Chapter

Vitamin C, Aged Skin, Skin Health

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Abstract

Vitamin C is an essential nutriment for humans. Vitamin C is known for its antioxidant potential. Vitamin C acts as a potent water-soluble antioxidant in biological fluids. Thus, topical vitamin C will not only reduce the risks of development of photoaging but also could reduce the risk of carcinogenesis. In addition to its antioxidant properties, vitamin C is essential for collagen synthesis. Vitamin C stimulates or restores several mechanisms which are either deficient or disturbed. Topical application of vitamin C partially restores the anatomical structure of the epidermal-dermal junction in young skin. A clinical trial confirmed for the first time that topical application of 5% vitamin C over a period of 6 months significantly improves the clinical appearance of photodamaged skin when compared to the vehicle alone. In inflammatory skin diseases, that is, atopic dermatitis and psoriasis, vitamin C levels into the dermis are reduced. Moreover, a randomized double-blind comparative study conducted in patients with Bateman purpura showed a significant improvement that vitamin C is probably one of the main topical anti-aging agents. In addition, the use of photo-protective sunscreen after UV irradiation prevents the decrease of acid ascorbic dermis concentration. Indeed, the ingestion of vitamin C has different benefits on skin such as wound healing, cutaneous aging, and prevention of skin cancer.

Keywords: vitamin C, dermatoporosis, Bateman purpura, wound healing, collagen, healing

1. Introduction

Vitamin C is an essential nutriment for humans. The name “ascorbic” is derived from ascorbutic (scorbutus: scurvy). This disease was described by the ancient Egyptians, Greeks, and Romans and during the crusade in the thirteenth century. Its deficiency is responsible for scurvy. It is characterized by altered functions of the connective tissue, such as perifollicular hemorrhages and defective healing. In the eighteenth century, Lind, a British naval surgeon, found that ingestion of lemon juice cured this disease [1]. Since the discovery in the 1930s that vitamin C is the antiscorbutic factor, much works have been undertaken to elucidate its mechanisms of action. L-enantiomer is the most bioactive form of ascorbic acid. It is natural compound produced in most plants and animals; however, humans lack the enzyme L-gulono-gamma-lactone oxidase, which is necessary to produce it. Vitamin C is an alpha-ketolactone [2]. Humans are one of the few species that require dietary supplementation of ascorbic acid for survival.

2. Photo aging

Bearing in mind the mechanisms of photo damages, the beneficial role of vitamin C or L-ascorbic acid has been raised. Thus, vitamin C is the major aqueous phase antioxidant agent in humans. In addition to its antioxidant properties, vitamin C is essential for collagen synthesis. Vitamin C stimulates or restores several mechanisms, which are either deficient or disturbed.

The environmental damage, particularly ultraviolet irradiation (UV), induces human skin aging or photoaging. Indeed, 80% of facial aging is believed to be due to chronic sun exposure [3]. In addition, it is well known that UV from the sun has deleterious effects in human skin, including sunburn, immune suppression, and cancer [4, 5]. UVA radiation (λ, 320–400 nm) and infrared and even visible light may produce skin damage like a UVB radiation (λ, 290–320 nm) [6]. However, erythema, aging, and carcinogenesis are still assigned mainly to UVB and UVA [7]. The UV irradiation like other several exogenous (pollution, stress, and smoking) and endogenous factors (normal metabolic processes) invokes a complex sequence of specific molecular responses that damage skin connective tissue, particularly production of reactive oxygen species (ROS).

The photochronological generation of ROS is a primary mechanism by which UV irradiation initiates molecular responses in human skin. These ROS contain superoxide anion, peroxide, and singlet oxygen. Further, some evidence suggests that free radicals induce alterations in gene expression pathways, which in turn contribute to the degradation of collagen and the accumulation of elastin emblematic of photoaged skin [8]. The ROS activate cell surface receptors such as epidermal growth factor, interleukin (IL1), insulin, keratinocytes growth factor, and tumor necrosis factor (TNF α) *in vivo.*

The UV irradiation actives NADPH oxidase that is responsible for generating hydrogen peroxide too. The NADPH oxidase catalyzes the reduction of molecular oxygen to superoxide anion [9]. The hydrogen peroxide is distinct from photochemical generation of ROS, which occurs only during UV exposure and abates following UV exposure. Hydrogen peroxide is less damaging to cells and can be converted to other ROS, such as hydroxyl radical and singlet oxygen [9]. It is known that UV irradiation significantly upregulates the synthesis of several types of collagen-degrading enzymes known as matrix metalloproteinases (MMPs). The UV irradiation activates protein kinase-mediated signaling pathways. The activated kinase upregulate expression and functional nuclear transcription factor, AP-1. It follows the stimulation of transcription of genes for matrix-degrading enzymes, such as metalloproteinase (MMP) 1 (collagenase), MMP-3 (stromelysin 1), and MMP-9 (92 Kd gelatinase) [10, 11]. The UV-induced MMP-1 initiates cleavage of fibrillar collagen (type I and III in skin) at a single site within its central triple helix. This collagen can be further degraded by elevated levels of MMP-3 and MMP-9 [12]. The MMP induced by UV degrades skin collagen, disrupting the structural architecture of dermis. In addition, UV irradiation alters the synthesis of collagen, primarily through downregulation of type I and type III pro-collagen gene expression [13]. Collagen I is the most abundant protein in skin, and type I and type III collagen fibrils provide strength and resiliency to skin. Photoaged skin contains an abundance of degraded, disorganized collagen fibrils and has a reduced production of type I and type III pro-collagen. Fibroblasts are elongated and collapsed [14].

