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Visible light (>395 nm) causes micronuclei formation in mammalian cells without generation of cyclobutane pyrimidine dimers

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Abstract

Solar radiation gives rise to DNA damage in mammalian cells not only directly by excitation of DNA, which generates predominantly pyrimidine dimers, but also indirectly by the excitation of endogenous photosensitizers, which causes oxidative DNA modifications. The latter mechanism has a low quantum yield, but it is the only one proceeding in the visible range of the spectrum. To investigate its relevance for the genotoxicity of sunlight, we have analysed the generation of micronuclei associated with the induction of oxidative DNA damage by visible light in melanoma cells and primary human skin fibroblasts. Similar yields of light-induced oxidative DNA base modifications sensitive to the repair glycosylase Fpg (7,8-dihydro-8-oxoguanine and other oxidative purine modifications) were observed in the normal fibroblasts and the malignant melanoma cells of the same donor. When irradiations were carried out at intervals to compensate for a photodecomposition of the endogenous chromophore, a significant generation of micronuclei was observed in both cell types. Cyclobutane pyrimidine dimers could be excluded to be responsible for the micronuclei induction at wavelengths >395 nm. Experiments with a cut-off filter indicate that the ratio of pyrimidine dimers and Fpg-sensitive oxidative modifications in irradiated cells not only reflects the relative contributions of direct and indirect mechanisms, but is also similar to the ratio by which the two mechanisms contribute to the generation of the micronuclei. The results suggest that indirectly generated oxidative DNA modifications can contribute significantly to the adverse effects of sunlight.

Keywords: Oxidative DNA damage; Visible light; Genotoxicity; Melanoma cells

1. Introduction

Solar radiation generates DNA modifications in mammalian cells by two different mechanisms, i.e. directly by excitation of DNA and indirectly by exci-

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tation of other endogenous chromophores, which act as photosensitizers [1,2]. The first mechanism gives rise to an efficient formation of cyclobutane pyrimidine dimers (CPDs) and (6–4) photoproducts. All other DNA modifications such as oxidative base modifications, single-strand breaks (SSB) and sites of base loss (AP sites) are only minor by-products, i.e. are produced in at least 100-fold lower yields [3,4]. The wavelength dependence of the direct DNA damage generation and of its consequences (the so-called action spectra) basically follows the DNA absorption spectrum with an exponential decrease in the UVB and UVA range [5–7].

The indirect generation of DNA modifications via excitation of endogenous chromophores is much less efficient than the direct damage and therefore probably only biologically relevant at wavelengths at which DNA absorption is negligible, i.e. in the UVA and visible range of the solar spectrum. Mechanistically, most photosensitizers generate predominantly oxidative DNA modifications [1,2,8]. These are formed either following a direct reaction of the excited chromophores with the DNA (type I reaction) or via electron transfer or energy transfer to molecular oxygen, which yields superoxide and singlet oxygen as primary products (type II reaction). In addition, a Förster-type energy transfer from an excited chromophore to DNA can take place and generate CPDs, but not (6–4) photoproducts [9,10]. The selective generation of cyclobutane thymine dimers by UVA (with low yields of (6–4) photoproducts and CPDs involving cytosine) has been suggested to result from such an energy transfer from unidentified chromophores [4,11]

We have previously reported an action spectrum for the generation of CPDs and oxidative purine modifications sensitive to the repair glycosylase Fpg and calculated that most of the oxidative purine modifications induced by solar radiation in mammalian cells are attributable to wavelengths between 400 and 450 nm [3]. This was confirmed in experiments with natural sunlight for various cell types [12]. The number of these oxidative DNA modifications was approximately 15% of that of CPDs in human keratinocytes exposed to natural sunlight and their generation was not inhibited when UV below 400 nm was excluded by a cut-off filter. This suggests that the oxidative modifications are formed by endogenous photosensitizers, which have not yet been identified chemically [12].

The contribution of the visible light-induced oxidative DNA damage to the various adverse effects of solar radiation is not yet known, but it may have been underestimated in many studies for two reasons. Firstly, irradiation at high dose rates causes a rapid saturation of damage generation, probably because of photodecomposition (photobleaching) of the endogenous photosensitizer. Therefore, in genotoxicity tests with high doses applied at high dose rates, the relative contribution of the indirect damage is probably much smaller than under conditions that are relevant for humans. Secondly, the higher penetration of visible light compared to UVB should favour the oxidative DNA damage relative to the generation of pyrimidine dimers in all target cells deeper in the skin. For the etiology of human melanoma, a causal role of oxidative DNA damage is a particular attractive hypothesis, since the incidence of melanoma appears not to correlate with the cumulative UV dose, but rather with the incidence of sunburns, i.e. with conditions of high inflammation-driven oxidative stress [13]. In addition, in melanomas and in melanocytes of melanoma patients an abnormal expression of proteins involved in antioxidant defense and enhanced lipid peroxidation has been observed [14–16] and a defective repair of oxidative base modifications was observed in some melanomas (unpublished results).

To assess the consequences of light-induced oxidative DNA damage, we have quantified both DNA modifications and micronuclei formation in melanoma cells and skin fibroblasts exposed to light from a halogen lamp. The results indicate that visible light generates micronuclei in melanoma cells without induction of pyrimidine dimers, demonstrating – to our knowledge for the first time – genotoxic consequences of solar radiation in this range of the spectrum.

2. Materials and methods

2.1. Cells and enzymes

M8,99 melanoma cells were isolated from a skin metastasis of a 58-year old male patient. From the same person, F8,99 primary skin fibroblasts were obtained. Both cell types were cultured in RPMI 1640 medium with 5% L-glutamine, 10% fetal calf serum, penicillin (100 U/ml) and streptomycin (100 μg/ml).