Free Radical Biology and Medicine 107 (2017) 101–109

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Review Article

Oxidatively-generated damage to DNA and proteins mediated by photosensitized UVA☆

Reto Brem, Melisa Guven, Peter Karran*

The Francis Crick Institute, 1, Midland Road, London NW1 1AT, UK

ARTICLE INFO

Keywords:
UVA
Photosensitizer
DNA lesions
Thiopurines
Thiopyrimidines
Protein oxidation

ABSTRACT

UVA accounts for about 95% of the solar ultraviolet (UV) radiation that reaches Earth and most likely contributes to human skin cancer risk. In contrast to UVB, which comprises the remaining 5% and is absorbed by DNA nucleobases to cause direct photodamage, UVA damages DNA indirectly. It does this largely through its interactions with cellular chromophores that act as photosensitisers to generate reactive oxygen species. Exogenously supplied chemicals, including some widely-prescribed medicines, may also act as photosensitisers and these drugs are associated with an increased risk of sun-related cancer. Because they amplify the effects of UVA on cells, they provide a means to investigate the mechanisms and effects of UVA-induced photodamage. Here, we describe some of the major lesions induced by two groups of UVA photosensitisers, the DNA thionucleotides and the fluoroquinolone antibiotics. In thionucleotides, replacement of the oxygen atoms of canonical nucleobases by sulfur converts them into strong UVA chromophores that can be incorporated into DNA. The fluoroquinolones are also UVA chromophores. They are not incorporated into DNA and induce a different range of DNA damages. We also draw attention to the potentially important contribution of photochemical protein damage to the cellular effects of photosensitised UVA. Proteins targeted for oxidation damage include DNA repair factors and we suggest that UVA-mediated protein damage may contribute to sunlight-induced cancer risk.

1. Introduction

UVA (wavelengths 320–400 nm) comprises more than 95% of the solar UV radiation that reaches Earth, making it far more abundant than UVB (280–320 nm) that accounts for the remainder. Most of UVB and all of UVC (wavelengths below 280 nm) are removed by the ozone layer and these shorter wavelengths are not present in incident sunlight. UVA is classified as “probably carcinogenic to humans” by WHO IARC [1] although, unlike UVC and UVB, it is absorbed poorly by canonical nucleotides and therefore causes much less damage to cellular DNA [2]. UVA-mediated DNA damage occurs partly by indirect mechanisms *via* interactions with cellular chromophores that act as photosensitisers to generate DNA-damaging reactive oxygen species (ROS). Depending on the distance between the chromophore and the target, UVA irradiation can also result in one-electron abstraction and the formation of a reactive radical cation. Importantly, UVA-generated ROS damage other biomolecules including proteins and lipids, and this non-DNA photodamage may be an important contributor to the biological effects of UVA such as carcinogenesis and photoaging.

Endogenous UVA chromophores have not been fully characterized, although porphyrins, flavins [3], melanin [4] and UVB photoproducts of tryptophan (6-formylindolo[3,2-*b*]carbazole, FICZ) [5] are among the potential candidates. Studies employing exogenous UVA chromophores that mimic and amplify the effects of their endogenous counterparts, provide a useful strategy to investigate events associated with UVA photosensitisers. Many of these chemicals have been used in various aspects of nucleic acid research. More importantly, some are widely-prescribed pharmaceuticals and their use is associated with an increased skin cancer risk. All these drugs have significant UVA absorbance and sensitise the formation of a variety of DNA and protein lesions. Although generally non-toxic, their foremost unifying feature is an extreme cytotoxicity in combination with low doses of UVA. This review will discuss DNA damage induced by photoactivation of thiopurines, thiopyrimidines and the fluoroquinolone group of antibiotics. We will also consider potentially important effects of photochemical damage to the proteome – particularly to DNA repair proteins.

