Stability, transdermal penetration, and cutaneous effects of ascorbic acid and its derivatives

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Summary

Topically applied antioxidants exert their benefits by offering protection from damaging free radicals and over-the-counter cosmeceuticals incorporating antioxidants are among the most popular anti-aging products available. One potent antioxidant of particular note, vitamin C, has been extensively utilized because it possesses a variety of other cutaneous benefits including photoprotection from UV A & B, neocollagenesis, inhibition of melanogenesis and improvement of a variety of inflammatory skin disorders. However, the instability of this water-soluble vitamin, together with difficulties associated with its topical delivery, has presented issues for the formulation chemist. This article reviews the scientific data and clinical studies that underpin the stability, percutaneous absorption, and cutaneous effects of vitamin C together with its commonly utilized, commercially available derivatives.

Keywords: ascorbic acid, vitamin C, topical, photoprotection, melanogenesis, neocollagenesis

Introduction

L-Ascorbic acid (AA, 1; Scheme 1) has many important biochemical functions but is most typically identified as the primary water-soluble nonenzymic antioxidant in human tissue.1 As such much of the research on AA has focused on its role as a free-radical scavenger. Antioxidants, like AA, act by scavenging and reducing free radicals to less reactive species, thus reducing oxidative damage to critical cellular components. In this process, AA is oxidized to dehydro-L-ascorbic acid 2 which can be re-reduced to AA by the endogenous antioxidant pool or decay irreversibly to diketogulonic acid 3.

Stability and percutaneous delivery

Due to AA’s inherent hydrophilicity, penetration of AA across the skin might be expected to be poor. Nevertheless, significantly enhanced cutaneous levels of AA have been recorded after topical application.2–7 For optimal penetration of the epidermal barrier,6 aqueous formulations of AA (including its sodium salt) must be at a pH which is below the pKa of AA itself (pKa 4.2), thus reducing the charge density on the molecule. In porcine skin models, under these conditions, application of up to 20% AA in an aqueous formulation increased the underlying AA concentration 20-fold.6 The resultant tissue saturation was achieved with only 3 days of application and the half-life for AA decline was approximately 4 days.

Unfortunately, the beneficial antioxidant effect of AA also underscores the primary challenge in developing AA formulations, ensuring its stability. Oxidation of AA is triggered by its ionization in aqueous solution. Other factors that increase the rate of degradation of AA are high pH or temperature, the presence of dissolved oxygen, and catalytic amounts of metal ions.8–10 Indeed, the degradation of AA, most often accompanied by a yellowish discolouration, can proceed rapidly depending on formulation, packaging and storage conditions.11–14 Various stabilization strategies can be attempted to address the issue, such as exclusion of...
oxygen during formulation, oxygen-impermeable packaging, encapsulation, low pH, minimization of water, and inclusion of electrolytes and other antioxidants.\textsuperscript{15–17} Despite these strategies, AA stability remains a challenge, and some of these approaches can lead to cost and difficulty in formulation. A summary of the salient stability, percutaneous absorption, and therapeutic dermalogical characteristics of AA and its derivatives is given in Table 1.

Ascorbyl 2-phosphates, usually formulated as their sodium (SAP) and magnesium (MAP; 4) salts, are stable in solution at ~pH 7. Introduction of the phosphate group in the second position of the cyclic ring protects the enediol system of the molecule from oxidation; ascorbyl phosphate salts are therefore not in themselves antioxidant agents.\textsuperscript{13} As a consequence, their effectiveness depends upon their conversion \textit{in vivo} to AA (presumably accomplished by an alkaline phosphatase). Confirmation of trans-dermal penetration and conversion to AA has been demonstrated for MAP.\textsuperscript{18} Although the stability of the ascorbyl phosphate salts is significantly greater than for AA, with their increased charge density, skin penetration presents an even greater challenge. Indeed, it has been demonstrated that topically applied ascorbyl phosphate salts are, at very best, poorly absorbed in comparison with AA.\textsuperscript{3,6} In one study, SAP was not observed to penetrate the epidermis.\textsuperscript{18} In another experiment, only AA and not MAP improved skin barrier function \textit{in vivo} suggesting a difference in rates of skin penetration.\textsuperscript{19} However, skin treatment with lasers, microdermabrasion, or by sonophoresis has been shown to dramatically improve topical delivery of both vitamin C and derivatives that normally demonstrate poor dermal penetration.\textsuperscript{20–22}

