ORIGINAL ARTICLE

Vitamin C pharmacokinetics of plain and slow release formulations in smokers

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Summary

Background & aims: Combination of the antioxidants ascorbic acid in slow release formulation and \( \alpha \)-tocopherol can retard the progression of atherosclerosis. In order to determine if differences in formulation could explain some of the different results in the intervention trials we determined selected pharmacokinetics for two different formulations of ascorbic acid together with \( \alpha \)-tocopherol.

Methods: Single-blinded, randomised, placebo-controlled intervention study with 48 healthy men, aged 20–65 years, smoking \( \geq 5 \) cigarettes/day. Subjects received 250 mg plain release ascorbic acid and 91 mg plain release \( \alpha \)-tocopheryl acetate, 250 mg slow release ascorbic acid and 91 mg plain release \( \alpha \)-tocopheryl acetate or placebo twice daily for 4 weeks. A series of blood samples were collected after administration of the first dose and repeated after 4 weeks of supplementation.

Results: The fluctuation of ascorbic acid plasma concentrations decreased significantly (\( P = 0.003 \)) after 4 weeks supplementation in the slow versus the plain release group.

Conclusions: This study shows that there were pharmacokinetic differences between plain and slow release formulations of ascorbic acid. However, these effects are small and unlikely to be of significant clinical importance.

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Introduction

Vitamin C, or ascorbic acid, cannot be synthesised by the human body. \(^1\) Man therefore depends on dietary intake to maintain vital biological functions dependent on ascorbic acid. While it is well accepted that ascorbic acid intake in doses of about 20–90 mg per day are sufficient to prevent scurvy, \(^2,3\) considerable controversy exists about the effects of so-called mega doses, i.e. \( \text{“gram” doses, on the major diseases in the western society: Cancer and atherosclerosis. A few small focused trials}^{4-6} \) and larger preventive trials \(^7\) of up to a few years duration have shown benefits from antioxidant supplementation, e.g. reduction of the atherosclerotic progression. These positive findings from intervention studies are at variance with larger and longer preventive trials where it was not...
possible to find positive effects of ascorbic acid in combination with vitamin E and β-carotene.8–15

Recently, the antioxidant supplementation in atherosclerosis prevention (ASAP) study showed a beneficial effect on atherosclerotic progression of ascorbic acid and α-tocopherol in combination using a slow release formulation of ascorbic acid.4,5 The significant effect as shown by a reduced progression of atherosclerosis was only seen for the smoking male and no effect was found in female smokers as well as non-smokers of both genders. Furthermore the ASAP study did not show any effect with ascorbic acid or α-tocopherol individually. The rationale for using a slow formulation of ascorbic acid was to reduce fluctuations in the plasma concentrations of ascorbic acid. Earlier studies had indicated that this was achieved after a single dose.16 However, more detailed pharmacokinetic studies were not conducted and little is known about the basic pharmacokinetics of ascorbic acid in plain versus slow release formulation. The bioavailability of ascorbic acid in non-smoking men has been found to be dose-dependent and doses exceeding 500 mg are largely excreted or not absorbed.17 Another pharmacokinetic study did not find any significant effect of smoking on the bioavailability, maximum plasma concentration or elimination for erythorbic acid, a stereo isomer of ascorbic acid.18

To further investigate the mechanism that retarded the progression of atherosclerosis in the ASAP study, we have studied the possible pharmacokinetic differences between plain and slow release ascorbic acid in smoking men and expanded these studies to the steady state situation following 4 weeks of supplementation.

Materials and methods

Subjects

Healthy men 20–65 years of age and smoking five or more cigarettes per day were recruited from the Copenhagen area by newspaper advertisements. Exclusion criteria were uncontrolled hypertension (diastolic blood pressure >105 mmHg), abnormal weight (body mass index <20 kg m⁻² or >30 kg m⁻²), insulin dependent type 1 diabetes, simultaneous participation in other clinical trials, any condition limiting mobility, severe disease shortening life expectancy or other disease or condition worsening the adherence to the measurements or treatment. Although no restrictions regarding ethnicity were made, the study population was exclusively Caucasian. A written informed consent was obtained from all subjects. The Independent Ethic Committee of the City of Copenhagen approved the study protocol and the study was conducted according to the Declaration of Helsinki II and the guidelines of GCP with the exception of external monitoring, as it was a single site, small investigator initiated study. Sixty-six men completed an in-house screening and 50 of these men completed the first trial visit at the Department of Clinical Pharmacology, Copenhagen University Hospital in accordance with the study protocol. Four weeks later 48 subjects completed the second visit. Two volunteers dropped out because of lack of time from newly acquired jobs.

