European Journal of Pharmaceutics and Biopharmaceutics 117 (2017) 195–202

Contents lists available at ScienceDirect

European Journal of Pharmaceutics and Biopharmaceutics

journal homepage: www.elsevier.com/locate/ejpb

Research paper

Antioxidant-based topical formulations influence on the inflammatory response of Japanese skin: A clinical study using non-invasive techniques

Tais A.L. Wagemaker [a], Patrícia M.B.G. Maia Campos [a,*], Kenji Shimizu [b], Daiki Kyotani [c], Daisuke Yoshida [b]

[a] Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Avenida do Café, s/n, Ribeirão Preto, São Paulo, Brazil
[b] NIKKOL Group COSMOS Technical Center Co. Ltd., 3-24-3 Hasune, Itabashiku, Tokyo, Japan
[c] NIKKOL Group Nikoderm Research Inc., 1-6-14 Azuchimachi, Osaka, Chuo-ku, Osaka, Japan

ARTICLE INFO

Article history:
Received 3 February 2017
Revised 30 March 2017
Accepted in revised form 31 March 2017
Available online 3 April 2017

Keywords:
Topical antioxidants
Skin inflammation
Biophysical techniques
Reflectance confocal microscopy
Interleukin

ABSTRACT

Cutaneous irritants exposure induces an excess of ROS in the skin and can ensue an inflammatory response. Topical antioxidant-based formulations can help to counteract ROS generation. This study evaluated the influence of antioxidant-based topical formulations on the inflammatory response of skin, using a combination of *in vivo* real-time non-invasive techniques. Nine test areas were defined on each volar forearm of the 25 Japanese volunteers. Measurements were performed before and after treatment with 15 μL of a 5% sodium dodecyl sulfate solution and 15 μL of the same based formulation or the vehicle with 1% of the antioxidants. Volunteers without antioxidant treatment showed more pronounced erythematous areas. Transepidermal water loss of areas treated with green tea polyphenol (GTP)-based formulation showed fully recovered skin. Skin barrier damage caused by repeated applications of SDS showed characteristic alterations, detectable by *in vivo* confocal microscopy such as desquamation, spongiosis and inflammatory infiltrates. The majority of confocal microscopy inflammation signs were found in skin without treatment followed by the vehicle. Ascorbyl tetraisopalmitate, Coenzyme Q_{10}, GTP- and Resveratrol-based formulations reduced the anti-inflammatory cytokines release and attenuated inflammatory signs. The combination of techniques provides results that highlight the importance of antioxidant-based formulations for rapid skin recovery.

1. Introduction

Reactive oxygen species (ROS), such as peroxides, super-oxide anion, hydroxyl radical and singlet oxygen are free radicals. These free radicals can cause protein denaturation, lipid peroxidation, and oxidative damage to DNA. All these changes contribute to adverse effects on the skin, expressed as erythema, edema, wrinkling, photoaging, hypersensitivity and keratinization abnormalities [1]. Thus, ROS are considered an important factor contributing to aging and age-related diseases [2]. According to Masaki [3], the skin treatment with some antioxidants, such as vitamin C and polypehnols are effective to enhance resistance to oxidative stress and improve skin aging.

Skin inflammation is a fundamental and beneficial response to initiate the healing process aiming to restore skin homeostasis. Modern life-styles with intensive sun exposure and cutaneous irritants induce an excess of ROS in the skin and, ensue an inflammatory response to this imbalance [4]. In addition, during the early inflammatory phase, ROS are generated, and at high concentrations, ROS can induce severe tissue damage. It is a known consequence of free radicals directly acting on cytokine and growth factor receptors in dermal cells and keratinocytes [5].

An extensive crosstalk between epithelial and immune cells regulates immune responses in the skin to maintain or restore skin homeostasis [6]. Cytokines represent one possible signaling mechanism that coordinates methabolic response to permeability barrier disruption [7].

IL-1 is an essential pro-inflammatory cytokine and a mediator of the acute phase of inflammation. Among the many types of IL-1, IL-1α can be cleaved to produce an active 17 kDa C-terminal polypeptide that is responsible for initiating the inflammatory cascade [8].

* Corresponding author at: Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo, Avenida do Café, s/n, Ribeirão Preto, São Paulo 14040-903, Brazil.

E-mail addresses: taisalw@uol.com.br (T.A.L. Wagemaker), pmcampos@usp.br (P. M.B.G. Maia Campos), k.shimizu@ns-cosmos.co.jp (K. Shimizu), kyotani@nikoderm.com (D. Kyotani), yoshida@ns-cosmos.co.jp (D. Yoshida).

http://dx.doi.org/10.1016/j.ejpb.2017.03.025
0939-6411/

Aiming to restore the homeostasis the skin generates IL-1Ra. IL-1Ra is an anti-inflammatory cytokine usually produced by the same cells that also express IL-1α or IL-1β, such as monocytes, macrophages, dendritic cells, neutrophils and epithelial cells [9]. It is synthesized and released in response to the same stimuli that drive IL-1α release and neutralizes the effects of IL-1α [10]. The cascade of cytokines released on skin causes the cardinal signs of inflammation: redness, heat, pain, swelling and loss of function of the inflamed tissue.

Many models of inflammation have been developed to evaluate the mechanism and the effects of new drugs for the treatment of skin inflammatory process. Common cutaneous irritants like sodium dodecyl sulfate (SDS), with sufficient concentration or duration of exposures, can be used as a model of inflammation [11]. Furthermore, SDS application in reconstructed epidermis models using chemiluminescence probe MCLA showed ROS generation [12].