Alterations in elastic fibers are so strongly associated with photoaged skin that "elastosis," an accumulation of amorphous elastin material, is considered pathognomonic of photoaged skin. The UV irradiation induces a thickening and coiling of elastic fibers in the papillary dermis.

In addition, other alterations of many important structural components of the dermal extra cellular matrix are observed such as modifications in the structure and composition of anchoring fibrins, proteoglycans, and glycoaminoglycans [15, 16].

It is well known that skin pigment provides a significant degree of protection against actinic damage. Therefore, in lighter skin color after UV irradiation, erythema is greater and tanning is less in darker skin pigment. In addition, the effect of skin color on UV-induced MMP-1 gene expression and formation of thymine dimmers were investigated in human skin *in vivo*. UVB and UVA exposure resulted in substantial induction of MMP-1 mRNA and formation of thymine dimmers in lightly pigmented subjects group. In contrast, twice the average exposure of the lightly pigmented group produced only modest MMP-1 mRNA induction or DNA damage in the darkly pigmented group [9].

Skin photoaged induces variable changes that affect the sun-exposed areas (face, neck, forearms, and dorsum of hands), such as deep wrinkles not erased by stretching, roughness, yellow hue, leathery appearance [17–19], atrophy and laxity, a focal depigmentation (guttate hypomelanosis) and/or hyperpigmentary changes (bronzing, ephelides, and actinic lentigines), purpura, telangiectasia or venous lakes, comedoes (Favre-Raccouchot disease), and a neoformation on photoaged skin (benign and premalignant melanocytic, epithelial neoformation, and basal and squamous cell carcinomas). These aspects change among individuals. Degree of photoaging is great in individuals who have outdoor life styles, live in sunny climates, and are lightly phototype [20]. Although wrinkles can be found almost everywhere in an aged skin, they develop preferentially on photo-exposed areas and are thus largely visible by others. Little is known even about the pathogenesis of wrinkles [21]. In fact, skin laxity, due to decreased volumes of the inner-layers, and loss of intra-dermal tensional strength are presumed to play a major role in wrinkling [22]. In addition, it is claimed that the decreased dermal area compared to epidermis area, due to the disappearance of the dermal papillae and the weaker attachment by an altered dermo-epidermal junction (DEJ), is one of the physical prerequisites that allow wrinkle formation. The *stratum corneum* of the wrinkle is said to be thickened by an accumulation of corneocytes, forming a real horny plug at the bottom of the wrinkle. The spinous layer of a wrinkle was shown to be thinner at the base than at the flanks [23], and fewer keratohyaline granules are present in the wrinkle base as compared to the flanks. Furthermore, filaggrin decreases at the bottom of wrinkle. DEJ has also been shown to contain less collagen IV and VII at the bottom of the wrinkle than in its flanks or in the surrounding skin. Concerning dermis, Tsuji et al. earlier have reported [24] a decrease of actinic damage at the bottom of wrinkle compared to its sides or adjacent skin; this shows an accumulation of highly damaged elastotic material. In addition, oxytalan fibers have nevertheless been reported to be sparse or absent at the bottom of the wrinkle, and chondroitin sulphate GAGs have also been shown to be reduced under the wrinkles.

3. Vitamin C (ascorbate): the main potent antioxidant vitamin

Vitamin is known for its antioxidant potential. Vitamin C acts as a potent water-soluble antioxidant in biological fluids by scavenging physiologically relevant reactive oxygen species and reactive nitrogen species. It is the main water soluble nonenzymatic antioxidant in aqueous compartments. It does not absorb light in the UVB and UVA range, but it exerts its effects by neutralizing oxygen free radicals (superoxide, hydroxyl and water soluble peroxyl radical, singlet

oxygen, and hypochlorous acid) [25], as well as nonradical species such as hypochlorous acid, ozone, singlet oxygen, nitrosating species, nitroxide, and peroxynitrite. It is acknowledged that vitamin C works in extracellular fluids, as blood plasma and respiratory tract lining fluid. Low vitamin C blood levels are associated with enhanced oxidative stress, specifically reported in smoking and inflammatory diseases as rheumatoid arthritis and adult respiratory distress syndrome. In addition, vitamin C attenuates oxidative damage by generating small antioxidant molecules, such as α-tocopherol, glutathione, urate, and β-carotene. α-tocopherol may be regenerated at the expense of vitamin C at the interface between a lipid membrane and aqueous phase and therefore is protected from peroxidation. The mode of interaction between vitamin C and α-tocopherol related to antioxidant capacities is complex, depending on the *in vivo* context.