The most frequently commercially utilized nonsalt derivatives of AA such as ascorbyl 6-palmitate\textsuperscript{23,24} (AA-Pal; 5), ascorbyl 2-glucoside\textsuperscript{25} (AA-2G; 6), and tetra-isopalmitoyl ascorbate\textsuperscript{26,27} (VC-IP; 7) to a greater or lesser extent all demonstrate superior stability and ease of formulation to AA. Unlike the ascorbyl phosphate salts, AA-2G and VC-IP (which are all modified at the carbon 2 of the cyclic ring), the chemical modification of AA-Pal is located on carbon 6 and as such provides no protection to AA from oxidative degradation. AA-Pal is therefore less stable than these other forms in solutions and topical formulations. The stability of AA-Pal over AA therefore depends largely on the structural properties of the formulations in which it is incorporated.\textsuperscript{13,21}
With the addition of a lipid-soluble group, it might be expected that AA-Pal should provide a marked increase in AA levels in the skin compared to topically applied AA alone. However, one study has shown that topical application of AA-Pal did not increase skin levels of AA. Although AA-Pal appears to readily enter the skin, and microemulsions were able to deliver AA-Pal to the skin, it is possible that its conversion to AA may be limited.

However, this observation is incongruous with those made with a similar derivative, ascorbic 2-phosphate 6-palmitate (APPS, 8). APPS has been shown to penetrate the skin and appears it can be converted to ascorbic acid after delivery. Nonetheless, like SAP, the ability of APPS to penetrate the skin by topical application does seem very limited.

In contrast to AA-Pal, the stable AA derivative AA-2G is hydrolyzed by cellular ζ-glucosidase to release AA. Topically applied AA-2G has also been shown to exhibit the physiological effects of AA, but the rate of penetration and in vivo conversion to AA has not yet been determined. In comparison, and possibly unsurprisingly, the permeability of the lipophilic derivative 3-O-ethyl ascorbate (EAC) appears to be greater than for AA-2G. The 3-alkyl-ascorbic acids like EAC have been tested as antioxidants and were found to be potent chain-breaking agents with a high affinity for biomembranes.

The other stable AA analog used in cosmeceutical formulation is VC-IP in which four isopalmitic acid esters are located at the 2, 3, 5, and 6 carbon positions. When topically applied, it exhibited the physiological effects of AA which leads to the suggestion that, like AA-2G, it both penetrates the dermis and is converted in vivo to AA. In addition, in reconstructed skin models, VC-IP does penetrate and is converted efficiently to AA. However, a trade publication suggests percutaneous absorption on excised human skin (on a molar

### Table 1
Summary of the stability, percutaneous absorption, and therapeutic characteristics of AA and its derivatives.

