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A new hair follicle-derived human epidermal model for the evaluation of sunscreen genoprotection

D. Bacqueville [a,*,1], T. Douki [b,c,1], L. Duprat [a], S. Rebelo-Moreira [b,c], B. Guiraud [a], H. Dromigny [a], V. Perier [a], S. Bessou-Touya [a], H. Duplan [a]

[a] Service de Pharmacologie et Pharmacocinétique cutanée, Centre R&D Pierre Fabre, Toulouse, France
[b] Univ. Grenoble Alpes, INAC, LCIB, LAN, F-38000 Grenoble, France
[c] CEA, INAC, SCIB, LAN, F-38000 Grenoble, France

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ABSTRACT

Induction of skin cancer is the most deleterious effect of excessive exposure to sunlight. Accurate evaluation of sunscreens to protect the genome is thus of major importance. In particular, the ability of suncare products to prevent the formation of DNA damage should be evaluated more directly since the Sun Protection Factor is only related to erythema induction. For this purpose, we developed an *in vitro* approach using a recently characterized reconstituted human epidermis (RHE) model engineered from hair follicle. The relevance of this skin substitute in terms of UV-induced genotoxicity was compared to *ex vivo* explants exposed to solar-simulated radiation (SSR). The yield of bipyrimidine photoproducts, their rate of repair, and the induction of apoptosis were very similar in both types of skin samples. In order to evaluate the protection afforded by sunscreen against DNA damage, bipyrimidine photoproducts were quantified in tissue models following SSR exposure in the presence or absence of a SPF50+ formula. A rather high DNA protection factor of approximately 20 was found in RHE, very similar to that determined for explants. Thus, RHE is a good surrogate to human skin, and also a convenient and useful tool for investigation of the genoprotection of sunscreens.

1. Introduction

Excessive exposure to sunlight is at the origin of a number of dermopathological effects in skin such as sunburn, ageing and cancer [1,2]. Limiting the damage induced by solar UV radiation is thus a major issue in public health. Unfortunately, in spite of numerous campaigns to promote excessive sun avoidance, the exposure of the general public is still increasing in industrialized countries for recreational and esthetic purposes. Outdoor work is another large source of occupational exposure to solar radiation. As a consequence, the incidence of skin cancers, including melanoma and carcinomas, is in constant increase since the last three decades. The use of sunscreens appears as an appropriate photoprotection strategy because it reduces the UV dose reaching both the dermis and epidermis, and thereby limits the onset of the biological and biochemical processes leading to pathological conditions [3]. A wide variety of photoprotection products is available on the market. They consist in different mixtures of organic and/or inorganic filters. As a consequence, the efficiency of these formulas against skin damage is variable. Thus, it rapidly appeared that a common evaluation protocol was needed to compare the photoprotective efficacy of sunfilters. This led to the definition of the Sun Protection Factor (SPF) [4] which measures the capacity of a product to limit the appearance of cutaneous erythema following exposure to UV light. Because erythema is mostly due to UVB (280–315 nm) and that UVA radiation (315–400 nm) is an important issue in sunlight deleterious effects, the photoprotection in this latter wavelength range is also evaluated, mostly through the persistent pigment darkening test [5].

Although these approaches for the evaluation of sunscreens are convenient and widely used, they suffer from some limitations.

Abbreviations: 64PP, pyrimidine (6-4) pyrimidone photoproducts; 8oxodGuo, 8-oxo-7,8-dihydro-2′-deoxyguanosine; CPD, cyclobutane pyrimidine dimer; DEW, Dewar valence isomer; HPLC-MS/MS, HPLC associated with tandem mass spectrometry; MED, minimal erythema dose; RHE, reconstituted human epidermis; SPF, Sun Protection Factor; SSR, solar-simulated radiation.

* Corresponding author at: Service de Pharmacologie et Pharmacocinétique cutanée, Centre R&D Pierre Fabre, 3, avenue Hubert Curien, BP 13562, 31035 TOULOUSE Cedex 1, France.
E-mail address: daniel.bacqueville@pierre-fabre.com (D. Bacqueville).

[1] Both authors contributed equally to this work.

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First, erythema is a threshold process and therefore does not take into account the effect of low and chronic UV exposure. In addition, both evaluation techniques are based on skin responses which are not related to tumorigenesis. Consequently, they do not provide enough information on the protection of the suncare products against skin cancer. Moreover, determination of SPF is a tedious and time-consuming process that requires participation of human volunteers. Numerous works have been devoted to the development of *in vitro* techniques as complementary tools to SPF [6–15]. These approaches mostly rely on the induction of UVB and/or UVA-activated molecular pathways in cutaneous cells as endpoints for the evaluation of the photoprotection afforded by sunscreens following topical application. In spite of their increasing use in the testing of new photoprotective and phototoxic compounds,*in vitro* models have rarely been used in order to investigate the efficacy of sunscreens at preventing DNA damage.