Vitamin C is an effective donor antioxidant, and its active form is ascorbate (reduced form). It loses electrons in antioxidant reactions and becomes oxidized to the short lived ascorbyl radical. The ascorbyl radical is relatively unreactive due to resonance stabilization of the unpaired electron; it readily dismutes ascorbate and dehydroascorbic acid (DHA) by NADH-dependent semidehydroascorbate reductase. DHA can be recycled back to ascorbate by NADPH-dependent thioredoxin reductase or glutathione-dependent DHA reductase.

Vitamin C plays a crucial role in various hydroxylation reactions. It acts as a cofactor in the enzymatic synthesis of collagen, carnitine, catecholamine, and neurohormones. As a cofactor for hydroxylase and oxygenase metalloenzymes, vitamin C is thought to work by reducing the active metal site, resulting in reactivation of the metal-enzyme complex or by acting as a co-substrate involved in the reduction of molecular oxygen. Vitamin C uses iron and copper as cofactors, and it enhances intestinal iron absorption. The reduction of iron by vitamin C is involved in the enhanced dietary absorption of nonheme iron.

It should be noted, however, that ascorbate might also act as a pro-oxidant. Hydroxyl radicals and lipid alkoxyl radicals can be generated in the presence of reduced metal ions with hydrogen peroxide and lipid hydroperoxides. These interactions involve redox reactions called the Fenton chemistry. Although this Fenton chemistry occurs easily *in vitro*, its relevance *in vivo* is still a matter of controversy. The effects of ascorbate depend on the *in viv*o availability of catalytic metal ions. In healthy individuals, iron is largely sequestered by iron binding proteins such as transferrin, ferritin, and ceruloplasmin. Instead of this, during tissue injury, metal ions may be released and could interact with ascorbate. Therefore, the ability of vitamin C to modulate metal ion metabolism will be discussed.

Fenton reaction mediated by vitamin C. (1) Vitamin C reduces ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}). (2) Ferrous ion reacts with oxygen to produce superoxide. (3) Dismutation of superoxide leads to hydrogen peroxide. (4) Hydrogen peroxide reacts with ferrous ions to form hydroxyl radicals.

1. $Fe^{3+} + AscH_2 \rightarrow Fe^{2+} + Asc^{-} + 2H^{+}$

2. $Fe^{2+} + O_2 \rightarrow Fe^{3+} + O_2^{-}$

3. $2O_2^{-} + 2H^{+} \rightarrow H_2O_2 + O_2$

4. $H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$

4. Effects of vitamin C on the skin

In addition to its antioxidants properties, vitamin C and some water-soluble derivatives are essential for collagen biosynthesis [26]. In fibroblasts cultures, vitamin C stimulates collagen biosynthesis. Its absence results in structurally unstable collagen, which is not secreted from cells at a normal rate [27]. The role of vitamin C in the hydroxylation of collagen molecules is well characterized [28]. In fact, ascorbic acid is an essential cofactor for the enzyme, prolyl, and lysyl hydroxylases, catalyzing the synthesis of hydroxyproline and hydroxylysine in collagen. Hydroxyproline acts to stabilize the collagen triple helix. Its absence results in structurally unstable collagen, which is not secreted from cells at a normal rate. Hydroxylysine is necessary for cross-linking one collagen molecule to another, providing tissue strength [29, 30]. This occurs concurrently with a decrease in elastin production, and the elastin protein is often overproduced in response to photodamage [31]. Vitamin C also increases the proliferation rate of fibroblasts, a capacity that is decreased with age [32].

Specifically, vitamin C has been shown to stabilize collagen mRNA, thus increasing collagen protein synthesis for repair of the damaged skin [33]. Further, vitamin C stimulates DNA repair in cultured fibroblasts [34].

The epidermal dermal junction of aged and photoaged skin is flattened due to the loss of rete ridges and the disappearance of papillary projections. In extremely aged skin, papillae virtually disappear, and the junction with the atrophic epidermis is a straight line versus undulations in younger skin with a thin papillary dermis along with a loss of capillaries. The corneocytes in aged skin become larger as a result of decreased epidermal turn over.

