Preparation Properties and Antioxidative Potency of Preparations Containing Ascorbic Acid

Yuri Ikeuchi-Takahashi*1, Katsumi Kido*1, Hisao Matsumura*1, Kenichi Sako*1 and Yusuke Hori*1

Department of Drug Delivery Research, Hoshi University*1,
Department of Prescription Analysis, Daichi University of Pharmacy*2,
Education Center for Integrative Medicine, Nihon Pharmaceutical University*1,
Division of Molecular Therapy, The Institute of Medical Science, The Advanced Clinical Research Center,
The University of Tokyo*1

Many preparations containing ascorbic acid are commercially available as over-the-counter (OTC) drugs and dietary supplements. In this study, we compared the properties of various OTC preparations and prescriptions for the medical use of ascorbic acid, and investigated their antioxidative potencies. In each preparation, the total vitamin C content corresponding to the indicated amount was confirmed. Ascorbic acid release from the preparations was not influenced by the pH of the release media. Based on the results of the drug release examination, the dosage form and additives were assumed to control the drug release from the preparations. The ratio of the amount of dehydroascorbic acid to that of total vitamin C at 120 min after the start of the release test was about 4-7 and 6-12% in the first and second fluids respectively (pH 1.2 and 6.8), respectively, and the oxidation of ascorbic acid increased over time. The total antioxidant capacity in the tested medium of the drug release experiment corresponded to the concentration of ascorbic acid. Based on these results, it is considered that the total amount of ascorbic acid absorbed into the bloodstream is an important influence on the antioxidative potency in vivo when preparations containing ascorbic acid are orally ingested.

Key words —— ascorbic acid, dehydroascorbic acid, over-the-counter drug, antioxidative potency, preparation property

Introduction

Recently, the frequency of complementary and alternative medicine (CAM) use has increased exponentially in Europe and the United States. The rise of self-decisions by patients in medical treatment and the spread of the Internet are promoting the use of CAM in Japan. The most popular type of CAM in Japan is dietary supplements.1) It is assumed that many consumers take over-the-counter (OTC) drugs and dietary supplements containing the vitamins and the minerals. Ascorbic acid is a powerful antioxidant that contributes to the formation and health of blood vessels, tendons, ligaments, bones, teeth, and gums.2–5) It helps the body absorb iron and recover from wounds and burns.5) It is reported that the hydrogen peroxide generated by the antioxidative effect of ascorbic acid is harmful to carcinoma cell, but does not influence healthy cells.6) The intake of ascorbic acid is closely related to a decrease in the incidence of some cancers.7–9) Certain nutrients were identified to play a critical role in the normal functioning of the skin, particularly when nutrient deficiencies are apparent, e.g., ascorbic acid in collagen synthesis.10–11) Several studies have observed improved protection of the skin against sun damage by dietary supplementation with vitamins C and

* 2-4-41 Ebara, Shinagawa-ku, Tokyo, 142-8501 Japan
Furthermore, higher intake of ascorbic acid and linoleic acid and lower intake of fats and carbohydrates are associated with better skin-aging appearance. Many preparations containing ascorbic acid are commercially available as OTC drugs and dietary supplements, and it is expected that preparations containing ascorbic acid are used by the younger and older generations because of their various functions. OTC drugs containing ascorbic acid are classified into the low risk drugs. Hence, a customer must choose these drugs by self-decisions without positive information supply. The dosage forms of these preparations include powders, granules, tablets, and chewable tablets. Preparations containing sodium or calcium ascorbate are also commercially available.

In this study, we compared the drug release properties of various OTC preparations and prescriptions for medical use of ascorbic acid. The time courses of the oxidation ratio and the antioxidative potencies in the dissolution medium on the release test were also investigated. Then, we tried to provide information to utilize effectively the preparations containing ascorbic acid by clarifying the characteristics of the preparations.

