Relative Bioavailability of Liposomal Vitamin C – 'VitaLip-C'

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Abstract

Background: Vitamin C is a water-soluble vitamin and one of the most commonly used vitamins. With increasing vitamin C intake, the plasma steady state concentration reaches a maximal level of about 70–80 µM. However, from the available literature, it appears that a daily intake of about 200–400 mg of vitamin C ensures saturation of the blood in healthy individuals. Bioavailability can be increased by various methods, such as encapsulation, micronisation, etc. VitaLip-C is a liposomal form of vitamin C, which is shown to be clinically proven and evaluated by Transmission Electron Microscopy (TEM) for the presence of liposomes.

Materials and Methods: In the present study, authors have studied the relative bioavailability of VitaLip-C with the marketed formulation of vitamin C in animals.

Results and Conclusion: It has been observed that VitaLip-C has 88.76% higher relative oral bioavailability in comparison to marketed formulation claiming 500 mg of vitamin C.

Keywords: Vitamin C, liposome, relative bioavailability, pharmacokinetics, VitaLip-C

Conflict of Interest: Dr. Yogesh Dound is Propreitor, & Shivani Agawane and Khushbu Gupta are Research Associates from Shreepad Shree Vallabh SSV Phytopharmaceuticals.

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Introduction

Vitamin C is a water-soluble vitamin known chemically as L-ascorbic acid (as kore' bik as' id). Vitamin C is found in many foods, particularly citrus fruits, green vegetables, tomatoes and potatoes. Vitamin C deficiency is the cause of scurvy, which is marked by fatigue, spongy gums, loss of teeth, ecchymosis, petechiae and excessive bleeding including bleeding from the gums, into joints and into internal organs.

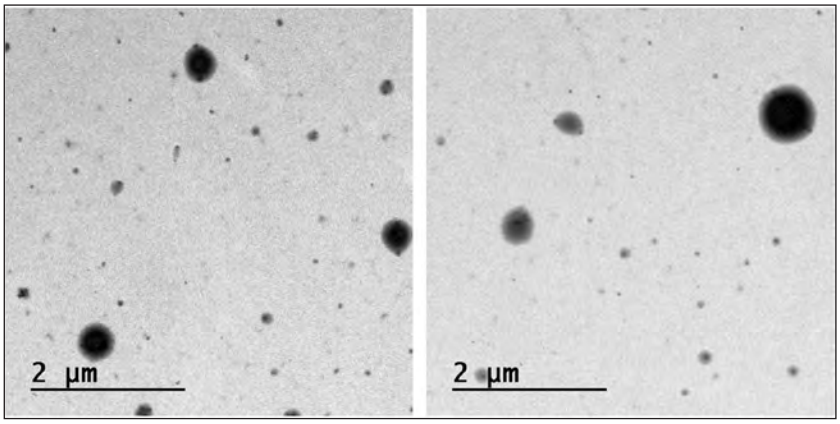

Figure 1: TEM images of VitaLip-C showing the presence of vesicles formation.

The biological benefits of the water-soluble molecule vitamin C (l-ascorbic acid or ascorbate) have been well documented.[1-4] Based on its unique chemistry, the biological role of ascorbate is to act as a reducing agent, donating electrons in various enzymatic and non-enzymatic reactions.[5] It is a co-factor for at least eight enzymatic reactions involved in key bodily processes including the production of collagen, preventing harmful genetic mutations, protecting white blood cells and the production of carnitine, vital for energy.[6,7] Ascorbate is reversibly oxidized with the loss of two electrons to form dehydroascorbic acid (DHAA).

For healthy individuals, it is possible to get sufficient amounts of vitamin C through the diet provided it contains high amounts of vitamin C-rich sources.[8] However, in many diseases and in people with very poor vitamin C status, the dietary intake may be insufficient to provide adequate amounts of vit C.[9,10]

Following ingestion, subsequent vitamin C bioavailability is largely determined by rates of intestinal absorption and further influenced by renal reabsorption and excretion. With increasing vitamin C intake, the plasma steady state concentration reaches a maximal level of about 70–80 µM.[11,12] From the available literature, it appears that a daily intake of about 200–400 mg of vitamin C ensures saturation of the blood in healthy individuals.[13] During periods of altered distribution due to temporary physiological needs such as pregnancy or increased turnover during disease or smoking, higher intakes are needed to maintain sufficient levels.

Several attempts have been made to bypass the maximum steady state plasma concentration of about 70–80 µM achievable through oral administration. An approach to increase the maximum achievable plasma concentration through oral administration has been via liposomes. Different strategies, such as liposomes, solid dispersion, complex, emulsion, micelles, nanogels and microspheres have been employed to overcome poor absorption and other limitations of Vitamin C.

