Liposomal-encapsulated Ascorbic Acid: Influence on Vitamin C Bioavailability and Capacity to Protect Against Ischemia–Reperfusion Injury


Abstract

Intravenous administration of vitamin C has been shown to decrease oxidative stress and, in some instances, improve physiological function in adult humans. Oral vitamin C administration is typically less effective than intravenous, due in part to inferior vitamin C bioavailability. The purpose of this study was to determine the efficacy of oral delivery of vitamin C encapsulated in liposomes. On 4 separate randomly ordered occasions, 11 men and women were administered an oral placebo, or 4 g of vitamin C via oral, oral liposomal, or intravenous delivery. The data indicate that oral delivery of 4 g of vitamin C encapsulated in liposomes (1) produces circulating concentrations of vitamin C that are greater than unencapsulated oral but less than intravenous administration and (2) provides protection from ischemia–reperfusion-mediated oxidative stress that is similar to the protection provided by unencapsulated oral and intravenous administrations.

Keywords: liposome, oral, intravenous, oxidative stress, thiobarbituric acid reactive substances

Introduction

Infusion of vitamin C, via arteries or veins, has been reported to decrease oxidative stress and, in many instances, improve physiological function in adult humans. Reported beneficial effects include, but are not limited to, increased baroreflex sensitivity, improved endothelial function and vascular conductance, augmented inotropic and thermogenic response to beta-adrenergic stimulation, decreased systemic inflammation and reduced fluid requirements during recovery from thermal injury, fatigue resistance in clinical populations, and cancer cell-specific toxicity. Compared with vitamin C infusion, oral vitamin C administration is typically less effective, in part due to inferior vitamin C bioavailability.

Following ingestion, subsequent vitamin C bioavailability is largely determined by rates of intestinal absorption and further influenced by renal reabsorption and excretion. Sodium-dependent vitamin C transporters 1 and 2 are the primary mediators of intestinal absorption and renal reabsorption; Sodium-dependent vitamin C transporter 2 also facilitates entry of vitamin C into most metabolically active cells and tissues. Direct infusion of vitamin C bypasses the dependency on intestinal absorption.
吸収；したがって、高濃度の循環を速やかに達成する。Vitamin C の投与は、一般的な公衆にとって実用的な投与方法とし、また感染、不快感、静脈炎のリスクを伴います。したがって、有効な Vitamin C の送達の代替方法が注目になる。これについては、Vitamin C を含有するリポソームの口腔内摂取が有望である。

リポソームは、脂質双子層を有する直径数ミクロンの球状の空洞貯料物質である。薬物や栄養補助剤を充填すると、高効率の薬物/補助剤送達方法である。口腔内摂取して、薬物の薬動力学的特性が正常な吸収パターンを凌駕する。すなわち、Vitamin C を包含するリポソーム内でも薬物の吸収が加速される。18-19 一致する考えに従い、現在の調査の目的は、Vitamin C を口腔内摂取する Liposome 内と同等の用量を口腔内摂取する Liposome 内の循環濃度を比較することであった。そこで、リポソーム内に包含された Vc の薬物産物を有効性に影響する機能的な推定を追求するために、二酸化炭素による筋肉血流の障害-再灌流障害の程度を定量した。高い循環性の推定を考慮に入れると、リポソーム Vc は未包含 Vc と比較して、血流の障害-再灌流がより有意に緩和されることが期待される。

**Methods**

**Research participants**

我々は、11人の成人男性を研究対象者に選択し、研究対象者の体格特性は Table 1 に示されている。入力基準は、年齢が45–70歳の範囲で、または体重指数が25kg/m²以上の肥満度を有する者であるとした。脂質過酸化は、この年齢範囲での肥満の成人に高率である。18-19 排除基準は、3ヶ月前に薬物、栄養補助剤、または増強する薬物使用歴がある者、妊娠中の者、既往のアレルギー反応またはリポソームへの高反応性、既往の腎石、または2年前の喫煙歴、または他の研究に参加している者である。実験的プロトコルの概要は、ヘルシンキ宣言1975年に、1983年に改訂版がなされたものと一致し、コロラド州立大学の倫理委員会の承認を得た。各研究対象者の参加を説明し、合意に達するまでに書面による承認書が得られた。
Table 1

Selected physical characteristics of research participants ($n = 11$).

