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Mechanism of guanine-specific DNA damage by UVA and its role in photocarcinogenesis and photoaging

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Solar UV light is a well-known carcinogen. UVA radiation is probably carcinogenic to humans. In addition, recent investigations point to the importance of UVA irradiation in the photoaging. We investigated the mechanism of sequence-specific DNA damage using ^{32}P-labeled DNA fragments in relation to carcinogenesis and aging. Furthermore, we investigated whether UVA accelerates the telomere shortening in human WI-38 fibroblasts. The exposure of double-stranded DNA fragments to 365 nm light in the presence of endogenous sensitizers produced sequence-specific cleavage at the 5' site of 5'-GG-3' and 5'-GGG-3' sequences. In addition, HPLC analysis revealed that sensitizers plus 365 nm light increased the 8-oxodG content of double-stranded DNA. We discuss the mechanisms of guanine-specific DNA damage caused by excited photosensitizers in relation to carcinogenesis and aging.

Key words: UVA, DNA damage, carcinogenesis, aging, telomere

1. Introduction

Solar UV light containing UVA and UVB is the major source of human exposure to UV radiation. UVB is directly absorbed by the DNA molecule to form cyclobutane pyrimidine dimers and pyrimidine (6-4) pyrimidone photoadducts, resulting in mutation and carcinogenesis. Recent studies on UV carcinogenesis have revealed that UVA is also mutagenic and carcinogenic [1]. Although carcinogenic potential of UVA is much smaller than that of UVB, UVA, which comprises approximately 95% of solar UV radiation, may play an important role in carcinogenesis. UVA-induced DNA damage presumably involves indirect mechanisms by which UV-absorbing substances (photosensitizers) are activated to produce reactive species causing DNA damage [2, 3], because only little UVA can be absorbed by the DNA molecule. Therefore, solar carcinogenesis would involve UVA-induced oxidative DNA damage. Repeated exposure of human skin to solar UV radiation leads to not only skin carcinogenesis but also photoaging. Here we discuss the mechanisms of guanine-specific DNA damage caused by excited photosensitizers in relation to carcinogenesis and aging.

2. Photocarcinogenesis

2-1. Type I mechanism

The Type I mechanism involves electron transfer through the interaction of an excited photosensitizer with DNA base. This mechanism is dependent on the oxidation potential of the DNA base and the reduction potential of the excited photosensitizer. We have demonstrated that 365-nm UVA radiation in the presence of endogenous sensitizers, riboflavin (vitamin B_2) [4, 5], pterin and its derivatives [6], causes damage to double-stranded DNA fragment specifically at the 5'-G of GG sequences whereas little or no damage was found at single guanines. Nalidixic acid (NA) is a quinolone antibacterial used for the treatment of urinary tract infections. NA has been reported to cause skin tumors in experimental animals exposed to UVA. We have found that NA causes damage to DNA fragments obtained from the human c-Ha-*ras*-1 protooncogene and the *p53* tumor suppressor gene upon UVA irradiation [7, 8]. DNA damage was observed only when DNA fragments were treated with piperidine,

suggesting that the damage is due to base modification with little or no strand breaks. NA caused photodamage in double-stranded DNA particularly at the 5'-G in 5'-GG-3' sequence. In single-stranded DNA, guanines were specifically damaged, but the site specificity for consecutive guanines was not observed. The measurement of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) with an HPLC coupled with an electrochemical detector (HPLC-ECD) showed that 8-oxodG was formed more efficiently in double-stranded DNA than in single-stranded DNA. These results suggest that the double-helical structure contributes to efficient NA-induced DNA photodamage and determination of its site specificity. The energy level of the highest occupied molecular orbital (HOMO) of guanine is highest among the four DNA bases, and therefore, guanine is most likely to be oxidized. Recently, more detailed calculations have revealed that a large part of HOMO is concentrated and electron-loss centers are localized on the 5'-G of GG doublets in the B-form double-stranded DNA, and that stacking of two guanine bases significantly lowers the ionization potential. Therefore, electron transfer occurs specifically at this site to produce the guanine cation radical. Oxidative damage to guanines mediated by electron transfer can be followed by long-range electron transfer leading to oxidation of guanines at remote sites [9].