<table>
<thead>
<tr>
<th></th>
<th>Ascorbic acid (AA)</th>
<th>Sodium ascorbyl phosphate (SAP)</th>
<th>Magnesium ascorbyl phosphate (MAP)</th>
<th>Ascorbyl palmitate (AA-PAL)</th>
<th>Ascorbyl tetraso-palmitate (VC-IP)</th>
<th>Ascorbyl glucose 6-palmitate (AA-2G)</th>
<th>Ascorbyl 2-phosphate 6-palmitate (APPS)</th>
<th>3-O-Ethyl ascorbate (EAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stability</td>
<td>If pH &lt; 3.5 in aqueous solutions; Anhydrous</td>
<td>Yes at pH7</td>
<td>Yes at pH7</td>
<td>Similar to AA</td>
<td>Yes pH &lt; 5</td>
<td>Yes at pH7</td>
<td>Yes at pH7</td>
<td>No data</td>
</tr>
<tr>
<td>Percutaneous absorption</td>
<td>Yes Human ex vivo (as solution or microparticles)</td>
<td>Yes Animal ex vivo (but limited)</td>
<td>Yes Animal ex vivo (but limited)</td>
<td>Yes Animal in vivo (formulation dependent)</td>
<td>Yes Human ex vivo &gt; MAP (trade publication)</td>
<td>Yes Animal in vivo</td>
<td>Yes Animal in vivo</td>
<td>Yes Animal ex vivo &gt; AA-2G</td>
</tr>
<tr>
<td>Conversion to AA</td>
<td>N/A No data</td>
<td>Yes in vitro</td>
<td>No data</td>
<td>Yes in vitro</td>
<td>Yes in vitro</td>
<td>Yes in vitro</td>
<td>No data No data</td>
<td>No data No data</td>
</tr>
<tr>
<td>Photoprotection</td>
<td>Yes Human in vivo</td>
<td>Yes Human in vivo &lt; AA</td>
<td>No data</td>
<td>Yes Animal in vivo</td>
<td>Yes in vitro data</td>
<td>Yes Human in vivo &lt; SAP</td>
<td>No data No data</td>
<td>No data No data</td>
</tr>
<tr>
<td>Cutaneous neocollagenesis</td>
<td>Yes Human in vivo</td>
<td>Yes in vitro &lt; MAP</td>
<td>Yes in vitro</td>
<td>Yes in vitro</td>
<td>Yes in vitro</td>
<td>Yes in vitro</td>
<td>No data No data</td>
<td>No data No data</td>
</tr>
<tr>
<td>Inhibition of melanogenesis</td>
<td>Yes Human in vivo</td>
<td>Yes Human in vivo (trade publication)</td>
<td>No data</td>
<td>Yes Human in vivo</td>
<td>Yes in vitro (trade publication)</td>
<td>Yes Human in vivo</td>
<td>Yes Human in vivo</td>
<td>Yes Human in vivo</td>
</tr>
</tbody>
</table>
Photoprotection

Among the many important biochemical functions of AA, it is its potent antioxidant ability which is principally acknowledged. The generation of reactive oxygen species and other free radicals by normal cellular processes, UV irradiation and pollutants are associated with carcinogenesis and aging. In this regard, several clinical studies showing the benefits of topically applied vitamin C against the biochemical and physically identifiable processes of aging due to oxidative stress have recently been reviewed.\(^7,37\)

As noted earlier, the topical application of AA has been shown to significantly elevate cutaneous levels of this vitamin, and this correlates with protection of the skin from UVB-induced oxidative damage as measured by a decrease in UVB erythema and sunburn cell formation.\(^3,38,39\) However, as already suggested, AA does not act independently of other cellular processes in its role as an antioxidant. Importantly AA is also capable of recycling photo-oxidized \(\alpha\)-tocopherol thereby regenerating vitamin E and contributing to an antioxidant reservoir in the skin.\(^40\) AA therefore not only acts through scavenging reactive oxygen species itself but also potentiates the antioxidant activity of \(\alpha\)-tocopherol for protection against free-radical damage.\(^31-43\) In fact, topical AA combined with \(\alpha\)-tocopherol provided notably increased photoprotection against UV-induced erythema and diminished the number of sunburn cells as compared to that seen for each antioxidant individually\(^39\) which underlines the synergistic effect, particularly in protection against UV-induced oxidative damage. The presence of \(\alpha\)-tocopherol in formulations may also have a stabilizing effect on the photo-degradation of ascorbic acid.\(^44\)

Like AA, AA-Pal is itself biologically active. It can directly generate the ascorbyl radical, an action that also drives the radical scavenging process by AA.\(^45\) However, topical treatment with AA-Pal was not as effective as AA in providing protection against UVB radiation exposure in mouse models.\(^28\) although when applied to porcine skin did provide protection against ultraviolet-induced free radicals.\(^29\) However, another study in human keratinocyte cell culture has suggested that despite its antioxidant properties, AA-Pal may potentiate skin damage following UVB irradiation.\(^46,47\)

The protective effect of SAP on UVB-induced cutaneous damage in mouse skin \textit{in vitro} is due to the maintenance of a normal AA level by conversion of SAP to AA in skin tissue.\(^48\) However, topically applied SAP was less effective than identically applied AA in reducing oxidative stress in human skin \textit{in vivo}.\(^11\) Nonetheless, MAP did repress mortality of UVB irradiated mouse keratinocytes \textit{in vitro} more markedly than AA.\(^49\) Keratinocytes are known to actively uptake vitamin C and possess efficient systems to maintain high intracellular levels of vitamin C.\(^50\) The apparent difference between the \textit{in vitro} and \textit{ex vivo} effects of ascorbyl phosphate salts may therefore further underscore the difficulty associated with their dermal penetration.