<table>
<thead>
<tr>
<th></th>
<th>MEAN ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>2/9</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53 ± 2</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.62 ± 0.02</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>89.0 ± 2.9</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>34.1 ± 1.0</td>
</tr>
<tr>
<td>Baseline plasma vitamin C (mg/dL)$^a$</td>
<td>0.84 ± 0.05</td>
</tr>
<tr>
<td>Baseline TBARS ($\mu$M MDA)$^a$</td>
<td>5.82 ± 0.81</td>
</tr>
</tbody>
</table>

Note:

$^a$After 12-hour fast, prior to placebo treatment administration.

**Abbreviations:** TBARS, thiobarbituric acid reactive substances; MDA, malondialdehyde.

Experimental design and overview

Following screening, research participants reported to the laboratory on four randomly ordered separate occasions, separated by a minimum of seven days. Research participants were administered an oral placebo or 4 g of vitamin C encapsulated within liposomes, unencapsulated, or via intravenous infusion. The rationale for 4 g dose of vitamin C was based on previous studies that have demonstrated favorable outcomes pertinent to oxidative stress and physiological function.$^{1,4,5,9,22}$ Three hours after administration, participants completed a forearm ischemia–reperfusion protocol. Venous blood was sampled at baseline and throughout the experimental visit for the determination of circulating concentrations of vitamin C and markers of oxidative stress.

Screening

The screening visit comprised completion of a medical history questionnaire, measurement of height (via a stadiometer) and body mass (via a physician’s scale), calculation of body mass index [body mass/height$^2$ (kg/m$^2$)], and familiarization with the forearm ischemia–reperfusion protocol.

Treatment visits

Participants reported to the laboratory on four randomly ordered separate occasions, separated by a minimum of seven days, each following a 12-hour fast and 24-hour abstention from exercise (Fig. 1). An intravenous catheter was placed in an antecubital vein for repeated blood sampling. The catheter was kept patent with a saline drip. Following baseline blood collection, participants were administered one of the following four treatments: (1) placebo, oral consumption of 16 mL of water; (2) oral consumption of unencapsulated vitamin C, 4.25 g of 94% sodium ascorbate (equivalent to 4 g of
vitamin C) dissolved in 16 mL of water; (3) oral consumption of vitamin C encapsulated in liposomes (equivalent to 4 g of vitamin C) suspended in 16 mL of ultrahigh purity water (see below for more details); and (4) intravenous administration of vitamin C (Bioniche Pharma), 4 g dissolved in 100 mL of saline and administered by automated infusion pump (Harvard Apparatus) over one hour (1.667 mL/min, equivalent to 67 mg/min).

Preparation of liposomes

The liposomes were provided by Empirical Labs. The formula consisted of 136 mg of mixed natural phospholipids and 284 mg of USP sodium ascorbate (NaA), in a total volume of 1 mL with ultrahigh purity water (conductivity <0.065 μS/cm). All of the ingredients used in the formulation were classified by the United States Food and Drug Administration as Generally Recognized as Safe and had no known toxicity. Particle size was determined via microparticle tracking (Microtrac S3500; Microtrac) and confirmed with dynamic light scattering.

