbate interferes with accurate glucose measurement, most likely because of the structural similarity of ascorbate and glucose, and interference of ascorbate with catalytic oxidation-based reactions [16, 17]. We became intrigued with the idea that this interference could be used as a quick estimation for the blood ascorbate level after IV ascorbate infusions as an alternative to HPLC. We hypothesize that the difference between FSBG readings before and after IV ascorbate infusion could be used as a rough estimate for blood ascorbate concentration in clinical IV ascorbate use.

MATERIALS AND METHODS

Subjects, blood sampling, and blood glucose readings taken from FSBG

A total of 33 subjects were included in this analysis. All study participants were consented prior to the study. Healthy and diabetic subjects were originally recruited for a study evaluating biomarkers of oxidative stress and IV ascorbate pharmacokinetics (KUMC IRB protocol HSC# 11119). For 19 of these subjects (9 healthy, 10 diabetic), blood samples for ascorbate and glucose were taken before IV ascorbate infusion, at the completion of IV ascorbate infusion, and then at 2 hrs, 2.5 hrs, 3 hrs, 3.5 hrs and 4 hrs post-infusion, in addition to the FSBG readings. The 14 oncology participants were receiving high-dose IV ascorbate treatment as part of outcome studies (KUMC IRB HSC#7823 and HSC#10006). For 13 of the oncology participants, blood samples for ascorbate were taken only at the completion of the IV infusion, in addition to the FSBG readings. For the remaining 1 oncology participant, blood samples for ascorbate were taken both before and at the completion of IV ascorbate infusions, in addition to the FSBG. The blood samples were put on ice and immediately transferred to the research laboratory and centrifuged at 4°C at 1500 × g for 10 min. Plasma was taken and stored at -80°C until analysis by HPLC for detection of ascorbate. Fingerstick glucose monitors (Accu-Chek by Roche, and Onetouch Ultra 2 by Lifescan) were used at each time point when blood was drawn, and readings were recorded.

HPLC analysis of blood ascorbate

The sample processing and HPLC analysis were performed according to a method established by Levine et al [11,12]. Plasma was diluted 5~1000 times in 90% methanol with 1 mM EDTA, vortex, and then centrifuged at 4°C at 20,000 × g for 15 min. Supernatant was analyzed by Waters e2695 HPLC (Waters, Milford, MA) with electrochemical detection (ESA, Chelmsford, MA). The column used was an ODS-DABS UI TraspHERE, 5μ, 4.6 mm × 25 mm (Beckman Coulter, Fullerton, CA). Mobile phase was run at a flow rate of 1 mL/min, containing 30% methanol, 0.05 M sodium phosphate monobasic, 0.05 M sodium acetate, 189 μM dodecyltrimethyl-ammonium chloride, and 36.6 μM tetraoctylammonium bromide. The Empower II software (Waters, MA) was used for instrument control and data analysis.

Data analysis and Statistics

To estimate the blood ascorbate concentrations from the FSBG readings, the FSBG readings of each sample point were subtracted by the baseline glucose readings of the relative subject: $AA_{FSBG} = FSBG_X - FSBG_0$ (AA_{FSBG} is the estimated blood ascorbate concentration, $FSBG_X$ is the FSBG reading at a given time point, $FSBG_0$ is the FSBG reading before IV infusion of ascorbate).

A total of 114 paired data-points from 29 subjects (7 healthy, 8 diabetic and 14 cancer) were used for correlation analysis and ANOVA analysis. To assess if the new method FSBG is comparable to the standard HPLC method, a linear mixed effects models was performed on log-transformed concentrations with sample, method, and the interaction of sample and method as fixed effects, and measurements within each subject as repeated measures. Point estimates and 90% confidence interval (CI) for differences on the log scale was exponentiated to obtain estimates for ratios of geometric means on the original scale. HPLC method was used as the reference in the comparisons.

After a function was established from the correlation analysis, additional 22 pairs of data-points from 4 additional subjects (2 healthy, 2 diabetic) were used to test accuracy and reliability of the function.

RESULTS

Correlation and difference between the results obtained from FSBG measurement and HPLC detection

Ascorbate concentrations from the FSBG measurement were calculated using: $AA_{FSBG} = FSBG_X - FSBG_0$ (AA_{FSBG} is the estimated blood ascorbate concentration from FSBG, $FSBG_X$ is the FSBG reading at a given time point, $FSBG_0$ is the FSBG reading before IV infusion of ascorbate). Total of 114 pairs of data from 29 subjects (7 healthy, 8 diabetic and 14 cancer) were obtained by both FSBG method (AA_{FSBG}) and HPLC method (AA_{HPLC}). With 14 subjects, we were able to obtain both AA_{FSBG} and AA_{HPLC} values at all the indicated time points from 0 hr (beginning of ascorbate IV infusion) to 6 hrs after ascorbate IV infusion (Fig 1A). A similarity of the time-course curves is found between values from HPLC method and those from the FSBG method (Fig 1A).