2-2. Type II mechanisms

The major Type II mechanism involves energy transfer from an excited photosensitizer to molecular oxygen to produce $^1O_2$ with a relatively long lifetime. $^1O_2$-mediated DNA damage is specifically caused at every guanine. UVA radiation caused DNA photolesions at guanine residues through the generation of $^1O_2$ in the presence of hematoporphyrin [4, 10] and methylene blue [11]. Exogenous molecules may cause cancer through DNA photodamage in a similar mechanism. Fluoroquinolone antibacterials have recently been used for a variety of infectious diseases. Fluoroquinolones have been reported to cause skin tumors in animals exposed to UVA. LFLX induced a number of squamous cell carcinoma in animals, and is distributed to skin more efficiently than other fluoroquinolones. Our experiment showed that LFLX caused DNA damage specifically at guanines by generating $^1O_2$ [8].

Therefore, solar carcinogenesis would involve not only UVB-induced DNA photoadduct formation but also UVA-induced oxidative DNA damage mediated by excited photosensitizers.

3. Photoaging

Repeated exposure of human skin to solar UV irradiation leads to skin photoaging. Increasing evidence demonstrates that UVA, as well as UVB, contributes to photoaging. In humans, telomere shortening is believed to be associated with cell senescence. Telomere plays essential roles in chromosomal structure and function, including stabilization of the chromosome during DNA replication and possible prevention of aberrant chromosomal recombination. Telomere in vertebrates contains highly conserved repeats of a characteristic hexameric sequence (5'-TTAGGG-3'). In human fibroblasts, the telomere length is decreased by 50–200 base pairs (on average, approximately 90 base pairs) per cell division under the normal condition. Recently, Zglinicki *et al.* reported an increase of the rate of telomere shortening by oxidative stress in human fibroblasts [12]. Telomere is shortened by approximately 500 base pairs per cell division under hyperoxic conditions. However, the mechanism for the increase of telomere shortening rate by oxidative stress remains to be clarified. In this study, we investigated shortening rate of telomeres in human WI-38 fibroblasts exposed to UVA irradiation. We also examined the formation of 8-oxodG in human cultured cells by using an HPLC-ECD. Furthermore, we investigated the mechanism for increase of telomere shortening induced by UVA irradiation using ^{32}P 5' end-labeled DNA fragment including the telomeric sequence. The ^{32}P-labeled DNA fragment was exposed to UVA irradiation in the presence of riboflavin, as a model of endogenous compound, and subsequently treated with *E. coli* formamidopyrimidine-DNA glycosylase (Fpg). Fpg protein is a DNA glycosylase that removes 8-oxodG from DNA. We also

measured the photodynamic 8-oxodG formation with riboflavin in DNA fragments including the telomeric sequence by using an HPLC-ECD.

The terminal restriction fragment (TRF) from WI-38 fibroblasts irradiated with UVA decreased with increasing the irradiation dose [13]. Furthermore, UVA irradiation dose-dependently increased the formation of 8-oxodG in both WI-38 fibroblasts and HL-60 cells. UVA irradiation with riboflavin induced 8-oxodG formation in the ^{32}P-labeled DNA fragments containing telomeric sequence, and Fpg protein treatment led to chain cleavages at the central guanine of 5'-GGG-3' in telomere sequence. The amount of 8-oxodG formation in DNA fragment containing telomere sequence (5'-CGC(TTAGGG)$_7$CGC-3') was approximately 5 times more than that in DNA fragment containing non-telomere sequence (5'-CGC(TGTGAG)$_7$CGC-3') [14]. These results indicate that the photoexcited endogenous photosensitizer specifically oxidizes the central guanine of 5'-GGG-3' in telomere sequence to produce 8-oxodG probably through an electron transfer reaction. In GGG triplets, HOMO is mainly distributed on the 5'-G, and therefore, this guanine is easily oxidized by electron transfer. However, the guanine cation radical formed at the 5'-G is reduced by electron transfer from the middle guanine (hole migration), because the radical on the middle G is speculated to be the most stable in certain GGG triplets. Therefore, the middle G is most likely to be damaged. The guanine cation radical produced by electron transfer undergoes hydration, followed by subsequent oxidation to 8-oxodG. It is concluded that the site-specific damage in telomere sequence induced by UVA irradiation may participate in increase of telomere shortening rate.

