Effect of Poly-ADP-Ribose-Polymerase (PARP) in ultraviolet light induced skin damage

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1. Introduction

The radiation striking the earth is approximately 10% ultraviolet (UV). The UV spectrum is divided for convenience into UVA (320-400 nm), UVB (280-320 nm), UVC (100-290 nm) and vacuum UV (10-100 nm). Shortest UVB, UVC and vacuum UV are screened by the ozone layer. The remained UVA and UVB light reaches the earth surface and can cause photodamage. Photodamage is the specific damage produced in tissue by single or repeated exposure to ultraviolet light. The UVB component, which is directly absorbed by cellular macromolecules (including DNA), causes DNA single strand breakages. In contrast, UVA is weakly absorbed by most biomolecules but generated reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$) - via interaction with cellular chromophores -, hydrogen peroxide (H_2O_2) and superoxid anion. In humans, both acute and chronic exposure to sunlight are associated with various physiological and pathological states. The acute response leads to immediate effects such as erythema, sunburn, pigmentation, hyperplasia, immunosupression. The chronic response leads to delayed effects such as cataract, skin aging, premalignancies (solar keratosis) and malignancies, including melanoma and non-melanoma (basal cell and squamous cell carcinomas) skin cancer. It is known that most of these effects depend upon the duration and frequency of exposure, the intensity of solar radiation and the reactivity of skin based on genetically determined constitutive skin color and skin phototype. Microscopically changes are also detectable. Epidermal changes include intracellular edema, vacuolization and swelling of melanocytes and the development of "sunburn cells".

Sunscreens are now recognized to be an important strategy in the prevention or minimization of premalignant and malignant skin lesions. We would like to test the photoprotective ability of BGP-15M a novel poly-ADP-ribose polymerase **(**PARP) inhibitor against acute and chronic photodamage produced in skin by exposure to UV light.

The poly-ADP-ribose polymerase is a nuclear nick-sensor enzyme, becomes activated by recognizing DNA single-strand breaks. Upon activation, PARP cleaves NAD to nicotinamid and ADP-ribose. Excessive activation of PARP leads to depletion of NAD and ATP levels resulting in impaired cellular energy metabolism. „PARP-mediated

cellular suicide pathway" contributes to cell necrosis and tissue injury. ROS-induced impaired energy metabolism and cell death can partially reverted by PARP inhibitors, because these latter significantly decrease the rate of NAD^+ catabolism and reduce ATP utilization for the resynthesis of NAD^+. The fact that PARP inhibitors decrease the catabolism of cytoplasmatic NAD^+ and so possibly decrease ROS-induced mitochondrial NAD^+ loss suggest a connection between oxidative mitochondrial damage and PARP activation. Therefore it is possible that protection of cells from oxidative damage can be achieved by the potential of PARP inhibitors to reduce DNA injury and ROS production.

2. Scientific goals

I. Test the photoprotective ability of BGP-15M a novel PARP inhibitor against acute and chronic photodamage produced in skin by exposure to UV light.

II. Test the anti-photocarcinogenic effect of BGP-15M against UV light induced tumor formation.

3. Material and methods

3.1. Experimental animals

Investigations were carried out on hairless VAF/plus (CRL:hr/hr BR) mice, 5 weeks of age, purchased from the Charles River Hungary Co..

3.2. UV irradiation

UV irradiation carried out by the Waldmann UV 8001K light booth equipped with UV21 Philips lamps major peak at 313 nm (in UVB, 285-350 nm). The total irradiance

was measured by IL 700 spectroradiometer with cosine-corrected SEE 400 detector and WBS 320 filter.

3.3. Test substances

BGP-15M containing cream and lotion or vehicle in different concentration (5%, 10%, 15%) and Ambre Solaire cream (SPF 30) was applied at 2 mg/cm^2 to mouse skin prior to UV light exposure and removed immediately after it.

3.4. Determination of minimal erythema dose (MED)

Phototesting of mice skin: six unprotected skin surface areas (0.25 cm^2) of animals were exposed by increasing dose (0.07 – 0.32 J/cm^2) of UVB.
Clinical MED was determined 24 h after UVB exposure.

3.5. Experimental condition to determination of antierythematogenic dose of BGP-15M

Three groups of mice were used to study the antierythematogenic effect of BGP-15M-containing creams in a concentration of 5%, 10 % and 15%. Tests were carried out on uncovered skin areas (1 cm^2, separated from each other by a 0.5 cm wide range of covered skin) of ventral-thoracic region of animals, in form of self-control examination. The proximal test areas were treated by BGP-15M-containing creams (2 mg/ cm^2 skin surface) immediately before erythematogenic UVB exposure (4.4 MED) carried out by sunlight. The concentration of BGP-15M was 5% in group I., 10% in group II., 15% in group III. of mice. The distal test areas exposed to sunlight (in the same time) without any pretreatment and used as positive controls. Animals without UV exposure severed as negative controls. 20 hours following exposure to the sun the test areas were compared to each-other and the unexposed skin of control animal.