Some of the inflammation signs can be measured using skin biophysical techniques and Reflectance Confocal Microscopy (RCM). These techniques have occupied a prominent position in the real time and non-invasive evaluation to observe the superficial layers of the skin [13,14] and, the effectiveness of active ingredients and skin care products by objective, scientific methods [15–17].

RCM has also been an important tool to evaluate the skin and the effects of topical products. By interpreting the light reflectance indexes of the different skin structures, this technique allows for real time evaluation and is a non-invasive way to observe the superficial layers of the skin [18].

In this context, the combination of biophysical techniques, RCM and interleukin assays, contributes to the better understanding of the role of exogenous antioxidants in the skin inflammatory process. Thus, the aim of this study was to evaluate the influence of antioxidant-based formulations on the inflammatory response in skin using a combination of *in vivo* non-invasive techniques.

2. Materials and methods

Twenty-five Japanese volunteers, aged between 24 and 55 years, male (9) and female (16), were enrolled and divided into two groups (11 and 14 volunteers). Each subject received all the formulations under study (vehicle and formulations containing antioxidants). All subjects signed informed consent forms approved by the Cosmos Technical Center Inc. Co. and the NIKKOL Group, Japan, and the study protocol conformed to the principles set forth by the Declaration of Helsinki.

2.1. Studied formulations

All raw materials, excepted for polyethylene glycol (PEG) 400 and butylatedhydroxytoluene (BHT), were obtained from Nikko Chemicals, NIKKOL Group, Japan. The experimental formulations contained water, behenyl alcohol, polyglyeryl-10 pentastearate, sodium stearoyllactylate (Nikkomulese 41®), butylene glycol, PEG 400, glycerin, squalene (Nikkol Sugar Squalene®), thriethylhexanoin (Trifat 308®), cetylpalmitate (Nikkol N-SPV®), glyceryl stearate and PEG-60 glyceryl stearate (Nikkol MGS-150V), xanthan gum, phenoxyethanol and BHT. The formulation was complimented or not (vehicle) with 1% of the antioxidants: green tea polyphenol (GTP) (Polyphenon E®, Mitsui Norin Co. Ltd, Japan), resveratrol (R) (98% pure, Guilin Layin Natural Ingredients Corp., China), Coenzyme Q_{10} (Co) (Kaneka Corporation, Japan), magnesium ascorbyl phosphate (MAP) (VC-PMG®, Nikko Chemicals, NIKKOL Group, Japan) or ascorbyl tetraisopalmitate (AT) (VC-IP®, Nikko Chemicals, NIKKOL Group, Japan). Composition of the formulations are described in Table 1.

The formulations under study, the vehicle and the formulations containing antioxidants, were subjected to preliminary stability tests by centrifugation (B1710 DS, Kubota Corporation, Japan), pH determination (HORIBA Ltd., Japan), and a visual evaluation of the organoleptic characteristics under controlled temperatures (25 °C, 45 °C and −5 °C) for 28 days. Every 7 days, the viscosity was evaluated using a Viscometer TVB-10 (Toki Sangyo Co. Ltd., Japan). The stability of the formulations was also observed by microscopy.

2.2. Preliminary skin penetration tests

To evaluate the skin penetration of the formulations, a 2,2-diphenyl-1-picrylhydarzyl (DDPH) assay was used. DPPH was obtained from Wako Pure Chemicals Industries, Ltd., Japan. It is a stable free radical that can react with compounds able to donate H^+. For the measurement of the radical scavenging activity, each formulation was added to DPPH ethanolic solutions to the final concentrations 15.6–5000 μg/mL. The reaction mixture was incubated for 20 min at room temperature. The absorbance was measured at 550 nm against a corresponding blank. The antioxidant activity was calculated as previously described by Wagemaker et al. [19]: $AA\% = [(A_{DPPH} - A_{Sample})/A_{DPPH}] \times 100$, where AA_{DPPH} is the sample DPPH antioxidant activity and A_{Sample} is the absorption of DPPH. The tests were carried out in triplicate, and the formulation concentration providing 50% of the antioxidant activity ($IC_{50\%}$) was obtained by plotting the antioxidant activity against the formulation concentration. Pure α-tocopherol (TAMA BIOCHEMICAL CO., Ltd., Japan) at a concentration of 39.6–5000 mg/mL was used as a positive control. Three volunteers applied the formulations under study for three consecutive days to evaluate the skin penetration. On the third day, twenty tape strips (D-Squame® discs, CuDerm Corporation, USA) were taken, and their potentials to scavenge the DPPH radical were determined accordingly to the method described previously.

2.3. Study design

Using a randomized design eight equidistant circles (diameter = 8 mm) were defined on the volar forearm of each participant: one negative control (no treatment), one positive control (no formulation) and the other areas for the application of each test formulation and vehicle. The initial measurements were taken (T0). Then, 15 μL of 5% sodium dodecyl sulfate (Wako Pure Chemicals Industries, Ltd.; SDS) solution was applied onto skin for 30 min for four consecutive days using Finn Chambers® Tape (SanporEpitest Ltd., Finland), except onto negative control (no treatment). After four days treated with SDS, 15 μL of the vehicle or the formulations containing 1% antioxidants were applied on each demarked area once daily for seven days. On the negative control and positive control demarked areas were not applied any formulation.

2.4. Instrumentation

Instrumental measurements were conducted in an acclimatized room (temperature 20 ± 2 °C and humidity 45 ± 2%). Data acquisition was performed on each area before SDS induced skin irritation (baseline), after four days application of SDS (TSDS) and after seven days of the antioxidant treatments (TA).

The TEWL was measured using AS-CT1 (Asahi Biomed, Japan), and the values reported are expressed in $g\,m^{-2}\,h^{-1}$. The average values of three measurements were used for subsequent calculations. The SCWC was determined with a non-invasive SKICON