Design

The study was a single-blinded (study participants only), single-site, randomised, placebo-controlled intervention study with three treatment groups. The supplementation comprised tablets of (A) 250 mg plain release ascorbic acid and 91 mg plain release d-α-tocopheryl (as acetate corresponding to 100 mg), (B) 250 mg slow release ascorbic acid and 91 mg plain release d-α-tocopheryl (CellaVie®) or (C) identical placebo tablets. Two tablets were consumed daily, one with the morning meal and one with the evening meal. The trial period was 4 weeks, where steady state for ascorbic acid2,19–21 and α-tocopherol is achieved.22,23 The supplements were generously donated by Ferrosan A/S (Denmark) and contained by analysis 267 mg ascorbic acid and 104 mg d-α-tocopheryl acetate. The doses of the supplements were identical to the doses used in the ASAP study.4,5 Subjects were advised to stop supplement of vitamins and natural remedies containing antioxidants as soon as a written informed consent was obtained at the screening visit. Compliance with trial treatment was calculated by counting any remaining tablets at the second trial visit.

Pharmacokinetic trial

Twenty subjects from each supplement group were invited to stay for a 12 h pharmacokinetic study to test the absorption and distribution of ascorbic acid and α-tocopherol. The first blood sample (baseline) was drawn after overnight fasting. After administration of the supplement, twelve blood samples were drawn over the next 12 h with 45 min interval between the first nine samples and prolonged time interval for the last drawn blood samples. A diet with negligible ascorbic acid content was provided
during the pharmacokinetic sampling period. The pharmacokinetic study was repeated after a 4-week supplementation period with plain or slow release formulation. Nineteen subjects completed the second trial visit from each supplement group. To determine a possible seasonal variation, a placebo group consisting of ten subjects was included. We have previously shown that plasma ascorbic acid does not fluctuate among the placebo group\textsuperscript{18} and consequently no pharmacokinetic evaluation was conducted for this group.

**Measurements of antioxidants**

Blood samples were drawn from an indwelling venflon catheter in a forearm vein into pre-cooled evacuated tubes containing 5 ml anticoagulant Lithium heparin (Beckton Dickinson). The vacutainer was immediately centrifuged for two minutes at 2000 \times g (4°C). All standards were from Fluka (Milwaukee, IL).

Aliquot (500 \mu l) of plasma was immediately stabilized with an equal amount cold (10\%) metaphosphoric acid containing 2 mM EDTA. The precipitate was removed by centrifugation (1 min at 16,000 \times g). The supernatant was stored at –80°C for less than one month until analysis. It has previously been shown that ascorbic acid is stable under these conditions.\textsuperscript{24} Total plasma ascorbic acid (i.e. ascorbic acid + dehydroascorbic acid) was separated by reverse-phase ion-pairing high-performance liquid chromatography (HPLC) and quantified by coulometric detection as described elsewhere.\textsuperscript{25} Four observations were missing and were replaced after the following principle: if the first observation was missing; it was replaced by the last observation. Other missing observations were replaced by the preceding observation.

\(\alpha\)-Tocopherol and \(\gamma\)-tocopherol were measured as described previously.\textsuperscript{26} The conditions for the reversed-phase HPLC assay with electrochemical detection were specifically optimised for the separation of \(\alpha\)- and \(\gamma\)-tocopherol.\textsuperscript{27} The concentrations reported for \(\gamma\)-tocopherol also include those for \(\beta\)-tocopherol, which does not separate from the former under reverse-phase conditions but represents less than 10\% of the two forms in blood plasma.\textsuperscript{28}

**Statistical methods**

Pharmacokinetic parameters were determined by using WinNonlin software version 2.1 (Scientific Consulting, Inc.). A non-compartmental model type was used in combination with a linear/log trapezoidal calculation method. The area under the plasma concentration curve (AUC) was calculated from zero to the last observation. Data were further analysed by using Statistica version 6 (StatSoft, Tulsa, OK). Differences between plain release, slow release and placebo groups were analysed with one-way ANOVA. \(t\)-Test for independent samples by groups was used to examine the difference between the plain and slow release formulations while effects of supplemenations tested by using the \(t\)-test for dependent samples by groups. A two-tailed \(P\) value \(< 0.05\) was considered statistically significant.

**Results**

**Ascorbic acid**

The study start characteristics of the population are summarized in Table 1. There were no significant differences in the subjects' characteristics between the groups, except for lower compliance (tablet count) in the placebo group \((P < 0.001)\). When comparing compliance for the plain and slow release groups, no significant differences were observed.