3.6. Experimental conditions to comparison of BGP-15M-pretreated and Ambre Solaire cream (SPF 30)-pretreated sunexposed skin with that of sunexposed, untreated (positive control) and unexposed and untreated (negative control) ones

Four groups of mice were used to compare the photoprotective effect of BGP-15M-containing lotion (20%) and cream (15%) with that of Ambre Solaire cream (SPF 30). Tests were carried out on uncovered abdominal skin areas (1 cm^2, separated from each other by a 0.5 cm wide range of covered skin) of ventral-thoracic region of animals, in form of self-control examination. The proximal test areas were treated either by BGP-15M-containing cream or lotion (2 mg/ cm^2 skin surface) or Ambre Solaire cream (2 mg/ cm^2 skin surface) immediately before erythematogenic UVB exposure (2.0 MED) carried out by sunlight. The distal test areas exposed to sunlight (in the same time) without any pretreatment and used as positive controls. As negative controls, non-irradiated, sunscreen-treated groups of animals were used. 20 hours following exposure to the sun the test areas were compared to each other and to the skin surfaces of the negative and positive control animals.

3.7. Experimental conditions to determination of poly-ADP-ribose-polymerase (PARP) activity by ADP-ribosylation assay after UVB exposure in samples of BGP-15M-pretreated, untreated (positive control) and unexposed and untreated (negative control) skin

Determination of PARP activity was carried out by ADP-ribosylation assay using an anti-ADP-ribose monoclonal antibody and Western blot analysis. The experiment were carried out by 2.0 MED UVB dose, an erythematogenic exposure to the ventral-thoracic region of mice pretreated with 15% BGP-15-containing cream or without it an in the untreated control animals PARP activity was determined.

3.8. Histology

Tissue samples were fixed in 10% neutral-buffered formaldehyde and were embedded in paraffin. 3-4 □m tissue sections were deparaffinized, rehydrated in graded alcohol and stained with hematoxylin and eosin.

3.9. Immunostaining of tissue sections

The slides were incubated either with poly-ADP-ribose-specific polyclonal serum (Biomol Res.) or polyclonal IL-10 (goat anti-mouse) serum (Sigma Co.), staining was carried out according to the streptavidin-biotin-peroxidase method using the Immunotech Universal Kit.

4. Results

4.1. Phototesting of mice skin - determination of minimal erythema dose (MED)

The minimal erythema dose of UVB was determined. Erythema started developing at a dose of 0.24 J/cm^2.

4.2. Effect of single erythematogenic UVB irradiation to the mice skin

Using 2.0 MED UVB dose as erythematogenic exposure all the clinical signs of sunburn could be detected after a latency of 24 hours. The exposed skin area showed a red discoloration (erythema) with erosion. In the formalin-fixed, paraffin-embedded tissue sections acanthotic and exulcerated epidermis with an inflammatory infiltrate in the dermal connective tissue could be observed by histological examination.

4.3. Determination of antierythematogenic effect of BGP-15M

In test areas pretreated with equal or greater than 10% BGP-15M-containing creams erythema or other signs of sunburn could not be observed. The skin surface of these pretreated test areas were clinically same as the unexposed skin of control animals (negative control). In contrast to it, the distal, UV exposed test areas of the animals showed all the signs of sunburn inform of lilac, red discoloration with oedema.

4.4. Histology

Results of histological examination stained by Hematoxylin-Eosin showed exulcerated epidermis with inflammatory infiltrates in the dermal connective tissue of biopsy material taken from UV-exposed mouse skin without pretreatment of sunscreen.

In contrast to it in BGP-15M-pretreated (15%), UV-irradiated skin samples except a mild acanthosis, histologically normal mouse skin could be observed. In skin biopsy samples pretreated with 5% BGP-15M cream a mild acanthosis and an inflammatory reaction in the upper dermis without any ulceration could be detected.

Conclusion: Clinical observations and the result of histological investigation suggested that BGP-15M-containing cream in a concentration of 10 % functions as sunscreen.

4.5. Comparison of BGP-15-pretreated and Ambre Solaire cream (SPF 30)-pretreated sunexposed skin with that of sunexposed, untreated (positive control) and unexposed and untreated (negative control) ones

Test areas pretreated with 15% BGP-15M-containing cream or 20% BGP-15-containing lotto or Ambre Solaire cream (SPF 30) showed normal skin surface as it could be observed in animals used as negative control.
The skin areas with UV exposure without pretreatment of sunscreen were found erythematous clinically.
Comparison showed no difference between the photoprotective effect of Ambre Solaire cream with sun protection factor 30 and the used concentration of BGP-15M substances (cream and lotion).

Conclusion: It suggest that the 15% BGP-15M-containing cream and the 20% BGP-15M-containing lotto might have an SPF 30.

4.6. Determination of poly-ADP-ribose-polymerase (PARP) activity by ADP-ribosylation assay after UVB exposure in samples of BGP-15M-pretreated, untreated (positive control) and unexposed and untreated (negative control) skin

In skin samples without BGP-15M pretreatment and with an exposure of 2.0 MED UVB all the signs of sunburn could be detected clinically and histologically and elevated PARP activity was found in ADP-ribosylation assay. In contrast to it, in BGP-15M-pretreated skin samples no clinical signs of sunburn were observed with nearly the same PARP activity as in the unexposed control skin.

5. Conclusion

Topically applied BGP-15M containing cream

I. decrease the UV light-induced single-strand DNA breaks in skin

II. decreased the UV light-induced overactivation of self-ADP ribosylation of PARP in the skin

III. able to avoid the photoimmunosuppression

IV. is photoprotective against acut UVB damage

V. is as effective as a 30 SPF sunscreen

VI: protect the skin agains UV light induced chronic skin damages clinically, histologically, immunhistologically and ultramicroscopically

VII. protect the chronic UVB and UVA light induced photocarcinogenesis

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