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Global Dermatology 2018 -Topical Nutlin-3 Potentiates the UVB-induced p53 Response and Reduces DNA Photodamage and Apoptosis in Mouse Epidermal Keratinocytes in Vivo- Birthe Mørch Thomsen- Copenhagen University

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Abstract

Background/Aims: (-)-Nutlin-3 (nutlin) may be a cis-imidazoline analog, which activates p53 by antagonizing murine double minute (Mdm2) protein. We therefore conducted a study in hairless mice to analyze the effect of topical nutlin on murine epidermis after UVB-radiation.

Methods: Animals were euthanized 24 hours after irradiation, the dorsal, treated skin was biopsied and fixed in 4% formalin. Sections were incubated with the antibodies against p53, thymine dimers and terminal deoxynucleotidyl transferase-mediated dUTP nick and labelling (TUNEL).

Results: We showed here that nutlin was active after topical application in hairless mice and potentiated the p53 nuclear translocation in epidermal keratinocytes after ultraviolet B irradiation. Moreover, topical treatment with nutlin resulted in a decrease in the number of keratinocytes exhibiting positive nuclear staining for thymine dimers ($P<0.05$compared with the vehicle control) and decreased the frequency of apoptotic, TUNEL-positive cells ($P=0.02$).

Conclusion: We hypothesize that nutlin stimulates DNA photodamage repair in the epidermis and may prove useful for the chemoprevention of skin cancer.

Keywords: Nutlin-3; p53; Apoptosis; Thymine dimer; Hairless mice

Experimental Design:

Animals: Female C3.Cg/TifBomTac immunocompetent mice (age 15 weeks) were purchased from Taconic (Ry, Denmark). The mice were kept in an animal facility on a 12 h light/dark cycle at 23 - 24°C. Animal care and treatment followed Danish national guidelines.

Drug treatment, ultraviolet source and experimental design :
Mice were sedated with 0.05 ml HypDorm (fentanyl citrate 0.158 mg/ml, fluanisone 5mg/ml, midasolam 2.5mg/ml) and treated on one of the lateral halves of the back skin with 100 µl isopropanol solution of 43 mM nutlin [(-) 4-(4,5-bis(4-chlorophenyl)-2-(2-isopropoxy-4- methoxyphenyl)-4,5-dihydro-1H-imidazole-1-carbonyl)piperazin-2- one] (Cayman Chemical) 30 min before irradiation. The other half was treated with the same volume of isopropanol. The mice were then irradiated with 3 SED (100 mJ/cm2) UVB from a light source comprising an array of 6 TL12 tubes (Philips, Eindhoven, The Netherlands) and re-treated with nutlin or isopropanol, as above. Control mice were not irradiated. Animals were euthanized 24 hours after irradiation because studies in vitro in human skin and in vivo in mice skin suggest that after 24 hours about half of the CPD are repaired [5,6]. The treated dorsal skin was biopsied and fixed in 4% formalin.

Immunohistochemistry :

4 µm sections were cut from the paraffin embedded skin biopsies, deparaffinised with xylene, re-hydrated and incubated with the antibodies against p53 (rabbit, polyclonal, Novocastra Newcastle upon Tyne, UK) or peroxidase-conjugated monoclonal anti-thymine dimer antibody (Kamiya Biomedical, Seattle, WA). The antibodies were visualised using the LSAB+ System-HRP (Dako, Glostrup, Denmark). Terminal deoxynucleotidyl transferase-mediated dUTP nick and labelling (TUNEL) was performed using the DeadEnd Colorimetric TUNEL system (Promega, Madison, WI). Apoptotic, TUNEL-positive cells were counted in a 3 mm long section of epidermis. The extent of thymine-dimer positivity was assessed on an ordered categorical interval scale: 1) 0% stained nuclei, 2) 1-25%, 3) 26-50%, 4) 51-75% positive nuclei. In no samples the frequency of positive nuclei exceeded 75%.

Results :

As shown in Figure 1, the p53 staining of unexposed epidermis revealed moderate immunoreactivity in the cytoplasm but not in the nuclei of epidermal keratinocytes. After UVB irradiation the cytoplasmic staining was more intense and single cell nuclei were stained positive for p53. In non-irradiated skin nutlin did not induce p53. Since it is known that p53 is induced by DNA photoproducts and p53 is involved in the repair of CPD [7] we stained skin biopsies with the antibody against thymine dimers. Topical treatment with nutlin resulted in an overall decrease in the number of keratinocytes exhibiting positive nuclear staining for thymine dimers.Nutlin treatment significantly inhibited the UVB-induced apoptosis (Figure 2C-D). TUNEL staining revealed 9.0 ±2.6 (mean ±standard deviation) TUNEL-positive cells /

mm epidermal length in UVB-irradiated, vehicle-treated skin versus 1.7 ±0.5 TUNEL-positive cells /mm in the nutlin-treated skin (P=0.02, paired t-test).

Conclusions :

This study shows that the Mdm2 inhibitor, nutlin, activates p53 in the epidermis in UVB-irradiated mice. This was accompanied by a significant decrease in the frequency of the cells harbouring thymine dimers and diminished keratinocyte apoptosis. We suggest that the decreased apoptosis is caused by enhanced CPD repair due to p53 activation by topically applied nutlin. It is conceivable that nutlin may be used for chemoprevention of squamous cell carcinoma in humans.

Conflicts of Interest :

This study was financed solely by the Bispebjerg University Hospital and has not been supported by any pharmaceutical company.

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