☆ This article is one of a series of papers on the subject of oxidative DNA damage & repair that have been published as a special issue of Free Radical Biology & Medicine to commemorate the Nobel Prize won by Prof. Tomas Lindahl. A detailed introduction and synopsis of all the articles in the special issue can be found in the following paper by Cadet & Davies [96].

* Corresponding author.
E-mail address: peter.karran@crick.ac.uk (P. Karran).

http://dx.doi.org/10.1016/j.freeradbiomed.2016.10.488
Received 4 August 2016; Received in revised form 19 October 2016; Accepted 21 October 2016
Available online 27 October 2016

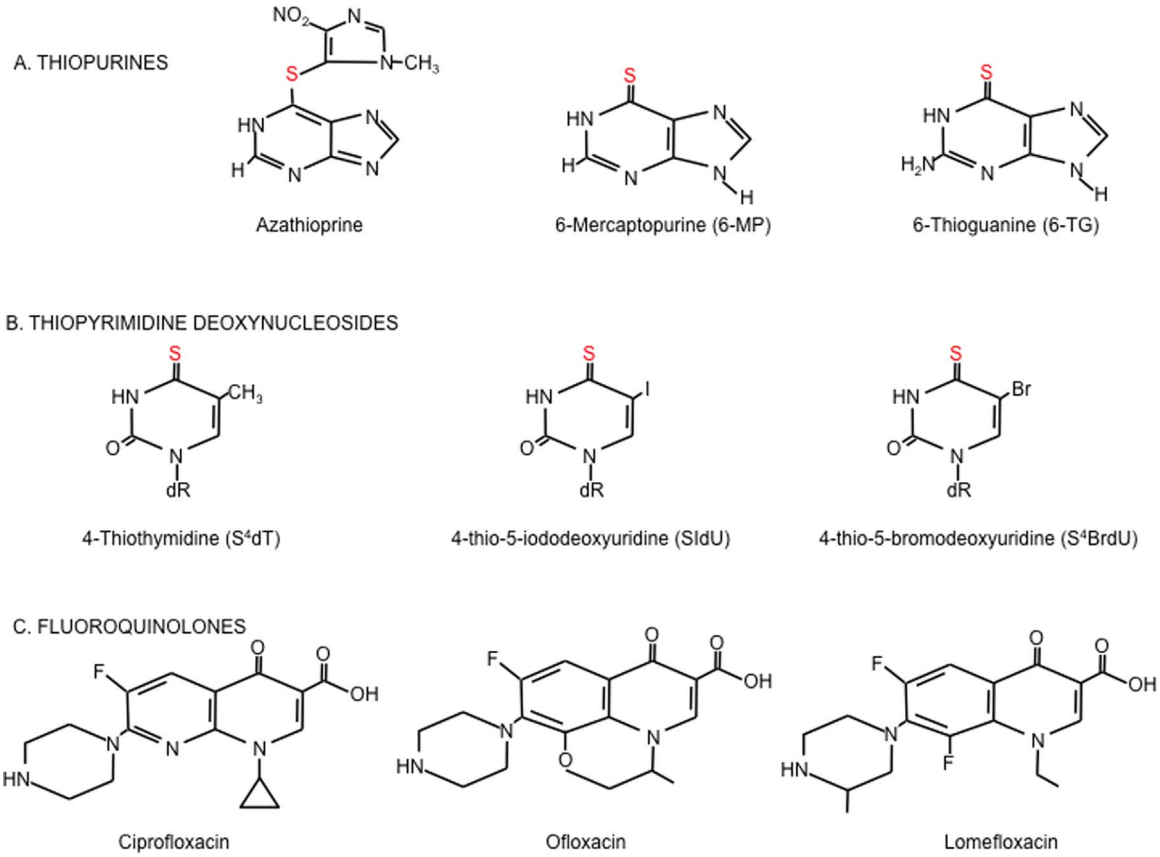

Fig. 1. Structures of UVA photosensitisers. Azathioprine, mercaptopurine and 6-thioguanine are all converted to 6-TG deoxyribonucleotides, which are in turn incorporated into DNA. This is a prerequisite for the clinical effectiveness of thiopurines. Thiopyrimidine deoxynucleosides are incorporated into DNA of cells *via* the TK-dependent pyrimidine nucleoside salvage pathway. The fluoroquinolone class of antibiotics acts as inhibitors of DNA topoisomerases and intercalate rather than incorporate into DNA.