Topical application of vitamin C partially restores the anatomical structure of the epidermal-dermal junction in young skin and increases the number of nutritive capillary loops in the papillary dermis close to the epidermal tissue in the aged skin of postmenopausal women. The increase of the density of papillae after vitamin C treatment is linked to a restructuring of the papillary dermis, as the top of the newly formed papillae and the capillaries are localized above the average height of the plane basal layer seen predominantly in aged skin. Moreover, new blood vessels are formed during the treatment with vitamin C. These vessels show a normal anatomical structure in confocal microscopical examination and are apparently integrated in a healthy vascular architecture. The mechanism, by which VC restores dermal papillae, is unknown. These results suggest that topical vitamin C may have important antiaging effects in correcting the structural and functional losses associated with skin aging [35].

Vitamin C protects keratinocytes from the damage produced by ultraviolet A [36]. Vitamin C transport proteins are increased in keratinocytes in response to UV light, suggesting an increased need for vitamin C uptake for adequate protection [37, 38]. UV light decreases vitamin C content of skin, an effect that is dependent on the intensity and duration of UV exposure [39–42]. In cultured keratinocytes, the addition of vitamin C reduces UV-related DNA damage and lipid peroxidation, limits the release of pro-inflammatory cytokines, and protects against apoptosis [36–42]. Vitamin C also modulates redox-sensitive cell signaling in cultured skin cells and consequently increases cell survival following UV exposure [43, 44].

4.1 Systemic administration of vitamin C

Vitamin C is important to the body, including skin. Therefore, as little as 10 mg per day is necessary to prevent scurvy. In the United States, the current

recommended daily allowance (RDA) is 60 mg. This rate increases for smokers and pregnant or lasting women. The supplementation of vitamin C has beneficial effects in human body, such as wound healing, prevention of cancer, cataract prevention, and atherosclerosis, and enhances immune mechanisms [45]. The saturated body store of vitamin C is approximately 20 mg/kg of body weight, corresponding to 0.9 mg/dl of a plasma ascorbate level [46]. The half-life of this vitamin is 10–20 days and is dependent on plasma levels [46]. In case of total deficiency, the organism reserves depleted approximately after 4 weeks. The cutaneous levels of vitamin C were well determined [47]. Ascorbate is distributed in all layers of the skin. However, in the epidermis, level of vitamin C is more than five times the level in the dermis (3.8 μmol/g skin versus 0.72 μmol/g skin). Those differences reflect the role of antioxidants in the epidermis and dermis.

The effect of aging on antioxidant capacity in murine skin is well known [39]. With age, the antioxidant activity in skin decreases, and UV enhances this phenomenon [48].

Vitamin C from oral supplements appears to accumulate in the skin, reducing the basal content of malonaldehyde and an oxidation residue of lipids. Oral supplementation with vitamin C (500 mg/day) has shown no evidence for an effect of the vitamin C on UVR-induced oxidative stress [49]. Fuchs and Kern have shown that a very high supplementation dose of 3 grams vitamin C daily increases significantly the minimal erythema dose [50].

Two observational studies found that higher intakes of vitamin C from the diet were associated with better skin appearance, with notable decreases in skin wrinkling [51, 52].

Many studies have proved the synergistic interaction of vitamin C and vitamin E (D-alpha-tocopherol) in antioxidant defense and in decrease of sunburn reaction. UV induces a chain reaction of lipid peroxidation in membranes rich in polyunsaturated fatty acids. Vitamin E protects the membranes by stopping the propagation reactions of lipid peroxyl radicals, while ascorbic acid simultaneously recycles alpha-tocopherol.

Vitamin C is also required to form competent barrier lipids in the epidermis [53] by stimulating the synthesis of ceramides. It has also been shown to stimulate the barrier function of the endothelial cells [54].

Besides, Vitamin C supplementation has been reported to inhibit skin, nerve, and lung and kidney carcinogenesis. Vitamin C has been shown to inhibit tumor cell carcinogen-induced DNA damage [55].

Its beneficial activity as a photo protectant [56] and anticancer agent [57] has been demonstrated by dietary supplementation in humans and in animal species even in those that can synthesize the vitamin.

5. Local vitamin C

Aging causes a decline in vitamin C content in both the epidermis and dermis [58, 59], and it has been demonstrated a correlation between decreasing ascorbic acid dermis level and age: vitamin C concentration is higher by young women [59]. Leveque et al. have shown that there is a direct relationship between iron and vitamin C concentrations in the human dermis and aging. Therefore, the level of vitamin C decreases linearly with age [60]. Moreover, the vitamin C concentration skin decreases after exposure to UV lights [61]. In addition, it was demonstrated that the use of photoprotective sunscreen after UV irradiation prevents the decrease of acid ascorbic dermis concentration [61]. Since sun exposure induces reduction in vitamin C levels in the dermis, it appears interesting to bring to the skin topical

vitamin C in such cases. Thus, topical vitamin C will reduce the risks of development of photoaging but also could reduce the risk of carcinogenesis. Animal experiments have demonstrated the UV photoprotective effect of topically applied vitamin C. Darr et al. [62] showed, in porcine model, that 10% aqueous vitamin C that was applied several times to the animal's skin, which was irradiated with 400 mJ/cm^2, reduced UV-induced skin photoinjury (erythema and sunburn cell formation) via its antioxidant potential. In the hairless mouse model, Bissett et al. [63] showed that a solution of ascorbic acid (5%) applied 2 hours before exposure and reduced chronic skin damage from UVB and UVA.