AA-2G reduced UV-induced damage of human skin keratinocytes and fibroblasts more effectively than ascorbyl 2-phosphate\(^51\) and demonstrated a photoprotective effect against UVB.\(^41\) VC-IP was found to suppress the elevation of intracellular peroxide after UVB irradiation and provide enhanced cellular tolerance against UVB and reactive oxygen species.\(^57\) It also showed antioxidant activity comparable with retinyl palmitate and tocopheryl acetate in an \textit{in vitro} chemiluminescence assay.\(^52\)

Neocollagenesis

Apart from its role as a potent antioxidant, it has also been demonstrated that AA functions as an essential cofactor for the enzymes lysyl hydroxylase and prolyl hydroxylase both of which are required for the post-translational processing of types I and III collagen. Because AA stimulates collagen production in the dermis (Fig. 1),\(^53-55\) and can cause a dramatic increase in fibroblast proliferation potentially resulting in greater collagen production,\(^56\) it might be presumed that AA possesses the potential to also increase collagen production for wrinkle appearance reduction.

AA-2G increased collagen production on normal human fibroblasts \textit{in vitro} to a significantly lesser extent than either ascorbyl 2-phosphate or AA, but did enhance three-dimensional collagen mesh reorganization at a lower concentration.\(^57\) However, an alternative study demonstrated that AA-2G successfully stimulated collagen synthesis in cultured human fibroblasts with an effectiveness comparable to that of AA.\(^15\) while VC-IP is purported to increase collagen synthesis greater than twofold over AA at the same dosage.\(^26\) In other cultured human fibroblast experiments, MAP was found to be equivalent to AA in stimulating collagen synthesis, while SAP required at least a tenfold greater concentration to produce the same effect as AA.\(^58\)
AA-Pal augmented human intestinal smooth muscle cells procollagen synthesis *in vitro* more effectively than AA but induced similar increases in the amounts of newly synthesized collagen secreted into the medium.\(^{59}\)

AA also plays a role in collagen synthesis at the level of gene expression.\(^{60,61}\) It has been shown to up-regulate collagen synthesis and increase the synthesis of the inhibitor of metalloproteinase-I\(^{62}\) which decreases UV-induced collagen degradation.

**Inhibition of melanogenesis**

Finally, AA has been shown to suppress melanin production by reducing orthoquinones (such as dopaquione) so that tyrosinase-dependent melanin formation is prevented.\(^{63}\) In this way, AA has proven to be useful for skin depigmentation.\(^{64}\) In the only comparative study available, the melanin synthesis in B 16 melanoma cells was inhibited more by AA-2G than by ascorbyl 2-phosphate.\(^{51}\) That these AA derivatives can be transformed into the active antioxidant is the most likely basis for their ability to suppress melanin biosynthesis. It is therefore not surprising that clinical effects have also been noted for MAP,\(^{64-67}\) VC-IP,\(^{26,27}\) APPS,\(^{68}\) and EAC,\(^{69}\) although there is no comparative data for these compounds.

**Conclusions and outlook**

Ascorbic acid and its derivatives have been ascribed antiaging effects in several cosmetic studies in which overall reduced facial photoaging (Fig. 2), improved skin texture (Fig. 3), and improvement in skin collagen and elastin have been well documented.\(^{11,52,70,71}\) Fundamental to these observations are the neocollagenic, skin lightening, and antioxidant properties of AA for which there is substantial evidence.

The instability and aqueous solubility of AA has historically presented issues for formulation. Nevertheless, the current understanding of AA now enables formulation for more than acceptable transdermal penetration. Unfortunately, it seems, many products in the market still fail even the most rudimentary analysis (e.g. rapid discoloration). However, the advent of chemically
modified analogs of AA has also helped to improve this formulation issue and, with the exception of AA-Pal, the oxidative stability of AA has been greatly improved. Still, on current available evidence in the scientific literature, the effective transdermal penetration of these analogs (especially the phosphate analogs), their conversion to active AA and, ultimately, their comparative clinical effectiveness still needs to be fully ascertained. These concerns aside, cosmeceuticals containing appropriately formulated AA or its analogs for topical application should play an essential role in the treatment of aging skin.

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