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Cyclobutane pyrimidine dimers are photosensitised by carprofen plus UVA in human HaCaT cells

K.S. Robinson, N.J. Traynor, H. Moseley, J. Ferguson, J.A. Woods *

The Photobiology Unit, University of Dundee, Ninewells Hospital and Medical School, Dundee DD1 9SY, UK

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ABSTRACT

Every year in the UK about 75,000 cases of non-melanoma skin cancer (NMSC) are registered, and about 9500 people are diagnosed with cutaneous melanoma (CM). The main risk factor for these cancers is exposure to sunlight. The effects of light on skin are wavelength dependent, with wavelengths in the UVB waveband (280–315 nm) being the most carcinogenic. UVB is directly absorbed by DNA, producing dimeric pyrimidine photoproducts including cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimodone photoproducts (6-4PP). However UVA (315–400 nm) can also produce CPD, induce skin tumours in mice, and has been shown to be mutagenic in cell culture. Although the precise role of UVA in human skin cancer remains to be elucidated, it comprises the major portion of solar UV radiation, transmits through window glass and can be delivered in high doses from tanning lamps. Non-steroidal anti-inflammatory drugs (NSAIDs), in particular the 2-aryl propionic acid derivatives, are a well-documented group of photosensitising chemicals producing clinical phototoxic and photoallergic reactions. We have used carprofen, a model compound from this group to see if it could amplify the effects of UVA and contribute to the formation of CPD by UVA. Preliminary work has shown that carprofen combined with low doses of UVA (λ_{max}: 365 nm; 5 J/cm^2) can produce both strand breaks (SB) and CPD in human skin or blood cells. CPD were detected indirectly by both an immunofluorescence method and as T4 endonuclease V sensitive sites in the comet assay. These findings show that compounds other than fluoroquinolones and psoralen derivatives may contribute to CPD formation in skin cells in combination with UVA.

1. Introduction

The effects of UVR on skin are wavelength dependent, with shorter wavelengths in the UVB waveband (280–315 nm) being the most mutagenic and carcinogenic. UVB is directly absorbed by DNA, producing dimeric pyrimidine photoproducts including cyclobutane pyrimidine dimers (CPD) and pyrimidine (6-4) pyrimodone photoproducts (6-4PP) (Halliwell and Gutteridge, 2007).

However UVA (315–400 nm) can also induce CPD in mouse and human skin (Mouret et al., 2006), and has been shown to be mutagenic in rodent cells (Agar et al., 2004; Ikehata et al., 2008; Rochette et al., 2003). UVA can also induce cancer in non-mammalian model systems (Setlow and Woodhead, 1994). Although the precise role of UVA in skin cancer is still to be explained (Runger, 2007; Wang et al., 2001), it comprises the major portion of solar UV radiation and can be delivered in high doses from tanning lamps. The CPD produced by UVA are at a much lower yield than that produced by UVB, appear to be dose-dependent, and are formed by a mechanism thought to be distinct, involving triplet energy transfer from an excited state endogenous chromophore (Besaratinia et al., 2005).

Exogenous chromophores can also photosensitise DNA damage. In fact cutaneous photoreactions are evoked when normally harmless doses of irradiation, usually in the UVA range, are absorbed by a photosensiting drug in the skin inducing the formation of a reactive species. Neither the drug nor the daylight exposure produces a reaction on their own. Drug-induced photosensitivity is responsible for a significant number of referrals to the Dundee Photobiology Unit, forming 7% of 5608 cases between the years 1970 and 2000. The question is whether clinical and subclinical exposure to light-reactive drugs can produce cellular and tissue changes that contribute to a risk of skin carcinogenesis, as has been suggested recently for tetracyclines and photosensitising diuretics (Jensen et al., 2008; Karagas et al., 2007).

Non-steroidal anti-inflammatory drugs (NSAIDs), in particular the 2-aryl propionic acid derivatives, are a well-documented group of photosensitising chemicals producing clinical phototoxic and photoallergic reactions (Holzle et al., 1991). We have used carprofen (Fig. 1), a model compound from this group to study the extent to which exogenous photosensitisers could amplify the effects of UVA and contribute to the genotoxic burden of skin cells. The

* Corresponding author. Tel.: +44 1382 32239; fax: +44 1382 646047.
E-mail address: j.woods@dundee.ac.uk (J.A. Woods).