An *in vitro* study realized by Boxman et al. [64] used the heat shock protein HSP 27 as a sensitive marker of skin irritation or cellular stress in reconstructed skin. Stress (exposure to sodium lauryl sulphate or UV light) generated, in reconstructed skin, relocalization of HSP 27 from the cytoplasm to the cell nucleus in the absence of vitamin C and no relocalization in presence of vitamin C. In this study, they suggested that vitamin C may control the response to exterior stress in reconstructed skin. Topical application of ascorbate exerts a protective effect on the inflammatory response to sun exposure and on the UV immunosuppression. Perricone [65] has shown that ascorbyl palmitate, applied after UV burning, reduced redness 50% sooner than areas on the same patient which were left untreated. In mice's study, Steenvoorden [66] demonstrated that a single topical application of vitamin C at 0.5–5 $\mu mol/cm^2$ protected the skin against UV-induced systemic immunosuppression. Many studies have proved the synergistic interaction of vitamin C and vitamin E (D-alpha-tocopherol) in antioxidant defense [67] and in the decrease of sunburn reaction.

UV induces a chain reaction of lipid peroxidation in membranes rich in polyunsaturated fatty acids. Alpha-tocopherol protects the membranes by stopping the propagation reactions of lipid peroxyl radicals, while ascorbic acid simultaneously recycles alpha-tocopherol.

The study led by Lin et al. [68] evaluated if a combination of topical vitamins C and E is better for UV protection of skin than an equivalent concentration of topical vitamin C or E alone. Results showed that the individual ingredients were associated with a twofold increase in the antioxidant protection factor compared with vehicle control, while the association of L-ascorbic acid and alpha-tocopherol produced a fourfold increase in the antioxidant protection factor. Another study [69, 70] showed that the addition of ferulic acid, an antioxidant, improves the chemical stability of vitamin C and E and produces a further doubling of the photoprotective effect (from four to eightfold).

On the basis of *in vitro* and *in vivo* studies, it has been postulated but also proven that vitamin C could be used topically for prevention and correction of skin aging. Lots of results show an improvement of the clinical appearance of photoaged skin with vitamin C topical application. Photoprotective properties of topically applied vitamin C have thus placed this molecule as a potential candidate for use in prevention and treatment of skin aging.

Vitamin C can reverse the ROS (reactive oxygen species)-induced skin damage. An *in vivo* antioxidant capacity of vitamin C was demonstrated after a week of topical application. Topical application of vitamin C before sun exposure limits the UV-induced oxidant stress.

The histological pattern of aging skin is the flattening of the dermal-epidermal junction which is accompanied by a reduction of the density of papillae in the dermal-epidermal transition zone [71]. In *in vivo* study, Sauermann et al. [72] determined the effect of a topical cream containing 3% vitamin C against the excipient alone using daily applications for 4 months on the forearm of 33 women. They showed with the confocal laser scanning microscopy method that topical

vitamin C may have therapeutically effects for partial corrections of the regressive structural changes with the aging process notably to enhance the density of dermal papillae, perhaps through the mechanism of angiogenesis.

Kameyama et al. [73] have studied the effect of a stable derivative of ascorbic acid and magnesium-ascorbyl-2-phosphate (MAP) on melanogenesis *in vitro* and *in vivo*. They showed that the topical application of MAP is effective in lightening the skin of some patients with hyperpigmentation disorders (melasma or solar lentigines). This vitamin C derivative suppresses melanin formation by influence of tyrosinase and melanoma cells.

Few studies [52, 74] have shown the role of ascorbate in the formation of *stratum corneum* barrier lipids. It seems that ascorbate normalizes epidermal lipid profiles, in particular glucosphingolipids and ceramides.

Lots of studies [75–77] have shown an improvement of the clinical appearance of photoaged skin with vitamin C topical application. Humbert et al. [78] evaluated the clinical effects and the modifications of skin relief and structure over a 6 month period of use of a cream containing 5% vitamin C on photoaged skin. They found modifications of the skin relief (wrinkles, roughness, and skin elasticity) and the skin ultrastructure, suggesting a positive influence of topical vitamin C on parameters characteristic for sun-induced skin aging.