A linear relationship was found between AA_{FSBG} and AA_{HPLC} for all the subjects at all time points (Fig 1B, $r^2=0.79$). The linear correlation held true in healthy subjects ($n=7$, $r^2=0.76$), diabetic subjects ($n=8$, $r^2=0.73$), and cancer patients ($n=14$, $r^2=0.71$) (Fig 1C). The linearity was identical between the three groups as tested by ANOVA analysis ($P>0.05$). All the data points were then log-transformed, and linear relationship still held true (Fig 1D, $r^2=0.88$). However, when $\ln(AA_{HPLC})$ was 2, equivalent to blood ascorbate concentration ~ 7.4 mg/dL or ~ 420 μ M, the FSBG method could not detect the ascorbate concentration (Fig 1D).

ANOVA analysis using a linear mixed effects model was performed on log-transformed data. The model assumptions were evaluated graphically, that the residuals were normally-distributed and had constant variance. Both assumptions were met. There was no interaction between sample and methods ($P = 0.54$). Method effect was not significant ($P=0.31$), whereas sample effect was highly significant ($P < 0.0001$). The difference between FSBG method and HPLC method on the log scale is -0.09 with 90% confidence interval (-0.38 , 0.18). Back-transformed to the original scale, the AA_{FSBG}/AA_{HPLC} estimated ratio was 0.90 with 90% confidence interval (0.69 , 1.20). Taken as a whole, the two different methods had no significant difference in detecting ascorbate concentration.

Correction of the AA_{FSBG} values

We hypothesized the difference between AA_{FSBG} and AA_{HPLC} could be corrected with the function obtained from the linear regression in Fig 1B: $y=0.9535x + 29.778$. The corrected AA_{FSBG} -corrected values were compared to AA_{HPLC} values. Because ANOVA analysis showed an AA_{FSBG}/AA_{HPLC} ratio of 0.90 , we also compared the $AA_{FSBG}/0.90$ values with the AA_{HPLC} values. The AA_{FSBG} -corrected values better agreed with the AA_{HPLC} values ($P=0.999$) than either AA_{FSBG} or $AA_{FSBG}/0.9$ ($P=0.11$). When compared to AA_{HPLC} , paired t-test showed that all the corrected values and the un-corrected AA_{FSBG} has no significant difference. The P value between AA_{FSBG} and AA_{HPLC} was 0.068 , between AA_{FSBG} -corrected and AA_{HPLC} was 0.999 , and between $AA_{FSBG}/0.90$ and AA_{HPLC} it was 0.112 . This indicates the corrected data resembles AA_{HPLC} more closely. When the 2 correction methods were compared to each other, they were identical as the P value was 0.548 . When the corrected values were compared to the un-corrected values, not significant difference was found either ($P= 0.548$ and 0.182). This no-difference before and after correction was also evident as shown in Fig 1A. This indicates that corrections were not necessary for the FSBG measurement for blood ascorbate concentration.

Validation of the estimation method of FSBG

To further validate this estimation method and the correction equation gained from the linear correlation, 18 pairs of data-points from 4 more subjects (2 normal and 2 diabetic) were

used. The same data were plotted as time-dependent plasma ascorbate values with FSBG method, HPLC method, and the corrections of FSBG values (Fig 1E). No significant difference between AA_{FSBG} and AA_{HPLC} ($P=0.14$) indicated again that the FSBG method can be a useful estimation. The corrections (both $AA_{\text{FSBG-corrected}}$ and $AA_{\text{FSBG}/0.9}$) improved the similarity to AA_{HPLC} by increased P values ($P=0.87$ and 0.45 respectively), but still showed no significant difference when compare to AA_{FSBG} ($P=0.60$ and 0.37 respectively).