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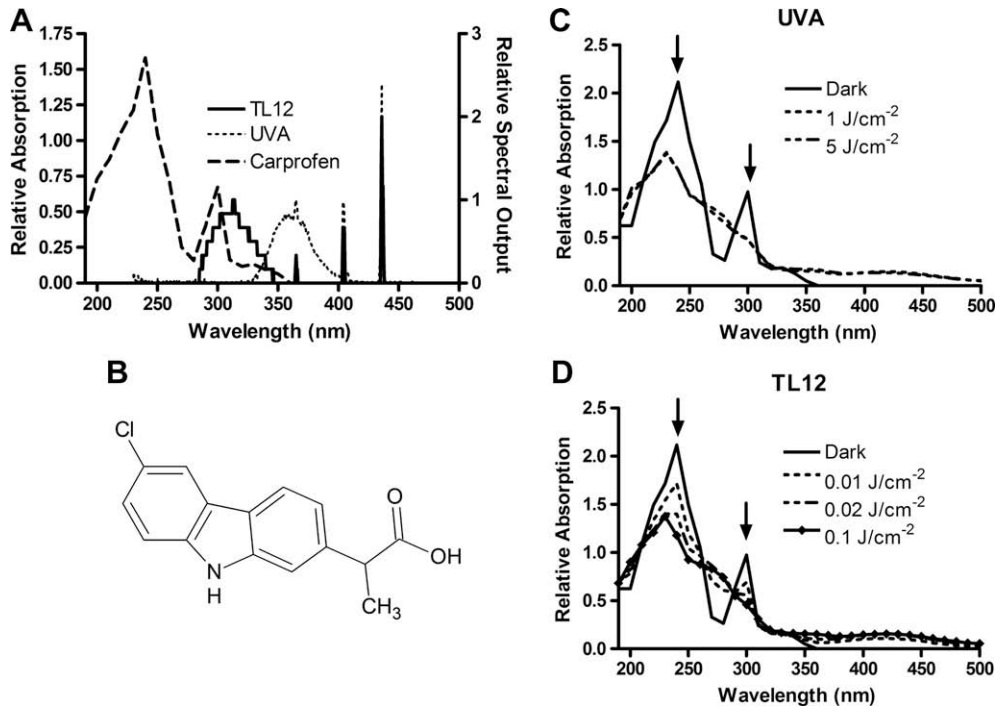

Fig. 1. (A) Absorption spectrum of carprofen in PBS, and the spectral outputs of the lamps used (UVA: dashed line; UVB: solid line). The UVA spectral output was established through the culture vessel lid and the filters. (B) Structure of carprofen. (C) Photodegradation of carprofen with UVA and, (D) UVB.

results show that carprofen combined with UVA can produce both strand breaks (SB) and CPD at doses where either alone is not genotoxic nor cytotoxic.

2. Materials and methods

2.1. Chemicals

All chemicals and cell culture reagents were obtained from Sigma–Aldrich Chemical Company Ltd. (Poole, UK) unless otherwise stated. Disposable sterile cell culture plastics were obtained from Greiner Bio-One (Cambridge, UK). An 'anti-cyclobutane pyrimidine dimer' antibody (Ab: Clone TDM-2 mouse IgG2a κ) against CPD was obtained from Caltag-MedSystems Ltd., (Buckingham, UK). This Ab was established from a mouse hybridoma cell line and binds to CPD in denatured DNA (TT, TC, CT and CC). T4 endonuclease V was obtained from AMS Biotechnology (Europe) Ltd., (Abingdon, UK). Chemicals were prepared on the day of the experiment. Drug solutions were wrapped in foil and experiments carried out under subdued lighting in a specially adapted photobiology laboratory.

2.2. Absorption spectrum and photostability

A stock solution of carprofen (1 mg/ml) was prepared in ethanol (EtOH) and further diluted in EtOH or phosphate-buffered saline (PBS) to give a final concentration of 10 μg/ml. The absorption spectrum of carprofen was measured with a double beam spectrophotometer (Hitachi; model U-3010). For the photostability studies the 10 μg/ml carprofen solution was irradiated with UVA (Cosmedico 1550 100 W, filtered. Irradiance was 2.7 mW/cm^2 as measured using a PUVA meter; Waldmann, Germany, Model 585100); or UVB (Philips TL12 100 W. Irradiance was 916 μW/cm^2 as measured using an International light meter; Model IL 1400A, with broadband filter and Quartz diffuser). UV meters were calibrated to the source in our UKAS accredited Optical Physics laboratory using a double-grating spectroradiometer (Bentham Instruments Ltd., Reading, UK) (Moseley et al., 2009; Traynor et al., 2005). The cuvette containing 10 μg/ml of carprofen was placed 10 cm away from the lamp and the solution stirred continuously throughout. The relative spectral output from the lamps, along with the absorption spectrum and structure of carprofen are shown in Fig. 1A and B.

2.3. Cell culture and maintenance

Human monocytic U937 cells derived from a histiocytic lymphoma (obtained from the European Collection of Cell Cultures) were maintained in RPMI media with 10% (v/v) foetal calf serum (FCS). Cultures were grown in a humidified incubator maintained at an atmosphere of 5% CO_2:95% air at 37 °C. Cells were seeded on the day of the experiment at a density of 2×10^5 cells/ml. HaCaT keratinocytes were a kind gift to the Photobiology Unit by Professor N. Fusenig (German Cancer Research Centre, Heidelberg, Germany) and were maintained in DMEM containing 5% (v/v) FCS and 1% (v/v) non-essential amino acids. HaCaT cells were seeded 18–24 h before the initiation of the experiment at a cell density of 6–7 $\times 10^4$ cells/cm^2. On the day of the experiment, cells were washed with PBS and incubated or not with carprofen or 0.1% DMSO for 1 h in EBSS (37 °C, 5%CO_2:95% air). After this time the cells were irradiated. Dark samples were wrapped in foil and sham irradiated. All cultures were maintained in the absence of antibiotics.

2.4. Monochromatic light irradiation

A stock solution of carprofen (1 mg/ml) was prepared in dimethyl sulfoxide (DMSO) and further diluted in Earl's balanced salt solution (EBSS) to give a final concentration of 1 μg/ml. Incubation of U937 cells with carprofen or 0.1% DMSO was in EBSS for 1 h at 37 °C, 5%CO_2:95% air. Two milliliters of the cell suspension was then transferred to a quartz cuvette containing a magnetic flea.