A double-blind randomized trial has been performed to evaluate the clinical effects and the modifications of skin relief and structure over a 6-month period of use of a cream containing 5% vitamin C on photoaged skin. The aim of the present study was to evaluate the effect on the dermal cells of vitamin C administrated by topical application on the skin of normal human volunteers by measuring the steady state level of the mRNAs of procollagens, their post-translational processing enzymes, the fibrillar structures-associated proteoglycan, decorin, the main components of the elastic fibers, elastin, and fibrillins 1 and 2, and the enzymes involved in the degradation of these matrix components. Such investigations performed on small biopsies were made possible by the use of quantitative RT-PCR controlled by original newly created internal standards of synthetic RNA. The mRNAs were measured by RT-PCR made quantitative by simultaneous amplification of internal standards of synthetic RNA.

Clinical assessments included evaluation at the beginning and after 3 and 6 months of daily treatment performed by the investigator and volunteer self-assessment. Skin relief parameters were determined on silicone rubber replicas performed at the same time points. Cutaneous biopsies were obtained at the end of the trial and investigated using immunohistochemistry and electron microscopy. Clinical examination by a dermatologist as well as self-assessment by the volunteers disclosed a significant improvement, in terms of "aging score," of the vitamin C–treated side versus control. A highly significant increase in the density of skin microrelief and a decrease of the deep furrows were demonstrated.

The results of this clinical trial confirmed for the first time based on a randomized controlled blind study that topical application of 5% vitamin C over a period of 6 months significantly improves the clinical appearance of photodamaged skin when compared to the vehicle alone. Significant favorable modifications of skin relief were induced, leading to the reappearance of an isotropic surface pattern. Significant reduction in small and coarse wrinkles and improvement in the overall aspect of the skin assessed by the volunteers were observed after 6 months of daily treatment. But such evolution of skin relief was already noticed 3 months after the beginning of treatment.

For the first time, this study disclosed fibroblast effects of topical vitamin C, with ultrastructural evidence of the elastic tissue to be repaired. Indeed 10 patients accepted a skin biopsy on their forearms, each being treated with either the vitamin

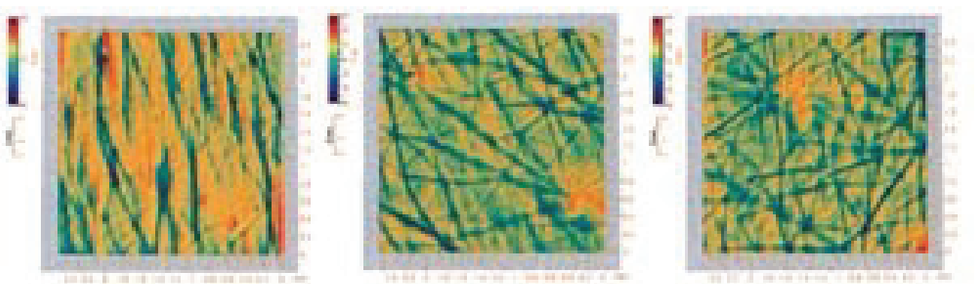
C emulsion or the placebo. Therefore, at the termination of the treatment, two 5-mm punch biopsies up to the hypodermis were collected under local anesthesia at the site of the topical application. One biopsy was used for classical morphology, and electron microscopy reported elsewhere (manuscript in preparation). The second biopsy was used for measurement of the mRNAs.

The mRNA of collagen type I and type III was increased to a similar extent by vitamin C. The mRNA of three posttranslational enzymes, procollagen N-, C-proteinases, and lysyloxidase, was similarly increased. The mRNA of decorin was also stimulated, but elastin, fibrillin 1 and 2, MMP1, 2, and 9 were not modified by the vitamin. The stimulating activity of topical vitamin C was most conspicuous in the women with the lowest dietary intake of the vitamin. The results demonstrate that vitamin C penetrates up to the dermis and further indicate that collagen synthesis is not maximal in postmenopausal women and can be increased.

These data clearly showed that topically applied vitamin C can have a beneficial effect for treatment of skin aging, the mechanism of action being related to an activation of the collagen metabolism, an in activation of a dermal synthesis of elastic fibers.

An example of skin relief improvement.

Same area in a patient at T0, after 3 months of treatment, and after 6 months of treatment.



Campos et al. [79] compared the effects of vitamin C and its derivatives on the skin after 2 and 4 week period daily applications of topical formulation containing ascorbic acid or derivatives (magnesium ascorbyl phosphate (MAP) and ascorbyl tetraisopalmitate (ATIP)). Results showed that vitamin C derivatives did not present the same effects of ascorbic acid on human skin. They obtained an enhancement in transepidermal water loss (TEWL) with ascorbic acid which due to enhancement of the epidermal cell renewal process on human skin and an increase of cutaneous hydration in the deeper cell layers with MAP.