Authors of the current study have developed natural Liposomal Vitamin C, 'VitaLip-C'. The TEM studies conducted on VitaLip-C has confirmed the special vesicle formation (Figure 1). Further a clinical trial with VitaLip-C was conducted in children aged 4 to 12 years suffering from URTI. It was observed that within 4-5 days of starting treatment with Liposomal Vitamin C, the score, as indicated by the Wisconsin Upper Respiratory Symptom Survey (WURSS) scale, had reduced. The symptoms also subsided, and no recurrence was observed till the end of 30 days. This result suggests that the VitaLip-C can be useful as an adjunct in the management of URTI, by improving immunity.[14]

Taking lead from the above studies and vesicular confirmation, authors of the current study decided to undertake a relative oral bioavailability study following single oral doses of 40 mg/2.5 ml of VitaLip-C and marketed formulation claiming 500 mg of Vitamin C, in rats.

Materials and Methods

Liposomal Vitamin C (VitaLip-C Liquid) was obtained from Shreepad Shree Vallabh SSV Phytopharmaceuticals, Mumbai, India. The source of plain Vitamin C was marketed vitamin C formulation of 500mg oral formulation. Male Wistar rats (290–340 g) were housed in a temperature (23 ± 1°C) and light-controlled room (12h light/dark cycle). They were allowed *ad libitum* access to food and water for 7 days. Rats were randomly divided into two groups of four animals each. The control group was given plain Vitamin C 400 mg/kg body weight of the rats and the treatment group was given Liposomal Vitamin

Table 1. Description of the dose of formulation in Treatment and Control groups

Group	Description	Dose [mg/kg]	Dose volume [ml/kg]	Time points for sampling post dose	No. of animals per time point
1	VitaLip-C liquid containing 40 mg of Ascorbic Acid/2.5 ml.	400	10	Pre-dose, 30 min, 60 min, 90 min, 120 min, 180 min, 240 min, 360 min, 480 min	4
2	Marketed vitamin C formulation of 500mg	400	10	Pre-dose, 30 min, 60 min, 90 min, 120 min, 180 min, 240 min, 360 min, 480 min	4

C at the dose of 400 mg/kg body weight. The details of the treatment are included in Table 1. The study was performed as per ethical practices laid down in the CPCSEA guidelines[15] for animal care and use at TheraIndx Lifesciences Private Limited, a CPCSEA approved laboratory (Registration number 1852/PO/Rc/S/16/CPCSEA). The study was approved by the Institutional Animals Ethics Committee (IAEC) of the test facility (Protocol No. IAEC/12/2019/141).

Method

All the formulations were orally administered by oral gavage by means of flexible plastic tubes having a length of 5 cm. At pre-determined intervals (Table 1) after administration, serial blood samples (~ 0.1 ml blood) were collected from the jugular vein (and terminal sampling was done by cardiac puncture. Blood was collected in 1.5 ml Eppendorf tubes containing 0.005 ml of 10% K_2EDTA, mixed gently and placed in ice before centrifugation. Blood samples were centrifuged at 2,000 p.m. for 10 minutes, plasma harvested and stored at –80°C till further bioanalysis. Compound concentrations were quantified in plasma by LCMS/MS using a partially validated bioanalytical method.[16]

Pharmacokinetic (PK) Analysis

PK analysis was performed using Non compartmental methods in WinNonlin.[17] The following PK parameters were estimated in plasma: C_{max}, T_{max}, K_e (elimination rate constant) terminal half-life [$t_{1/2}$], $AUC_{0-\infty}$ (Area Under the Concentration Time Curve up to infinity), apparent Clearance [CL/F], apparent Volume of Distribution [V/F]. The AUC of Ascorbic Acid in the test formulations was compared to the AUC of Ascorbic Acid. The significance of differences between group means were estimated through a t test using GraphPad Prism. The relative bioavailability was calculated by following formula:

$$\text{Relative Bioavailability} = \frac{AUC_{last,}\ \text{VitaLip-C} \times 100}{AUC_{last,}\ \text{Marketed formulation}}$$

Results

No apparent abnormalities were detected in animals post oral administration of 500 mg/kg of marketed formulation of Vitamin C and VitaLip-C. Following oral administration of Liposomal Vitamin C, it showed rapid absorption (T_{max}= 3 hours) with Peak plasma concentration (C_{max}), Area Under Curve (AUC_{last}) and half-life ($t_{1/2}$) of 194.5 µM, 1149.9 (h* µM) and 4.7 h, respectively. In the control group, following oral administration of marketed Vitamin C formulation, showed absorption (T_{max}= 1.5 hours) with Peak plasma concentration (C_{max}), Area Under Curve (AUC_{last}) and half-life ($t_{1/2}$) of 105 µM, 609.2 (h* µM) and 7.8 h, respective-