Therefore, we propose in the present work a strategy for the evaluation of the cutaneous protection against the genotoxicity of simulated sunlight using a recently characterized reconstituted human epidermis (RHE) grown from outer root sheath keratinocytes isolated from adult hair follicles [16]. This RHE model has the main advantage to be prepared from a non-invasive procedure since the keratinocytes are easily and quickly taken by hair plucking. It is of note that the commercial RHE models are usually reconstituted from keratinocytes isolated by surgical procedures (foreskin, mammoplasty, abdominoplasty). After 14 days culture at the air/liquid interface, the RHE model presented a similar morphology and three-dimensional architecture to those of native epidermis with the presence of the classical four skin layers (*stratum basale*, *stratum spinosum*, *stratum granulosum* and the outermost layer *stratum corneum*). The RHE model reconstruction was reproducible and the expression of proliferation and differentiation markers was close to the marketed epidermal substitutes and skin [16]. Moreover, the hair follicle-derived RHE is a convenient and easy-to-handle *in vitro* RHE model and a more reliable source of biological material than skin explants. However, since the gold standard tissue in photobiological research remains the human skin, experiments were also performed on *ex vivo*organotypic culture to validate the hair follicle derived RHE as a suitable model to investigate the genomic protection afforded by sunscreens. A first marker of genotoxicity was the formation of DNA dimeric pyrimidine photoproducts, including cyclobutane pyrimidine dimers (CPDs), pyrimidine (6-4) pyrimidone photoproducts (64PPs) and their Dewar valence isomers (DEWs). The three types of photoproducts were quantified by HPLC associated with tandem mass spectrometry (HPLC-MS/MS) [17,18]. The detection of CPDs was also achieved *in situ* by using immunohistochemistry [14,19]. Prevention of the formation of dimers by sunscreens has been shown by several groups [14,20–27]. We also recently provided quantitative data on the UVB and UVA protection of sunscreens in human skin explants based on the HPLC-MS/MS detection of CPDs [28]. These DNA lesions are interesting markers because they are at the origin of skin cancer and they also directly reflect the amount of UV photons that reach cutaneous cells after getting through the sunscreen and the upper skin layers. Since apoptosis is a major cellular response to the induction of DNA damage, SSR-induced cell death was also investigated through both sunburn cell detection and caspase-3 activation measurement in both *in vitro* and *ex vivo* skin models. To complete the comparison between the response of RHE model and skin explants to SSR, we also quantified the rate of DNA photoproduct repair. The new RHE model was finally validated as a reliable tool in the evaluation of sunscreen by comparison the photoprotection afforded by a SPF50+ product in both human skin models.

2. Material and methods

2.1. Preparation of reconstituted human epidermis and skin explants

Human epidermis was reconstructed *in vitro* from outer root sheath keratinocytes isolated from hair follicles obtained from healthy volunteers who had given their informed consent (Caucasian males and females, 28 ± 5 years). Tissue engineering was performed in-house according to the recently published protocol [16]. The 1.12 cm^2 epidermal equivalent consisted of airlifted, living, multilayered, normal human keratinocytes cultured for 16 days on an inert polycarbonate filter. Tissue culture was performed at 37 °C using growth medium changed three times a week and containing DMEM/Ham's F12 (3:1) supplemented with 10% fetal calf serum, epidermal growth factor, hydrocortisone, adenine, tri-iodothyronine, insulin, amphotericin and antibiotics.

Normal humanskin was obtained from abdominoplasty obtained from Caucasian female donors with their informed consent (43 ± 9 years), and *ex vivo* organ culture was performed as previously described [19]. Briefly, explants were punched into 14-mm discs and seeded in culture inserts in 6-well plate prefilled with 1.5 mL of culture medium based on DMEM supplemented with pyruvate, glutamine, essential and non essential amino acids, amphotericin and antibiotics.

2.2. Exposure to simulated sunlight in the presence of sunscreen

Solar-simulated radiation (SSR) was performed using a Suntest CPS$^+$ chamber (ATLAS) equipped with an NXE1500 xenon lamp and fitted with a UV filter to eliminate wavelengths below 290 nm [14,19]. Irradiance in UV spectra, measured by a recognized organization (Opto.cal gmbh), was about 70 W/m^2 from 290 to 400 nm (Supplementary material Fig. S1). The proportion of UVB was 0.67% of the total energy whereas the proportion of UVA was 9.93%. The UVA/UVB ratio was about 15 according to the method for *in vitro* determination of UVA protection [4].

Skin models were exposed to a single UV dose and harvested just after irradiation or 24 h after SSR exposure. Acute SSR irradiation was done by using an irradiation dose of 5.5, 11 and 16.5 J/cm^2 whereas tissue models were exposed to 1.37, 2.75 and 5.5 J/cm^2 for DNA repair experiments. SSR-induced genotoxicity and apoptosis were evaluated from at least 3 independent donors for each tissue model and 3 independent RHE/explants were used for the irradiation data points.

Sunscreen photoprotection was performed by SSR exposure with or without addition of a SPF50+ sunscreen. The latter was a broad-spectrum UVB + A photoprotective system, commercially available from Avène Laboratories (Boulogne, France), and this emulsion was made of Avene aqua, C12–15 alkyl benzoate, methylene bis-benzotriazolyltetramethylbutylphenol [nano], aqua, cetearylisononanoate, diisopropyladipate, isodecylneopentanoate, bis-ethylhexyloxyphenolmethoxyphenyltriazine, diethylhexylbutamidotriazone, aluminum starch octenylsuccinate, butyl methoxydibenzoylmethane, potassium cetyl phosphate, decyl glucoside, C10-18 triglycerides, acrylates/C10-30 alkyl acrylate crosspolymer, benzoic acid, caprylic/capric triglyceride, caprylyl glycol, disodium EDTA, glyceryl behenate, glyceryl dibehenate, glyceryl laurate, propylene glycol, silica, sodium hydroxide, tocopheryl glucoside, tribehenin and xanthan gum (INCI nomenclature). The formulation was topically applied at 2 mg/cm^2 1 h before SSR irradiation according to Cosmetics Europe recommendations. In some experiments, a placebo containing all components except the active UV filters was spread onto the tissue surface as a control.