Changes in ascorbic acid plasma concentrations are illustrated in Fig. 1 and Table 2. At study start, no significant differences in plasma ascorbic acid concentrations were observed between groups. After ingestion of a plain release tablet plasma ascorbic acid concentration increased 66\% \((P < 0.001)\) to a maximum concentration of 74.2 ± 24.3 \mu mol/l at 4.0 h \((T_{\text{max}})\). \(T_{\text{max}}\) for the slow release group was 5.0 h and the plasma concentration increased 38\% \((P < 0.001)\) to reach maximum concentration \((C_{\text{max}})\) corresponding to 70.2 ± 18.3 \mu mol/l \((P < 0.05)\). No significant difference was observed in the span (maximum concentration subtracted the minimum concentration) between the groups. Furthermore, there was no difference in AUC between the groups.

After 4 weeks of supplementation, plasma ascorbic acid \(C_{\text{max}}\) reached 76.9 ± 17.3 and 82.4 ± 14.6 \mu mol/l for plain and slow release group, respectively. The plasma concentration in the placebo group decreased to 39.0 ± 17.9 \mu mol/l. There was a significant difference \((P < 0.001)\) in the trough ascorbic acid concentration between the groups receiving supplements and placebo, while no difference was found between the plain and slow release groups. At the second visit, the ascorbic acid concentration increased more moderately; 26\% \((P < 0.001)\) (96.9 ± 18.4 \mu mol/l at \(T_{\text{max}}\)
2.1 h; plain release) and 11% (\( P < 0.001 \)) (91.6 ± 13.6 μmol/l at \( T_{\text{max}} \) of 2.4 h; slow release), respectively. The \( C_{\text{max}} \) was higher in the plain compared to the slow release group and a significant difference for the span between the two groups was observed (\( P < 0.005 \)).

**Effect of supplementation**

Four weeks of supplementation resulted in significantly increased plasma concentrations of ascorbic acid for both plain and slow release formulations (\( P < 0.001 \); Table 2). The ascorbic acid concentration decreased 25% in the placebo group (\( P < 0.001 \)). A decrease in \( T_{\text{max}} \) of about 30% could be observed in both the plain and slow release groups (\( P < 0.01 \)). Moreover, AUC increased 66% and 42% in the plain and slow release groups, respectively, after 4 weeks of supplementation (\( P < 0.001 \)). The maximum ascorbic acid plasma concentration (\( C_{\text{max}} \)) also increased significantly in both supplemented groups (\( P < 0.01 \)). There was no significant change in the span from study start to after the 4 weeks supplementation period for the two active treatment groups.

### Table 1  Characteristics of subjects. Characteristics of the subjects for the plain release, slow release and the placebo groups.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Plain release (n = 19)</th>
<th>Slow release (n = 19)</th>
<th>Placebo (n = 10)</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
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<tr>
<td>Age (yr)</td>
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<tr>
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<tr>
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<tr>
<td>DBP (mmHg)</td>
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<tr>
<td>Pack year (yr, smoking 1 pack/d)</td>
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<tr>
<td>Alcohol (drinks/wk)</td>
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<td>12</td>
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<tr>
<td>Compliance (tablet)</td>
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<td>93.8***</td>
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<td>Wash out period (d)</td>
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<tr>
<td>Trial period (d)</td>
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<td>1.7</td>
<td>28</td>
</tr>
</tbody>
</table>

***P < 0.001 by one-way ANOVA test for plain release vs. slow release vs. placebo.

![Figure 1](https://example.com/figure1.png)

**Figure 1**  Plasma ascorbic acid concentration. Changes in plasma ascorbic acid concentration after intake of one tablet of plain release ascorbic acid (250 mg) and plain release \( \alpha \)-tocopherol (91 mg) (plain release) or slow release ascorbic acid (250 mg) and plain release \( \alpha \)-tocopherol (91 mg) (slow release) at study start (closed circles and squares, respectively) and after 4 weeks of supplementation (open circles and squares, respectively). Error bars are SEM.
Tocopherol

When analysing the plasma \( \alpha \)-tocopherol concentration for the first ten subjects (six subjects receiving plain release and four receiving slow release), no absorption, distribution or elimination could be observed within the 12 h of blood sampling from either visits (data not shown). Therefore, it was decided to analyse only the baseline samples of all subjects.

The mean study start concentrations of \( \alpha \)-tocopherol and \( \gamma \)-tocopherol were similar in all groups. After 4 weeks of supplementation, \( \alpha \)-tocopherol concentration increased 32% (plain release) and 25% (slow release) (\( P < 0.001 \)). During the same period the concentration in the placebo group decreased 24% (\( P < 0.001 \)). In contrast, the \( \gamma \)-tocopherol concentration decreased 69% after 4 weeks of supplementation with plain release tablets, 81% for subjects receiving slow release tablets and 42% for controls. The \( \gamma \)-tocopherol concentration was significant different for the plain release versus slow release group (\( P < 0.01 \)). After 4 weeks of supplementation, a several-fold increase in the \( \alpha / \gamma \)-tocopherol plasma ratio was found in both supplemented groups, while a smaller increase was observed in the placebo group.