2. UVA photosensitisers

i) *Thiopurines*

The thiopurines azathioprine, mercaptopurine and 6-thioguanine (6-TG) (Fig. 1A) are effective anticancer, anti-inflammatory and immunosuppressant drugs (reviewed in [6]). They are all prodrugs that undergo enzymatic conversion that culminates in the formation of the thiopurine nucleotides that are an absolute requirement for their clinical effectiveness. Despite more than half a century of clinical use, the molecular events underlying thiopurine cytotoxicity are still not fully understood. Suggested mechanisms include inhibition of *de novo* purine synthesis resulting in an inadequate supply of purine nucleotides for replication and transcription [7] and interference with intracellular signalling pathways *via* competition for GTP binding by G proteins [8,9]. Thioguanine nucleotides are substrates for incorporation into DNA and to a lesser degree into RNA and the biological effects of thiopurines are at least partly dependent on the formation of DNA 6-TG [7,10]. DNA 6-TG may undergo *in situ* non-enzymatic methylation that can provoke ultimately lethal processing by DNA mismatch repair [11,12]. Alternatively, it can participate in the formation of DNA interstrand-crosslinks [13] that are highly toxic in a mismatch repair-independent manner. The methylated form of DNA 6-TG miscodes during replication and it is noteworthy that azathioprine treatment is associated with a perceptible increase in mutation frequency in circulating lymphocytes [14] and with an increased risk of leukemia [12]. Most striking, however, is the greater than 100-fold higher risk of skin cancer in immunosuppressed organ transplant patients [15], most of whom will have been prescribed azathioprine and whose skin contains detectable amounts of DNA 6-TG [16]. Its more intermittent use in the management of inflammatory bowel disease entails a lower, but still significant skin cancer risk [17–19]. Sunlight exposure is a contributory factor in thiopurine-related skin cancer. The skin of patients taking azathioprine is photosensitive to UVA but not to UVB, consistent with the absorbance maximum of DNA 6-TG at around 340 nm. This has led to the suggestion that the photochemical reactions of azathioprine or its metabolites [20] may contribute to skin cancer risk [21].

ii) *Thiopyrimidines*

4-Thiothymine (S^4T) is not currently used clinically although it has been proposed as a potential UVA photosensitiser for treatment of skin malignancies [22,23]. Like 6-TG, S^4T is derived from a canonical DNA base in which the replacement of a single oxygen atom by sulfur converts it to a UVA chromophore and S^4T has an absorbance maximum at 335 nm. The S^4T deoxyribonucleoside, 4-thiothymidine (S^4dT) (Fig. 1B), is a good substrate for thymidine kinase (TK) and S^4T is extensively incorporated into DNA of cells treated with S^4dT *via* the TK-dependent pyrimidine nucleoside salvage pathway [24]. Despite its accumulation to higher levels than DNA 6-TG and the ability to undergo facile *in situ* methylation, DNA S^4T is not detectably toxic. The absence of cytotoxicity has been ascribed to the preferential formation of structurally and thermodynamically good base pairs by both DNA S^4T and its methylated counterpart that obviates their engagement by DNA mismatch repair, the major contributor to DNA 6-TG toxicity [25]. DNA S^4T is, however, extremely cytotoxic in combination with low doses of UVA [24]. Recently, 2,4-dithiothymidine has been shown to be comparable or superior to 4-thiothymidine as a photosensitiser in solution and it is cytotoxic in combination with UVA [26,27].

The halopyrimidine nucleosides 5-iodo-2′-deoxyuridine (IdU)