Materials and Methods

1. Materials

Hicee® granules 25% (Hic-G) as prescribed for medical use were purchased from Takeda Pharmaceutical Co., Ltd. (Osaka, Japan). Vitamin C tablets 500 Iwaki (VC-Tab, Iwaki Seiyaku Co., Ltd., Tokyo, Japan), Yunker® C (YK-Chew, Sato Pharmaceutical Co., Ltd., Tokyo, Japan), Vitamin C chewable Iwaki (VC-Chew, Iwaki Seiyaku Co., Ltd., Tokyo, Japan), and Hicee® plus (Hic-Chew, Takeda Pharmaceutical Co., Ltd., Osaka, Japan) were purchased as OTC preparations. L(+)−Ascorbic acid and 2,6-dichloroindophenol sodium salt were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) as the purest grade available. Thiourea, metaphosphoric acid, acetic acid, and 2,4-dinitrophenylhydrazine were purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan) as the purest grade available. All other chemicals were obtained commercially at the purest grade available.

2. Determination of total vitamin C and dehydroascorbic acid

Determination of total vitamin C (the total amount of ascorbic acid and dehydroascorbic acid) and dehydroascorbic acid was performed according to the Methods of Analysis in Health Science. To determine total vitamin C, 3% (w/v) metaphosphoric acid solution containing 8% (v/v) acetic acid was added to test solutions containing 1-2 mg ascorbic acid, and the volume was adjusted to 50 mL. One drop of 0.2% (w/v) 2,6-dichloroindophenol sodium salt solution, 1 mL of 5% (w/v) metaphosphoric acid solution containing 2% (w/v) thiourea and 0.5 mL of 4.5 mol/L H₂SO₄ containing 2% (w/v) 2,4-dinitrophenylhydrazine were added to 1mL of the diluted test solution. Then, the solution was stored at 37 ℃ for 3 h. Eighty-five% sulfuric acid solution (2.5 mL) was added to it in ice water, and it was mixed and stored at room temperature for 30 min. Finally, the absorbance at 540 nm was measured. Ascorbic acid standard solutions were adjusted to 0, 12.5, 25, 50 μg/mL by dissolving L(+)−ascorbic acid into a 5% (w/v) metaphosphoric acid solution. The ascorbic acid standard solution of 1 mL was transferred to a test tube, and the same operation as for the above-mentioned diluted test solution was performed. A regression
equation between the absorbances and concentrations was obtained from the data of the standard solutions. The relationships between the concentration and the absorbance are expressed as \( y = 0.0097x \), where \( x \) is the concentration and \( y \) is the absorbance. The coefficient of determination \( (R^2) \) is 0.9991. The interassay coefficient of variation for quality control samples ranged from 0.9-4.6%. The concentration of ascorbic acid in the test solution was calculated in accordance with the above calibration curve. To determine dehydroascorbic acid, the diluted test solution of 1 mL was transferred to a test tube, and 1 mL of 5\% (w/v) metaphosphoric acid solution containing 2\% (w/v) thiourea and 0.5 mL of 4.5 mol/L H\(_2\)SO\(_4\) containing 2\% (w/v) 2,4-dinitrophenylhydrazine were added. Then, the solution was stored at 37 ℃ for 3 h. Eighty-five\% sulfuric acid solution (2.5 mL) was added to it in ice water. Finally, it was mixed and stored at room temperature for 30 min, and the absorbance at 540 nm was measured. The concentration of dehydroascorbic acid in the test solution was calculated in accordance with the above calibration curve.

3. Ascorbic acid content in preparations

The dosage form and components of the tested preparations are shown in Table 1. The content of ascorbic acid in the preparations was measured using the second fluid, 50 mM phosphate buffer (KH\(_2\)PO\(_4\)-NaOH) (pH 6.8), from the disintegration test in the Pharmacopoeia of Japan (JP) 15. The amount of preparations corresponding to 500 mg as ascorbic acid was stirred in 300 mL of the second fluid for 24 h at room temperature. Then, 5 mL of the tested solution was taken and filtered with a membrane filter (0.45 μm pore size). To determine total vitamin C, 3\% (w/v) metaphosphoric acid solution containing 8\% (v/v) acetic acid was added to 1 mL of the filtrate, and the volume was adjusted to 50 mL. The quantitative determination was performed according to the above-mentioned method.