6. How to manage topical vitamin C

The stratum corneum is the primary obstacle to efficient vitamin C absorption from external sources [80]. Although concentrations of vitamin C up to 30% have been used for animal studies, maximal absorption was achieved with a 20% vitamin C solution, with higher concentrations showing lower absorption [80]. Topical application of ascorbic acid will cross the epidermis into the underlying dermal layers. A major obstacle for L-ascorbic acid application in topical formulation is its low stability in aqueous media. The stability of vitamin C in topical solutions is a concern, as exposures to air, heat, and/or light may slowly degrade vitamin C. AA is a sensitive compound, which is degraded by oxidation. Several factors can negatively influence ascorbic acid degradation such as high storage temperatures, light, and high pH values. Catalytic amounts of metals, mainly iron, increase the rate of oxidation [81, 82].

The degradation of vitamin C proceeds within days and weeks depending on formulation, packaging, and storage condition. This loss of active substance is often accompanied by brownish discolorations of the formula, including an increased risk of skin incompatibilities and physical instabilities of the formula system itself. The instability of these compounds limits their application in pharmaceutical and cosmetics industries.

To solve this problem of stability, derivatives of vitamin C have been synthesized having an action similar to ascorbic acid but with improved chemical stability. Two derivatives are widely used in cosmetic products: lipophilic ascorbyl palmitate and hydrophilic ascorbyl phosphate salts. These differ in their ability to permeate the skin, as a result of their different hydrolipophilic properties. Many novel compounds as stabilized ascorbyl pentapeptide (SAP), which is much more stable than L-ascorbic acid in water [83], have been recently studied.

Formulation concepts for increase vitamin C stability have also been formulated on anhydrous system [38], in solution [39] or in other system as O/W/O emulsions [84] and micro emulsions with alkyl polyglucoside [85].

Topical application of vitamin C has been shown to elevate significantly cutaneous levels of vitamin E in pigs, and this correlates with protection of the skin from UVB damage as measured by erythema and sunburn cell formation [86]. A combination of both vitamins C and E provided very good protection from a UVB insult. This study confirms the utility of antioxidants as photoprotectants but suggests the importance of combining the compounds with known sunscreens to maximize photoprotection.

In a study done by Dreher [87], the topical application of combinations of both vitamins or of melatonin with vitamins enhanced the photoprotective response. The better protection was obtained by using the combination of melatonin with both vitamins.

From another hand, no significant protective effect of melatonin or the vitamins when applied alone or in combination was obtained when antioxidants were applied after UV radiation exposure. No improved photoprotective effect was obtained when multiple applications were done [88].

They investigate the effect of the use of high-frequency ultrasound together with coupling gel containing vitamin C and niacinamide as skin lightening agents. Ultrasound radiation enhanced the absorption of skin lightening agents in the stratum corneum in a radiation time-dependent manner. The data suggest that the use of high-frequency ultrasound radiation together with skin lightning gel is effective to reduce hyperpigmentation via enhancing transepidermal transport of vitamin C and niacinamide [89].

7. Bateman purpura

Bateman purpura is a noninflammatory hemorrhagic syndrome characterized by the presence of purpuric eruptions like a petechial or confluent ecchymosed, which was first described by Bateman in 1836. The skin tends to appear thin and wrinkly and almost look flimsy. It results in flat blotches, which start out red and then turn purple, darken a bit, and eventually fade away. It may also occur in the mucous membrane such as mouth and internal organs. The lesions appeared mainly along the outside of the forearm in successive dark purple blotches of an irregular form and various magnitudes. The lesions are also localized to legs, back of the hands, bridge of the nose in subjects wearing glasses, and on the parts of the body where the skin is lax and inelastic [90]. These are flat, irregular, and purple lesions which

appear on the skin as one gets older. Confirmed by Rayer in 1839, Unna described in 1895 six cases. All were aged women, and in all except one, the purpura was limited to the extensor surface of the forearm. Senile purpura frequency is around 10% in an elderly population between 70 and 90 years with a female predominance, and it is associated, in 90% of the cases, with pseudoscars. In 2007, Kaya and Saurat proposed the term "Dermatoporosis" to cover different manifestations and implications of the chronic cutaneous insufficiency/fragility syndrome [91]. The clinical manifestations of Dermatoporosis comprise morphological markers of fragility such as skin atrophy, senile purpura and stellate pseudoscar, and functional expression of skin fragility. They result of skin fragility from minor traumas, such as frequent skin laceration, delayed wound healing, and subcutaneous bleeding with the formation of dissecting hematomas leading to large zones of necrosis. Considering the special and pathognomonic patterns of Bateman purpura, such as skin atrophy, stellar scars, hemorrhages, and ecchymosis, it seemed to us that these symptoms are consistent with the diagnosis of localized scurvy. In order to support this assertion, Humbert et al. demonstrated few years ago that aged skin was deficient in vitamin C content [59]. Furthermore, it is noteworthy that the lesions occur on photo-exposed skin such as forearms, face, neck, etc. Sun exposure is known to deplete the skin in antioxidant vitamins, especially vitamin C [39]. A randomized double-blind comparative study (twice daily application of the active vs. the neutral cream) was conducted in patients with Bateman purpura. Clinical examination by experts showed a significant improvement on the vitamin C–treated side compared with the control. Twice-daily application of 5% topical vitamin C led to a clinically apparent improvement of the skin symptoms. These results confirmed the hypothesis of underlying of role vitamin C deficiency in the determinism of Bateman purpura [92, 93].