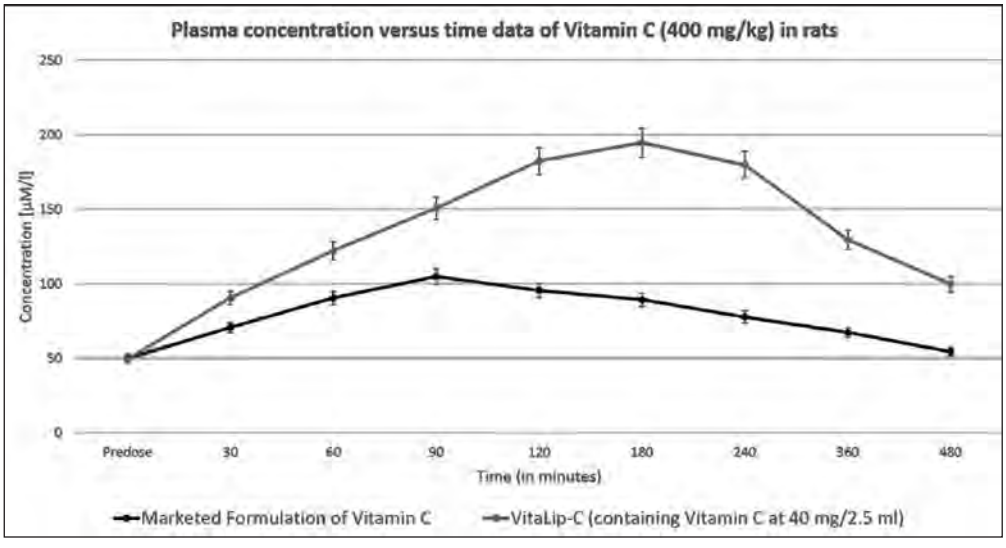

Figure 2: Pharmacokinetics of VitaLip-C and Marketed formulation of Vitamin C

ly. It has been observed that VitaLip-C showed 88.76% higher relative oral bioavailability in comparison to marketed formulation claiming 500mg of vitamin C. Figure 2 shows the relative bioavailability of VitaLip-C in comparison to marketed formulation of Vitamin C.

Discussion

A liposome is a spherical vesicle having at least one lipid bilayer. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs.[18] Liposomes can be prepared by disrupting biological membranes (such as by sonication).

Liposomes are most often composed of phospholipids, especially phosphatidylcholine, but may also include other lipids, such as egg phosphatidylethanolamine, so long as they are compatible with lipid bilayer structure.[19] A liposome design may employ surface ligands for attaching to unhealthy tissue.[20]

Liposomes are extensively used as carriers for numerous molecules in cosmetic and pharmaceutical industries. A very small number of dietary and nutritional supplement companies are currently pioneering the benefits of this unique science towards this new application. This new direction and employment of liposome science is in part due to the low absorption and bioavailability rates of traditional oral dietary and nutritional tablets and capsules. The low oral bioavailability and absorption of many nutrients is clinically well documented.[21] Therefore, the natural encapsulation of lypophilic and hydrophilic nutrients within liposomes has made for a very effective method of bypassing the destructive elements of the gastric system and aiding the encapsulated nutrient to be delivered to the cells and tissues.[22]

When ingested, the pharmacokinetic properties of liposome intestinal absorption override the usual absorption pattern of the encapsulated drug. That means the delivery of a drug/supplement with a typically slow or regulated pattern of absorption, such as vitamin C, is accelerated when encapsulated within a liposome.[23,24] 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.[23]

Hence, liposomes are useful vehicles to deliver drugs because of their ability to improve bioavailability and solubility of drugs. They can be used as a potential targeted delivery system by folate-ligand surface modification, gradual release of drugs, for e.g. curcumin in the body, and subsequent improved efficacy of treatment in cancer patients.[25,26] In a recently conducted study by one of the authors of the current publication, Dound *et al*, it was observed that liposomal Curcumin has higher bioavailability as compared to the various marketed formulations.[27]

Encapsulation of vitamin C in new types of liposomes causes the enhancement of vitamin C bioavailability on a physiological level, without compromising its potency on the cellular level. In a recently conducted study by M. Łukawski *et. al*, the enhanced bioavailability of vitamin C from the formulation was demonstrated through a medical experiment.[28]

The pharmacokinetic properties of a bolus of four grams of liposome-encapsulated Vitamin C were compared to those of plain vitamin C and placebo in eleven volunteers in a crossover trial.[29] The authors found a 35% increase in exposure (AUC_{0-4} hours) with a plasma C_{max} of about 200 μM after 3 h.

In a single blind study, plasma levels were measured in two subjects, following ingestion of tablets of liposomal Vitamin C. The reported plasma levels were higher than that usually seen with oral administration of vitamin C.[30]

In the current study, the liposomal Vitamin C, VitaLip-C, has been compared with marketed formulation of Vitamin C and was found to have 88.76% higher relative oral bioavailability. Bioavailability is a major concern for the fate of Vitamin C in the body. With the property of enhanced bioavailability, liposomal Vitamin C will improve its efficacy for various human applications.

Conclusion

The Liposomal Vitamin C, VitaLip C showed 88.76% higher relative oral bioavailability in comparison to marketed formulation claiming 500 mg of vitamin C. These pharmacokinetic values need to be studied in depth and correlated in humans. Further extensive clinical studies with liposomal vitamin C will prove its efficacy in various indications, in comparison to marketed formulations of plain Vitamin C.

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