Plasma ratios for ascorbic acid and \( \alpha \)-tocopherol were also examined (data not shown). Neither at study start nor after 4 weeks of supplementation a significant difference was observed for the plain and slow release groups versus placebo. There was no difference between the two supplemented groups.

### Discussion

The possible beneficial effects of antioxidant supplementation continue to be subject to much debate. Most preventive trials and a meta-analysis of randomised trials have not found a benefit of antioxidant supplementation, while a few smaller and more focused trials investigating people with pre-existing vascular disease such as the ASAP study have found significant beneficial effects. In most preventive trials where ascorbic acid was used as supplement, ascorbic acid was formulated as plain release. In contrast, the ASAP study used ascorbic acid in a slow release formulation. Thus, we wanted to investigate if the above discrepancy could relate to pharmacokinetic differences from different formulations of ascorbic acid.

In the present study, we examined the pharmacokinetics of the vitamin supplement used in the ASAP study (slow release formulation) and compared them with those of a similar plain release supplement and placebo. A larger span in plasma ascorbic acid concentration for the plain versus slow release group was expected.

After 4 weeks of supplementation with the slow release formulation, fluctuations in plasma ascorbic acid were below those found in the plain

| Table 2 Plasma concentrations at study start and after 4 weeks. Plasma concentration of ascorbic acid, \( \alpha \)-tocopherol and \( \gamma \)-tocopherol for the plain release, slow release and the placebo group. |
|-----------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                                   | Plain release   | Slow release    | Placebo         | \( P (t\text{-test for independent samples}) \) | \( P \) (one-way ANOVA) |
| Study start                       | Conc. (\( \mu \text{M} \)) | Conc. (\( \mu \text{M} \)) | Conc. (\( \mu \text{M} \)) | Conc. (\( \mu \text{M} \)) | Conc. (\( \mu \text{M} \)) | Conc. (\( \mu \text{M} \)) | Conc. (\( \mu \text{M} \)) |
| Ascorbic acid                     | 44.7            | 50.9            | 52.8            | 0.303           | 0.419           |
| \( \alpha \)-Tocopherol           | 31.1            | 30.2            | 31.3            | 0.797           | 0.945           |
| \( \gamma \)-Tocopherol          | 1.91            | 1.63            | 1.61            | 0.295           | 0.462           |
| \( \alpha / \gamma \)-Tocopherol | 18.3            | 21.2            | 21.9            | 0.282           | 0.435           |
| Ascorbic acid/\( \alpha \)-tocopherol | 1.60          | 1.83            | 1.78            | 0.380           | 0.636           |
| 4-weeks                           | Ascorbic acid   | 76.9 ***        | 82.4 ***        | 39.0 ***        | 0.295           | 0.000           |
| \( \alpha \)-Tocopherol           | 38.3 ***        | 36.1 ***        | 23.8 ***        | 0.435           | 0.000           |
| \( \gamma \)-Tocopherol          | 0.56 ***        | 0.31 ***        | 0.90 ***        | 0.018           | 0.000           |
| \( \alpha / \gamma \)-Tocopherol | 104.7 ***       | 210.0 ***       | 30.2 ***        | 0.013           | 0.000           |

\( * P < 0.05; ** P < 0.01; *** P < 0.001 \) by \( t\text{-test for dependent samples for study start vs. 4-weeks.} \)
release group. This finding is in agreement with an earlier smaller study. However, we found no difference in $T_{\text{max}}$ for the plain release group versus the slow release group after intake of the first tablet. After 4 weeks of supplementation a steady state was reached and $T_{\text{max}}$ decreased approximately 30% in both supplemented groups compared to study start. Such findings have been reported earlier, but no previous reported mechanism for the $T_{\text{max}}$ decrease has been suggested. During the 12 h pharmacokinetic studies, the AUC and $C_{\text{max}}$ values of ascorbic acid did not differ significantly between the two supplemented groups, neither at study start nor after 4 weeks of supplementation (Table 3).

This coherence was also found by Nyyssonen et al., while disagreement has been expressed in the area with respect to pharmacokinetic differences for the slow versus plain release formulation. One study observed higher AUC and $C_{\text{max}}$ values after the first dose in film-coated tablets compared with an enteric-coated pellet preparation, while at steady state the AUC and $C_{\text{max}}$ values of ascorbic acid did not differ significantly between the two supplemented groups, neither at study start nor after 4 weeks of supplementation (Table 3).

In conclusion, we found significant differences in fluctuation of plasma ascorbic acid when supplemented as a plain or slow release formulation, respectively, in combination with $\alpha$-tocopherol. However, the authors find it unlikely that the relative small differences observed between these two formulations be of major clinical importance.

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