Plasma vitamin C

Venous blood (~5 mL) was collected in prechilled heparin-coated tubes, placed immediately on ice, centrifuged within 30 minutes (3600 rpm for 10 minutes at 4°C) to isolate plasma, and then transferred to opaque tubes and stored at −80°C. During the laboratory visit when vitamin C was intravenously administered, extreme caution was used when collecting blood immediately postinfusion to avoid the contamination of samples with vitamin C. Preventive measures included flushing of the stopcock with ~10 mL of saline and discarding the first ~8 mL of blood. Within seven days of collection, plasma vitamin C concentration was determined via high-performance liquid chromatography with electrochemical detection by a commercial laboratory (Laboratory Corporation of America).
Noteworthy, the personnel of the commercial laboratory was naive as to treatments and conditions under which the samples were collected.

**Oxidative stress**

Blood samples to be used for measuring a marker of oxidative stress were collected in prechilled tubes containing ethylenediamine tetraacetic acid, placed immediately on ice, and centrifuged within 30 minutes (3600 rpm for 10 minutes at 4°C) to isolate plasma. Plasma was stored at −80°C until analysis. Plasma concentrations of thiobarbituric acid reactive substances (TBARS) were determined using calorimetric detection (Cayman Chemical). All samples were coded such that the research personnel analyzing the plasma was naive as to treatments and conditions under which the plasma was collected.

**Statistical analysis**

This was a randomized, placebo-controlled, repeated measures experimental design. Accordingly, analysis of variance (treatment × time) with repeated measures was used to examine the changes in circulating concentrations of vitamin C and MDA. Multiple comparisons of factor means were performed using Newman–Keuls test. One-way analysis of variance with repeated measures was used to compare areas under the circulating vitamin C and TBARS concentration curves. Areas under the curve were calculated via the trapezoidal rule. The level of statistical significance was set at $P < 0.05$. Data are reported as mean ± SE.

**Results**

**Research participants**

All 11 of the enrolled research participants completed the study. The vitamin C, irrespective of mode of delivery, was well tolerated, and there were no adverse events. Selected physical characteristics of the research participants are presented in Table 1. As a group, the research participants were obese, middle-aged, and older men and women.

**Plasma vitamin C concentration**

Baseline circulating concentrations of vitamin C did not differ between treatments. On average, the baseline concentrations were slightly lower than recommended values.\(^{15,23}\) Circulating concentrations of vitamin C prior to and following administration are presented in Figure 2A and B. Plasma vitamin C concentrations were greatest following intravenous administration at all time points compared with each of the other modes of delivery ($P < 0.001$). At two, three, and four hours, plasma vitamin C concentrations were greater after oral administration of vitamin C encapsulated in liposomes compared with placebo and unencapsulated vitamin C ($P < 0.01$); and unencapsulated vitamin C produced greater concentrations than placebo ($P < 0.01$). Consistent with these data, oral delivery of vitamin C encapsulated in liposomes (10.3 ± 0.9 mg/dL h) evoked a greater ($P = 0.002$) area under the curve than unencapsulated vitamin C (7.6 ± 0.4 mg/dL h) that in turn was greater ($P < 0.001$) than placebo (3.1 ± 0.4 mg/dL h).
Figure 2

Plasma concentrations of vitamin C (ascorbic acid) before (time = 0 minute) and after: (1) oral administration of placebo, (2) oral administration of 4 g of vitamin C encapsulated in liposomes, (3) oral administration of 4 g of unencapsulated vitamin C, and (4) intravenous administration of 4 g of vitamin C. (A) All treatments. (B) All treatments excluding intravenous administration. Twenty minutes of forearm ischemia was initiated at three hours. * $P < 0.001$ vs all other treatments; # $P < 0.001$ vs unencapsulated oral and placebo; and ^ $P < 0.001$ vs placebo. Data are mean ± SE (plasma vitamin C: 1 mg/dL = 56.78 μmol/L).

Lipid peroxidation
Baseline concentrations of plasma TBARS were not different between treatments, but they were somewhat variable. To better illustrate the influence of ischemia–reperfusion and/or vitamin C administration, these data have been presented as change in TBARS relative to baseline (Fig. 3). Forearm ischemia–reperfusion (20 minutes of occlusion) increased circulating TBARS 40 minutes postreperfusion \((P < 0.03)\); this increase was prevented by all of the vitamin C treatments and was unaffected by mode of delivery.