Consistent with results described above, the corrections did not improve the compliance with HPLC data, thus were not necessary in assessing ascorbate levels. When plasma ascorbate level was above 50 mg/dL, the error of FSBG methods was 13% ($\pm 8\%$) using HPLC results as standards. When plasma ascorbate levels were below 1 mg/dL (baseline), the error was large ($>100\%$) that the FSBG methods cannot detect the ascorbate concentration correctly. Moreover, there were 3 data pairs that had errors $>100\%$ in the range of 40 – 80 mg/dL, resulting in blood ascorbate determination discrepancy between the two methods of approximately 100 mg/dL. These data points were from one subject and may have been confounded because the subject ate just before these 3 samples were taken. Therefore, the blood glucose levels might be different from the baseline glucose level that was used to calculate the ascorbate levels.

Taken together, the AA_{FSBG} values can provide a rough estimation for blood ascorbate concentrations after IV ascorbate infusion. Corrections examined here did not improve the compliance with HPLC results, and thus were indicated not necessary. The FSBG method slightly underestimates blood ascorbate concentration compared to HPLC method ($AA_{\text{FSBG}}/AA_{\text{HPLC}}$ ratio is 0.90 from this study). The FSBG method works better at a high blood ascorbate concentration (> 50 mg/dL, or $2,800 \mu\text{M}$) after IV ascorbate infusion. When blood ascorbate concentration is below ~ 7.4 mg/dL (or $420 \mu\text{M}$), the FSBG method cannot detect the concentration correctly.

DISCUSSION

Recent studies have revealed the scientific basis and the scope for the use of vitamin C beyond its use as a nutritional supplement. IV ascorbate but not oral vitamin C produced plasma concentrations sufficiently high ($300 - 20,000 \mu\text{M}$) to encounter pro-oxidant effect in the extracellular milieu, thus is able to deliver treatment for diseases in which peroxide may have a therapeutic role, such as cancer or infections [7–9]. High-dose IV ascorbate is widely adopted in the CAIM community [1–6]. About 10,000 patients per year in US are using IV ascorbate, with an average dose of 28 grams/injection and 22 total doses per patients [4]. However the dosage used varies largely from 1 to 200 grams per treatment. A standard blood concentration level needs to be established for therapeutic purposes under different clinical conditions. Unfortunately, the recommended therapeutically effective blood levels of ascorbate are currently only dependent on limited human and animal pharmacokinetics data [8–10,18], and largely depend on CAIM practitioners' experience [4]. Clinical studies are needed to establish standards for administration of high-dose IV ascorbate.

Despite the lack of a standardized therapeutic level, the blood ascorbate monitoring in high-dose IV ascorbate therapy is vital in the planning and management of ascorbate dosing by healthcare providers. HPLC separation with electrochemical detection is the gold standard for determining blood ascorbate concentration, which is reliably accurate and reproducible. However, the HPLC detection for ascorbate demands instruments and specifically-trained personnel which are less likely to be available for private practices without access to a general clinical laboratory. Given that ascorbate is unstable and is rapidly oxidized in

aqueous systems [13–15, 19], it is recommended that blood samples containing ascorbate should be processed under 4°C immediately after blood draw, and plasma thus obtained should be immediately processed to extract ascorbate at 4°C [11,12]. If HPLC analysis of the plasma is not immediately available, plasma storage should be at –80°C [11,12]. Hemolysis, which could lead to ascorbate degradation during blood draw and/or sample processing, should also be carefully avoided [12]. Currently, many clinical samples are shipped to specific labs equipped with HPLC instruments for the ascorbate analysis. The turnaround time and handling conditions are not always ideal, which can lead to inaccurate readings.

Our study provides an easy, on-site measurement for blood ascorbate levels after high-dose IV ascorbate administration. Figure 2 represents a diagram for the different and simplified procedures of the new FSBG method, in comparison with the traditional HPLC method. As compared with the HPLC detection, FSBG test is simple and fast and blood draw and handling are avoided. Sample preparation and storage are not required, and the results are available in minutes. The results from the FSBG test hold a liner relationship to the results from HPLC detection. In our study, participants were divided into 3 groups – normal subjects, diabetic, and cancer patients. The FSBG worked in all three groups without notable difference (Fig 1D). There was only an in-significant difference in slopes of the liner regression. Studies have reported that in diabetic patients, the baseline blood ascorbate levels can be lower than healthy subjects [20]. However this difference cannot be detected in this study as the normal baseline levels of ascorbate is ~50µM and FSBG method is not sensitive and not meant for detection of ascorbate concentrations lower than 7.4 mg/dL (or 420µM) (Fig 1C). In fact, the in-sensitivity of FSBG to physiological ascorbate concentrations is desirable for the reliability of FSBG in measuring blood glucose. In contrast, when high-dose IV ascorbate is used, blood concentrations could easily elevate to > 50 mg/dL [4, 10]. Our study showed in these settings, the FSBG method could provide a quick and useful reference for practitioners to decide IV ascorbate dosing for a particular patient.