4. Drug release tests

The drug release experiment was performed according to the first method (rotation basket method) of the dissolution test in JP 15.\(^{17}\) The first fluid (pH 1.2) and the second fluid (pH 6.8) were used as release medium. An amount of each preparation corresponding to 500 mg of ascorbic

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Dosage form</th>
<th>Component</th>
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<tbody>
<tr>
<td>Hic-G</td>
<td>Granule</td>
<td>Ascorbic acid 250 mg/1 g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ascorbic acid 500 mg/tablet</td>
</tr>
<tr>
<td>VC-Tab</td>
<td>Tablet</td>
<td>Ascorbic acid 100 mg/tablet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium ascorbate 150 mg/tablet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(as ascorbic acid)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcium pantothenate 5 mg/tablet</td>
</tr>
<tr>
<td>YK-Chew</td>
<td>Chewable tablet</td>
<td>Ascorbic acid 250 mg/tablet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium ascorbate 250 mg/tablet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(as ascorbic acid)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Riboflavin butyric ester 3 mg/tablet</td>
</tr>
<tr>
<td>VC-Chew</td>
<td>Chewable tablet</td>
<td>Ascorbic acid 350 mg/tablet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Calcium ascorbate 150 mg/tablet</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(as ascorbic acid)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Riboflavin butyric ester 2.5 mg/tablet</td>
</tr>
</tbody>
</table>

Table 1. Dosage form and components of preparations containing ascorbic acid
acid was put into a basket, immersed completely in 900 mL of the dissolution medium pre-warmed at 37 ± 0.5°C so that the basket bottom was located 2.5 cm from the inner bottom of the container, and rotated at 60 rpm at 37 ± 0.5°C. At appropriate time points, 5 mL of the tested medium was taken and filtered with a membrane filter (0.45 μm pore size). Immediately after each sampling, 5 mL of fresh medium was added. To determine total vitamin C and dehydroascorbic acid, 3% (w/v) metaphosphoric acid solution containing 8% (v/v) acetic acid was added to 3-5 mL of the filtrate (the volume was decided according to the concentration of ascorbic acid), and the volume was adjusted to 50 mL. The quantitative determination was performed according to the above-mentioned method. As the release parameters, the area under the total vitamin C released – time curve (AUC) and the mean dissolution time (MDT) were calculated. AUC was calculated using the linear trapezoidal rule up to 90 min. MDT was calculated using the following equation:

$$\text{MDT} = \frac{\int_0^\infty t \frac{dm}{dt} dt}{\int_0^\infty \frac{dm}{dt} dt}$$  \hspace{1cm} (1)

where $m$ is the fraction of drug dissolved in solution at time $t$, $\frac{dm}{dt}$ is the dissolution rate.

5. Antioxidative potency

Antioxidative potency was measured by a spectrophotometric assay (OXY-adsorbent test, Free radical elective evaluator, Diacron, Italy) in the tested medium of the drug release experiment\(^{19-21}\). The second fluid (pH 6.8) was used as the release medium. This test is based on the capacity of HClO to oxidize antioxidants. Total antioxidant capacity can be obtained by evaluating the capacity to inactivate an oxidant solution (HClO) added in excess to the sample. Briefly, 10 μL of the tested medium was mixed with 1 mL of oxidant solution. Then, 10 μL of a chromogenic reagent was added after a 10 min incubation at 37°C. As HClO reacts with a chromogenic substrate, a colored complex develops that can be measured photometrically. The spectrophotometric measurement was determined at a wavelength of 546 nm. The concentration of the colored complex is directly proportional to the concentration of HClO and indirectly proportional to the antioxidant capacity. The results are expressed as μmol of HClO consumed by 1 mL of sample (μmol HClO/mL).