![Figure 3](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4915787/)

**Figure 3**
Change in circulating concentrations of TBARS from baseline (before) and after: (1) oral administration of placebo, (2) oral administration of 4 g of vitamin C encapsulated in liposomes, (3) oral administration of 4 g of unencapsulated vitamin C, and (4) intravenous administration of 4 g of vitamin C. Twenty minutes of forearm ischemia was initiated at three hours. *\(P < 0.046\) vs all other treatments. Data are mean ± SE.

**Discussion**

The new findings of this investigation were that the oral delivery of vitamin C encapsulated in liposomes: (1) produced circulating concentrations of vitamin C greater than those elicited by oral delivery of unencapsulated vitamin C but less than those following intravenous vitamin C administration and (2) provided protection from ischemia–reperfusion that was similar to the protection provided by oral unencapsulated vitamin C and intravenous vitamin C administrations.

To our knowledge, there has been only one other published human study evaluating oral delivery of vitamin C encapsulated in liposomes, and this was a pilot study with only two research participants. Circulating concentrations of vitamin C following oral delivery of 5 g of vitamin C encapsulated in liposomes were compared with concentrations following 5 g of unencapsulated vitamin C. No discernable differences were detected between the plasma concentrations; however, in light of its status as a pilot study, and with only two research participants, this lack of difference could be attributed to insufficient statistical power, that is, a type 2 error.
While human studies of liposomal vitamin C may be lacking, the concept and utilization of liposomes for pharmaceutical delivery are well established. At the time of submission, there were at least 15 medications currently approved for liposomal delivery, with several hundred clinical trials currently underway. The cancer drug, doxorubicin, is one example of a reasonably well-known medication routinely delivered via liposomal encapsulation. Advantages of liposomal encapsulation include accelerated intestinal absorption, increased stability of the pharmaceutical, protection of the gut from potentially irritating agents, and greater bioavailability of the pharmaceutical.

In the current investigation, we demonstrated greater bioavailability of vitamin C when delivered via liposomes compared with unencapsulated vitamin C. Blood is the primary transport medium of vitamin C, between the gut and the target tissues, thus circulating concentrations have long been accepted as a suitable expression of bioavailability. However, contingent on the dependent variable of interest and/or the specific role of vitamin C under investigation, in some instances, tissue/cellular concentrations may be more relevant than circulating concentrations. In light of the cost benefit and degree of invasiveness required for tissue sampling, we chose to analyze circulating concentrations.

To investigate a functional implication of enhanced vitamin C delivery/bioavailability, we quantified the magnitude of increase in circulating concentrations of biomarkers of oxidative stress following a forearm ischemia–reperfusion injury. Forearm ischemia–reperfusion injury has become an accepted model of oxidative stress-mediated damage, and several previous studies have examined the ability of antioxidant administration to attenuate increased oxidative stress following reperfusion. In the current study, forearm ischemia–reperfusion (20 minutes of occlusion) increased circulating TBARS 40 minutes postreperfusion; this increase was prevented by all of the vitamin C treatments and was unaffected by mode of delivery. While the protection provided by vitamin C was expected, we did not anticipate a similar degree of protection from all vitamin C treatments. The absence of difference in protection may, in part, be attributed to the magnitude of the vitamin C dose. A single (bolus) dose of 4 g of vitamin C is not trivial (approximately 40-fold greater than the minimum Recommended Dietary Allowance) and resulted in appreciably increased circulating concentrations of vitamin C (~3–30-fold) above baseline. The rationale for the 4 g dose was based on prior studies in which systemic doses ranging from 2 to 5 g were administered, and favorable outcomes reported pertinent to oxidative stress and/or physiological function. However, it may be that the exact dose required for protection from ischemia–reperfusion injury is less than 4 g. In the current study, a smaller dose of vitamin C may have produced discernable differences between modes of delivery. Future studies should incorporate consideration of a dose–response relationship.