4. Conclusion

A variety of reactive species mediate DNA damage and play critical roles in human diseases, particularly cancer. DNA damage causes mutations, which can lead to activation of protooncogenes and inactivation of tumor suppressor genes, resulting in carcinogenesis. The major oxidation product of guanine in DNA appears to be 8-oxodG in both mechanisms. It is interesting that recent study has shown the increase of 8-oxodG in epidermal cells of hairless mice after chronic UV exposure. It has been known that 8-oxodG is a miscoding lesion, which induces G→T transversion mutations [15]. As a consequence, it is assumed that, in Type I mechanism, the 5' G of contiguous guanines is the G→T mutation hotspot. Actually the mutations such as GGT→TGT and GGC→TGC has been observed in *ras* oncogenes [16]. These mutation may be explained by 8-oxodG formation at the 5' G of 5'-GG-3' and subsequent mutagenic replication (Fig. 1).

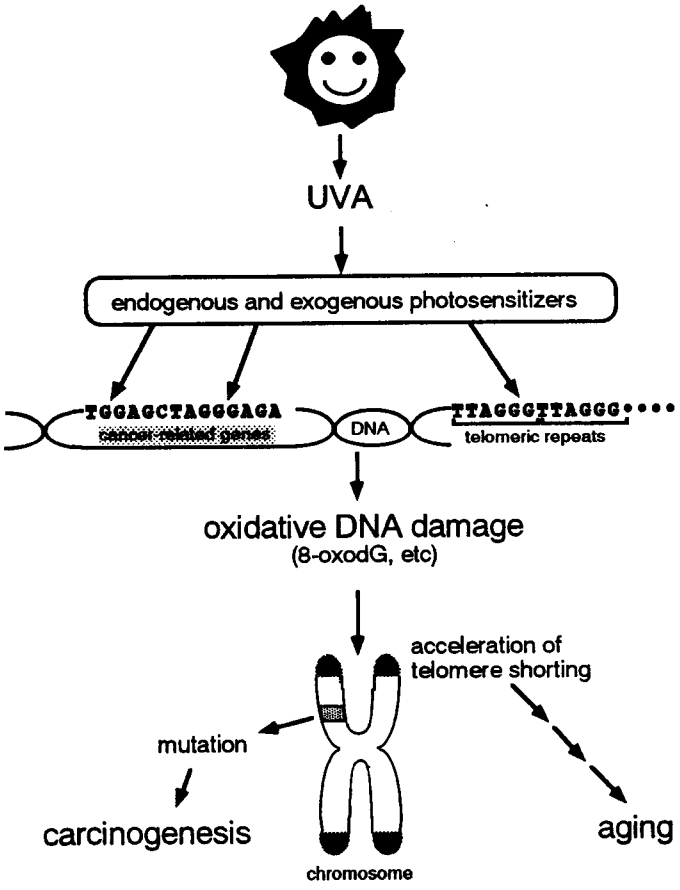

Fig. 1. Mechanism of oxidative DNA damage by UVA and its role in photocarcinogenesis and photoaging.

Oxidative stress may function as a common trigger for activation of the senescence program. UVA irradiation with riboflavin induced 8-oxodG formation in the DNA fragments containing the telomeric sequence, and Fpg protein treatment led to chain cleavages at the central guanine of 5'-GGG-3' in the telomere sequence. The site-specific damage in telomere sequence induced by UVA irradiation may participate in increase of telomere shortening rate (Fig. 1). Furthermore, it is concluded that the structure or location of telomeres in the nucleus may increase susceptibility to the DNA oxidation compared with other internal chromosome targets.

Although UVB has been believed to be responsible for solar carcinogenesis and aging, UVA-induced DNA damage in the presence of photosensitizers may play an important role in photocarcinogenesis and photoaging.

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