8. Atopic dermatitis and inflammatory skin diseases

Atopic dermatitis (AD) is defined as a chronic inflammatory cutaneous disease. It is caused by an immune response occurring in a genetically predisposed background. The mechanisms at the origin of AD are not totally elucidated but have three characteristics: Genetic: 50–70% of the patients have a relative who is affected; immunologic: allergy symptoms are frequent during AD; cutaneous: abnormalities of the cutaneous surface.

At cutaneous level, AD is characterized by repeated pruriginous outbreaks of acute eczema affecting mainly the skin folds. Clinical signs vary with age and according to the stage of the disease. It is possible to distinguish two phases: pruriginous outbreaks of acute eczema and remission. The chronic inflammatory reaction of the patient is associated with constitutive abnormalities or induced abnormalities of the cutaneous barrier, with an increase in the Trans Epidermal Water Loss (TEWL), abnormalities of the surface cutaneous lipids, and other clinical signs such as xerosis or dryness resulting from the deterioration of the barrier function. Besides, atopic dermatitis is an inflammatory skin condition, in which the beneficial role of antioxidant molecules can be underlined.

Vitamin C belongs to the defense mechanisms of the organism. As an example, vitamin C acts like a reducer against the free radicals of oxygen (harmful for the organism) released during the inflammatory phenomenon: it deactivates the radicals peroxides and becomes a stable ascorbyl radical. The mobilization of vitamin C during inflammation could explain the fall of its dermic concentration, particularly in atopic subjects [94], as well in psoriatic patients [95]. In theory, if

the anti-radicalizing vitamins (A, C, and E vitamins) applied topically arrive at the site of release of the free radicals, their intervention makes the reduction of the harmful effects of these radicals possible.

A clinical study was performed to assess the cutaneous effects of vitamin C applied topically to subjects with light to moderate atopic dermatitis who applied emulsion at 10% of vitamin C *versus* excipient on their forearms during 56 days. The barrier function parameters were assessed: TEWL, hydration, dryness, pH, and relief, and the clinical evolution of the cutaneous lesions was evaluated by SCORAD.

Overall, the clinical scores decreased over the 56 days of the study. Therefore, the eczema lesions tend to regress: SCORAD located on the forearm after and before treatment decreased of 10 and 4 units in the vitamin C and the excipient, respectively. The eczema lesions tended to regress but without significant difference between active and excipient.

The TEWL decreased and all the parameters evolved positively: increase of hydration, decrease of dryness, and improvement of cutaneous relief (unpublished data).

9. Vitamin C wound

There is evidence that topical vitamin C might be beneficial in several unrelated conditions. Topical vitamin C has been reported to improve wound healing [96]. As scurvy progresses, wound healing is impaired due to the loss of mature collagen, which allows wounds to remain open [97]. Skin lesions caused by vitamin C deficiency are remediated by an adequate intake of vitamin C. Studies on the effect of vitamin C supplementation on wound healing have reported somewhat mixed results. Data from laboratory animals and humans show that vitamin C deficiency results in poor wound healing, and vitamin C supplementation in deficient individuals shows significant benefits. Although vitamin C levels appear to increase collagen synthesis and decrease inflammatory responses at the site of the wound, neither vitamin C supplementation [98] nor increased plasma vitamin C status [99] increases wound closure time in otherwise healthy individuals. This suggests that vitamin C may only affect specific facets of the wound healing response. Topical ascorbic acid has not been properly evaluated prior to or during wound healing in humans.

10. Conclusion

Bearing in mind the mechanisms of photodamages and skin protection against aggressions, it should be desirable to take vitamin C orally or to apply it directly to the skin. In addition to its antioxidants properties, vitamin C and some water-soluble derivatives are essential for collagen biosynthesis. Vitamin C is a free radical scavenger by its antioxidant properties. Moreover, Vitamin C is probably one of the main topical anti-aging agents, with clinical proofs of efficacy. In addition, it was demonstrated that the use of photo protective sunscreen after UV irradiation prevents the decrease of acid ascorbic dermis concentration. Indeed, the ingestion of vitamin C has different benefits on skin such as wound healing, cutaneous aging, and prevention of skin cancer.

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