However in spite of its convenience, there are drawbacks for the use of FSBG test for ascorbate levels. It is not as accurate as HPLC method and it cannot detect low concentrations of ascorbate. Our data showed that the FSBG method can estimate ascorbate concentrations > 50 mg/dl (or 2,800 µM), but cannot correctly measure concentrations at baseline (< 1 mg/dL or 57 µM), or when blood ascorbate levels are only slightly elevated (e.g. < 7.4 mg/dL or 420µM). The minimum level that can be detected by the FSBG method cannot be concluded from these data, as concentrations between 7.4 mg/dl and 50 mg/dl were not tested. This was due to the original design of the human studies was limited to pre-decided high dosages of IV ascorbate and not using a moderate dose. Also, the FSBG method is an approximate estimation rather than a precise measurement. The benefit is for its accessibility, simplicity and speed.

In the use of the FSBG method for detection of high ascorbate concentrations, a correct glucose value for subtraction is very important. If blood glucose level might significantly change after the baseline glucose reading (e.g. due to food ingestion by the patient during the IV ascorbate infusion), then ascorbate measurement by FSBG could be confounded. A correct glucose level at the time of measurement should be detected by a conventional laboratory and be used rather than the baseline glucose level taken before.

Equally of great importance, practitioners should be aware that patients receiving IV ascorbate will have erroneous elevations of FSBG readings for several hours after infusions and should not be given insulin therapy based on these readings. This could result in a precipitous drop in blood glucose from insulin therapy based on an erroneous FSBG

reading. After IV ascorbate infusions, only blood levels sent to a conventional laboratory should be used to monitor blood glucose and practitioners should not rely on FSBG.

CONCLUSION

The FSBG method can be used as an approximate estimation of high blood ascorbate concentration after IV ascorbate (>50 mg/dL, or 2.8 mM). Corrections are not necessary. However this measurement is not accurate in detecting lower or baseline physiologic blood ascorbate. In regard to glucose monitoring, FSBG readings will be erroneously elevated following IV ascorbate use and insulin should not be administered to patients based on these readings.

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ABBREVIATIONS

CAIM	Complementary, Alternative and Integrative Medicine
FSBG	fingerstick blood glucose monitors
IV	intravenous
G6PD	glucose-6-phosphate dehydrogenises
HPLC	high performance liquid-chromatography
CI	confidence interval

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Fig. 1. Correlation and difference between FSBG and HPLC measurement for blood ascorbate concentrations

A. Time-dependent plasma ascorbate values after ascorbate IV infusion detected by fingerstick blood glucose monitor (FSBG) or by high-performance liquid chromatography (HPLC). The beginning of the IV infusion was defined as 0 hr. Each point represents data from 14 participants $\pm$ standard deviation (SD). The IV dose was 1g/kg body weight of the participant, and was complete approximately at 2 hrs. **B.** Linear correlation between blood ascorbate concentrations detected by FSBG (AA_{FSBG}) and by HPLC (AA_{HPLC}) in 29 subjects. **C.** Linear correlation between AA_{FSBG} and AA_{HPLC} in different groups of subjects. Dark circles, healthy subjects; open squares, diabetic subjects; and snow flaks, cancer patients. **D.** Log-transformed linear correlation between Ln (AA_{FSBG}) and Ln (AA_{HPLC}) in 29 subjects. **E.** Time-dependent plasma ascorbate values of 4 additional participants after ascorbate IV infusion for the validation of the FSBG method. FSBG_{corrected} represents the correction of AA_{FSBG} from previous regression analysis (function gained from Fig 1B: $AA_{FSBGcorrected} = 0.9535 AA_{FSBG} + 29.778$). FSBG/0.9 represents the correction of AA_{FSBG} using the AA_{FSBG}/AA_{HPLC} ratio of 0.9.

HPLC method

FSBG method

Fig. 2. Comparison of the procedures between HPLC method and FSBG method for detection of blood ascorbate concentration after intravenous ascorbate infusion

The HPLC method requires blood-draw, multi-step low temperature sample handling and HPLC equipment and software for data analysis. To avoid ascorbate degradation, hemolysis during blood-draw and sample handling should also be carefully avoided. The FSBG method takes pre- and post-infusion readings. Ascorbate concentration can be resulted simply by subtraction of pre-infusion reading from post-infusion reading.