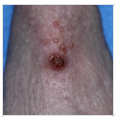

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Skin phtocarcinogenesis by sunbed in SKH-1 mice

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1 Works for me dx.doi.org/10.17504/protocols.io.bc45iyy6

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ABSTRACT

When SKH-1 mice are exposed to ultraviolet radiation three times a week for 60min each (a total of 80sessions), with a focus-skin distance of 20 cm, using the sunbed Philips Type HB 554/01/A with 8 Philips Performance S 100W tubes, all animals develope squamous skin carcinomas.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Antonio Cano Gomez, Francisco Jose Gomez Garcıa , Nuria Alvarez Sanchez, Paloma Sanchez-Pedreno Guillen adnd Vicente Vicente Ortega. Modelo defotocarcinogenesis cutanea en ratones SKH-1 por radiacion ultravioleta. Rev Esp Patol.2010;43(4):191–195

ATTACHMENTS

fotocarc SKH-1 rep.pdf

MATERIALS TEXT

Philips lamp (Type HB 554/01/A) with 8 Philips Performance S 100W tubes. The lamp has an emission spectrum of 220-425 nm with a maximum peak of 364 nm (98.6% UVA and 1.4% UVB).

1 Ultraviolet radiation: The mice were subjected to UV irradiation using a Philips lamp (Type HB 554/01/A) with 8 Philips Performance S 100W tubes. The lamp has an emission spectrum of 220-425 nm with a maximum peak of 364 nm (98.6% UVA and 1.4% UVB). The animals were exposed to radiation three times a week for a total of 80 sessions, each lasting 60 minutes. For this purpose, the animals were placed in PVC cages, with individual separators and a metallic lattice ceiling, which were placed under the ultraviolet lamp with a distance of 20 cm between the light source and the skin. The energy received in each session was 21.1 J/cm2, so that at the end of the experiment the total energy received by each mouse was 1688 J/cm^2.

2 Macroscopic study: A detailed macroscopic study of the dorsal skin was made after each session and digital photographs were taken on millimeter paper to measure the lesional areas at the end of the experiment (80 sessions). An application of the image processing and analysis software platform Leica Qwin was used to obtain a binary mask of the lesional areas, which were measured individually.

3 Microscopic study: At the end of the experiment, animals were sacrificed by CO_2 overdose. A necropsy was performed on the dorsal skin and viscera. The samples collected were embedded in paraffin and stained with haematoxylin and eosin for microscopic analysis. The presence of normal skin, actinic keratoses, dysplasia, carcinoma in situ and invasive carcinoma was examined under light microscopy (Leica DM 6000B). Immunohistochemical study of tumors was performed using the streptavidin-biotin method with following antibodies: proliferating cellular nuclear antigen (PCNA); p53; anti-metaloproteinase-9 (MMP-9); and anti-metallopeptidase inhibitor 1 (TIMP-1) (Dako, S.A, Barcelona, Spain). The sections were incubated overnight at 4°C with antibodies to PCNA (PC-10; Dako Corporation, Glostrup, Denmark); MMP; and TIMP-1 at a 1:200 dilution. Negative controls were treated with all reagents except the primary antibody. PCNA expression was quantified in high resolution images obtained from digitalized specimens by the microscopy scanning Leica SCN400F, using an image analysis module of Slidepath Digital Image Hub. Three 100 µm2 areas were traced on each image from the tumor invasion front (in the case of tumors) or the epidermis basal layer when no carcinoma was present. The software produced a count of the number of positive nuclei and the total number of cells, using the Nuclear Algorythm 3.0, which detects PCNA marking by means of a previously defined color pattern. Immunohistochemical quantification (PCNA and P53) was expressed as the mean percentage of each antiboy immunohistochemical positive cells, respect to total cells number(Ibrahim and Elwan, 2017). In semi-quantitative immunohistochemical analysis (TIMP, and MMP-9), scores were calculated by multiplying by the percentage of stained stroma surface (grade 0: no stained stroma, grade 1: less than 10% stained stroma, grade 2: 10-50% stained stroma, and grade 3: 51-100% stained stroma) by the graded intensity of stained stroma (grade 0-3)(Preidl et al., 2019). All microscopic analyses were carried out by two pathologists blinded to the results of the study.

4 Statistical analysis: Data were analyzed using the SPSS version 20.0 statistical package (SPSS® Inc., Chicago, IL, USA). A descriptive study was made of each variable. The associations between the different qualitative variables were studied using Pearson's chi-squared test. ANOVA and the Tukey test were applied to quantitative variables, in each case determining whether variances were homogeneous. Differences were regarded as significant if $p \leq 0.05$ and highly significant if $p \leq 0.01$.