We chose circulating TBARS as our expression of oxidative damage following ischemia–reperfusion. Recently, TBARS has been criticized for being a nonspecific marker of lipid peroxidation. We feel that TBARS was an appropriate outcome for the current study because: (1) the increase in TBARS is well correlated with the increase in isoprostanes, generally considered as a more specific marker of lipid peroxidation, during copper-mediated oxidative modification of low-density lipoprotein and peroxidation of docosahexaenoic acid and (2) TBARS proved to be of sufficient sensitivity to illustrate circulating responses to ischemia–reperfusion and an interaction between vitamin C and ischemia–reperfusion.

Noteworthy, in addition to its antioxidant properties, vitamin C has multiple other beneficial physiological effects. These include synthesis of catecholamines, formation of collagen, facilitation of iron absorption, and support with the processing of many hormones, enzymes, and amino acids. It is feasible that modes of delivery of vitamin C might influence one or more of these functions; however, detailed examination of these additional functions was beyond the scope of the current study.
In the current study, vitamin C was encapsulated in liposomes with a phospholipid bilayer composed of phosphatidylcholine. It is possible that the liposome itself, irrespective of its contents, may have some health benefits as phosphatidylcholine has been proposed to attenuate dementia progression, facilitate structure and function of the endoplasmic reticulum, and play a protective role during myocardial disease development. Again, detailed examination of these additional functions/benefits was beyond the scope of the current study. Future studies should incorporate an empty liposome as a control/comparison treatment.

We have demonstrated that oral delivery of vitamin C encapsulated in liposomes promotes greater bioavailability than unencapsulated vitamin C, while avoiding the risks associated with intravenous administration. This observation has favorable implications for both scientific study and clinical use. From a research tool perspective, several studies have shown that the physiological benefits of intravenous vitamin C administration are not always reproduced with oral administration, on account of lower bioavailability. Oral administration of encapsulated vitamin C may abrogate this limitation. From a clinical perspective, older adults and adults who smoke may have a greater need for vitamin C than their younger, nonsmoking counterparts. Oral administration of encapsulated vitamin C may easily meet this need while decreasing the risk of gastrointestinal upset. Further, oxidative stress has been proposed as a contributing factor to delayed recovery from elective surgeries, such as knee arthroplasty and some cardiac procedures. Presurgery ingestion of encapsulated vitamin C may help alleviate/prevent oxidative stress-mediated damage and promote accelerated recovery.

In summary, we have demonstrated that oral delivery of vitamin C encapsulated in liposomes (1) produced circulating concentrations of vitamin C greater than those elicited by oral delivery of unencapsulated vitamin C but less than those following intravenous vitamin C administration and (2) provided protection from ischemia–reperfusion that was similar to the protection provided by oral unencapsulated vitamin C and intravenous vitamin C administration.

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the Committee on Publication Ethics (COPE).

**Author Contributions**

Conceived and designed the experiments: JLD, RLS, EB, and CB. Collected and analyzed the data: JLD, HLP, JWB, SEB, GRG, RLS, MMS, EB, and CB. Wrote the first draft of the article: JLD and CB. Contributed to the writing of the article: JLD, HLP, JWB, SEB, GRG, RLS, MMS, EB, and CB. Agreed the article results and conclusions: JLD, HLP, JWB, SEB, GRG, RLS, MMS, EB, and CB. Jointly developed the structure and arguments for the article: JLD, EB, and CB. Made the critical revisions and approved the final version: JLD, HLP, JWB, SEB, GRG, RLS, MMS, EB, and CB. All authors reviewed and approved the final article.

**REFERENCES**


44. Hocker AD, Boileau RM, Lantz BA, Jewett BA, Gilbert JS, Dreyer HC. Endoplasmic reticulum