6. Statistical analysis

Statistical analysis was done using ANOVA with a post hoc Bonferroni-Dunn correction. The level of significance was taken as $p<0.005$. The relationship between two different parameters was obtained by simple regression analysis.

Results and Discussion

1. Contents of ascorbic acid in preparations

The contents of ascorbic acid in preparations are shown in Table 2. The contents of ascorbic acid are shown as a percentage of the indicated amount. Ascorbic acid powder, a pharmacopeial medicine, is described including L-ascorbic acid corresponding to 95-120% of the indicated amount in JP 15. The content was an appropriate

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Total ascorbic acid (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hic-G</td>
<td>102.2 ± 1.2</td>
</tr>
<tr>
<td>VC-Tab</td>
<td>102.8 ± 1.7</td>
</tr>
<tr>
<td>YK-Chew</td>
<td>101.0 ± 0.4</td>
</tr>
<tr>
<td>VC-Chew</td>
<td>101.8 ± 2.7</td>
</tr>
<tr>
<td>Hic-Chew</td>
<td>99.8 ± 0.0</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. (n = 3).
value in each preparation as compared with the standard of JP15.

2. Drug release tests

The drug release profiles of total vitamin C from the preparations in the first fluid (pH 1.2) and the second fluid (pH 6.8) are described in Fig. 1. Hic-G showed rapid drug release, and the drug release in the second fluid reached 80% within 10 min. However, the drug release in both the first and the second fluid did not reach up to 100% within 120 min. It was confirmed that a part of granules leaked from the basket, and stayed in the bottom of the flask. Consequently, the granules immersed in the bottom did not release the drug completely. YK-Chew showed relatively rapid drug release, and the drug release was completed within 30 min. The drug release from VC-Chew and Hic-Chew lasted for 120 min in both the first fluid and the second fluid. The release behavior in the first fluid and second fluid was similar in each preparation. Hence, it is suggested that the ascorbic acid release does not depend on the pH of the release media. Although YK-Chew, VC-Chew and Hic-Chew include sodium or calcium ascorbate, it is thought that the ascorbate does not influence drug release from the preparations. From the results, the possibility that the dosage form and additives controlled the drug release from the preparations was considered. The release parameters in the drug release experiment using the second fluid are described in Table 3. The decrease of AUC_{0-90} and the extension of MDT in VC-Chew and Hic-Chew were observed as compared with Hic-G, YK-Chew and VC-Tab. Since the sustained release was confirmed in VC-Chew and Hic-Chew, the influence on the drug absorption and the bioavailability was suggested. Time courses of the oxidation ratio (the ratio of the amount of dehydroascorbic acid to the amount of total vitamin C) in the first fluid and the second fluid on the release test are described in Fig. 2. In the first fluid, the oxidation ratio was about 4-7% at 120 min after the start of the release test. Since the oxidation ratio in the second fluid was about 6-12% at 120 min, the

![Fig. 1. Release profiles of total vitamin C from preparations in the first fluid of pH 1.2 (a) and in the second fluid of pH 6.8 (b)](image)

<table>
<thead>
<tr>
<th>Preparation</th>
<th>AUC_{0-90} (mean ± S.D.)</th>
<th>MDT (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hic-G</td>
<td>7666.8 ± 249.7 (^{b,c,d,e})</td>
<td>48.5 ± 0.6 (^{b,c,d,e})</td>
</tr>
<tr>
<td>VC-Tab</td>
<td>6195.4 ± 165.5 (^{c,d,e})</td>
<td>52.2 ± 1.2 (^{c,d,e})</td>
</tr>
<tr>
<td>YK-Chew</td>
<td>7914.4 ± 79.5 (^{b,d,e})</td>
<td>50.1 ± 0.3 (^{b,d,e})</td>
</tr>
<tr>
<td>VC-Chew</td>
<td>4512.4 ± 53.0 (^{c,c})</td>
<td>56.6 ± 0.2 (^{c,c})</td>
</tr>
<tr>
<td>Hic-Chew</td>
<td>4208.2 ± 96.7 (^{h,c})</td>
<td>55.4 ± 0.1 (^{h,c})</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. (n = 3-6).

a: \( p < 0.005 \) versus Hic-G
b: \( p < 0.005 \) versus VC-Tab
c: \( p < 0.005 \) versus YK-Chew
d: \( p < 0.005 \) versus VC-Chew
e: \( p < 0.005 \) versus Hic-Chew
oxidation in the neutral medium was slightly advanced as compared with the acid medium. From the results, since the oxidation of ascorbic acid in the release media increased with time, it was suggested to change into dehydroascorbic acid to some extent in the intestine after ascorbic acid preparations have been taken orally.

3. Antioxidative potency

The total antioxidant capacity in the tested medium of the drug release experiment is described in Fig. 3. The total antioxidant capacity was measured at 30 and 90 min after the start of the release test. The time-dependent changes of the antioxidant capacity were similar to that of the ascorbic acid concentration in the tested medium. The correlation between the amount of ascorbic acid and the total antioxidant capacity is described in Fig. 4. The amount of ascorbic acid was obtained by subtracting the amount of dehydroascorbic acid from the amount of total vitamin C. The determination coefficient ($R^2$) obtained by simple regression analysis indicated a strong correlation. Although the tested preparations include different additives and some preparations included calcium pantothenate or riboflavin butyric ester, the total antioxidant capacity corresponded to the amount of ascorbic acid. Hence, it is considered that antioxidative potency is influenced by the amount of ascorbic acid absorbed when ascorbic acid preparations are taken orally. Ascorbic acid is mainly absorbed by the active transport in the intestine. The bioavailability of ascorbic acid
after oral administration of 2 g of Hic-G described in the interview form prepared by Takeda Pharmaceutical Co., Ltd. is 63.3%. It is suggested that the sustained-release preparations causes the delay of absorption and influences on the bioavailability. It will be necessary to clarify if the results of in vitro dissolution test correlate to in vivo drug absorption in the future.

Conclusion

Preparations containing ascorbic acid of various dosage forms and including various components were investigated in this study. In each preparation, the content of total vitamin C corresponding to the indicated amount was confirmed. The release behavior in the first fluid and the second fluid was similar in each preparation. Hic-G and YK-Chew showed rapid drug release, while VC-Chew and Hic-Chew showed relatively sustained release. From the drug release results, it is suggested that the dosage form and additives controlled the drug release from the preparations. The ratio of oxidation in the tested medium on the drug release experiment was increased with time up to about 10% at 120 min. The total antioxidant capacity in the tested medium of the drug release experiment corresponded to the concentration of ascorbic acid. Hence, the bioavailability of ascorbic acid should influence the antioxidative potency of the preparation. Although the sustained-release preparations cause the delay of absorption and a possibility of influences on the bioavailability, the rapid drug release should be obtained when VC-Chew and Hic-Chew are ingested with chewing. Hence, it might be necessary to explain to the consumer that VC-chew and Hic-Chew should be taken with chewing. Ascorbic acid has been widely used in the pharmaceutical, cosmetic and food industry for its bioactivity and antioxidant properties. OTC preparations and dietary supplements containing ascorbic acid could be used by all generations because of their wide range of bioactivity, e.g., effect on skin and reduction in the incidence rates of some cancers. This study should contribute to effective utilization of the preparations containing ascorbic acid by clarifying the characteristics of the preparations.

Acknowledgments

The authors are grateful to Wismerll Company Ltd., Japan, for material support.

References


