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Mutation, DNA Strand Cleavage and Nitric Oxide Formation Caused by *N*-nitrosoproline with UVA & UVB

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N-Nitrosoproline(NPRO) is endogenously formed from proline and nitrite. NPRO has been reported to be nonmutagenic and noncarcinogenic. In this study, we have detected the direct mutagenicity of NPRO with UVA and UVB towards *S. typhimurium*. Formation of 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG), a mutagenic lesion, was observed in calf thymus DNA treated with NPRO plus simulated sunlight. Furthermore, the treatment with NPRO and sunlight induced single strand breaks in the superhelical replicative form of phage M13mp2 DNA. An analysis using scavengers suggested that both reactive oxygen species and NO radical mediate the strand breaks. The formation of nitric oxide was observed in NPRO solution irradiated with UVA. The co-mutagenic and co-toxic actions of NPRO and sunlight merit attention as possible mechanisms increasing the carcinogenic risk from UVA irradiation.

key words: *N*-Nitrosoproline, UVA, UVB, Mutation, NO formation, 8-oxodG, Carcinogenic risk

INTRODUCTION

There are many reports on the endogenous formation of *N*-nitroso compounds. Ohshima and Bartsch reported the formation of *N*-nitrosoproline (NPRO) in humans from sodium nitrite and proline, based on the finding of NPRO in the urine from male volunteers [1]. Greater amounts of NPRO are found in the smoker's urine at the level of 6 mg/ 24 hr.

Solar ultraviolet radiation (UV) is a causal factor for skin cancer in humans. Ultraviolet-A radiation (UVA) (320 nm-400 nm) is not absorbed very much by DNA, but tumor development was observed in the albino hairless mice exposed to UVA source [2]. UVA can reach to the subcutaneous area of the skin containing blood vessels. Earlier studies of our laboratory have shown that *N*-nitrosodialkylamines can be converted into directly mutagenic compounds on UVA irradiation and that oxidative species are produced during the photoreaction [3,4]. Although NPRO was reported to be noncarcinogenic and nonmutagenic in animal feeding experiments, perhaps because NPRO was not metabolized in vivo, we suspected that endogeneous NPRO in skin might work as a photosensitizer of sunlight UVA and play a role in photo-carcinogenicity.

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MATERIALS AND METHODS

N-Nitrosoproline (NPRO) was a gift form Dr. M. Mochizuki of Kyoritsu College of Pharmacy, who checked the purity as > 99% by high performance liquid chromatography. Irradiation and the detection of the mutagenicity produced were performed as described in our previous work [4]. 8-oxodG in calf tyhmus DNA treated with NPRO plus UVA and UVB were measured according to the previous work [3]. The DNA single strand breaks were detected by irradiating phage M13mp2 replicative form DNA (RF I) [4]. Nitric oxide in the irradiated solution of NPRO was measured as No_2^-.

RESULTS AND DISCUSSION

Figure 1 shows the direct-acting mutagenicity of UVA- and UVB-irradiated NPRO towards *S. typhimurium* TA1535. The mutagenicity formed from NPRO+UVA was higher than that from NPRO+UVB. The mutagenesis was irradiation time-dependent. When superhelical DNA (RF I) was irradiated with UVA in the presence of NPRO, a new band for nicked circular DNA (RF II) appeared (data not shown). Strand breaks occurred at an NPRO concentration as low as 10 μM. NPRO alone or sunlight alone did not induce these single strand breaks. On UVA irradiation of calf thymus DNA in the presence of NPRO, the formation of 8-oxodG occurred (data not shown). The 'NPRO+UVA' - mediated single strand breaks in M13mp2DNA were decreased by adding either an OH-

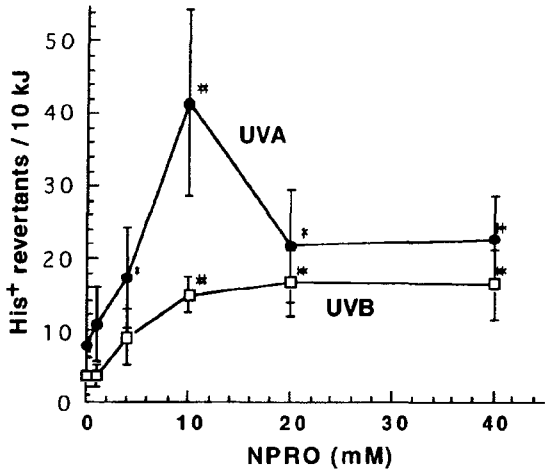

Figure 1. Formation of direct-acting mutagenicity from NPRO irradiated with UVA (●) and UVB (□). The intensity of UVA was 4.94 W/m^2 at 360 nm and that of UVB was 5.82 W/m^2 at 315 nm. *: $P<0.05$ and **: $P<0.01$; significantly different from the results in the dark-control (t-test).

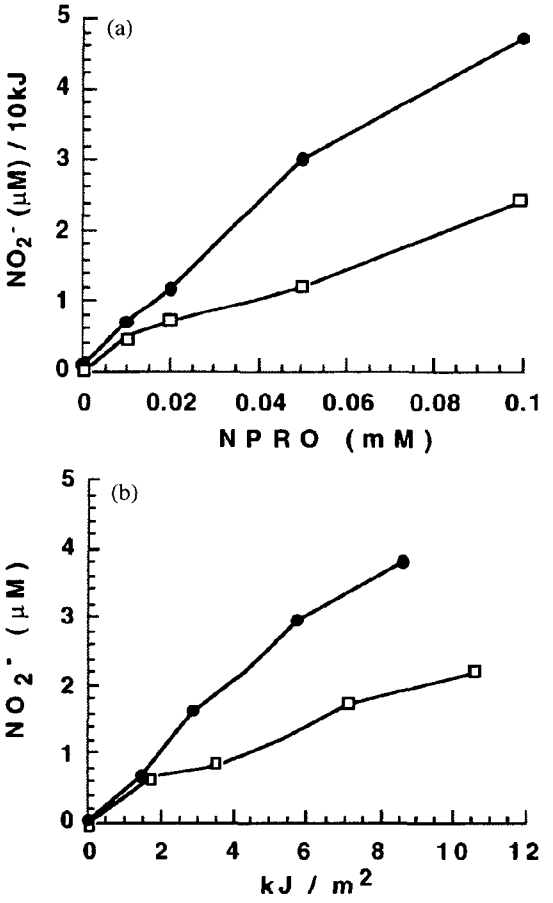

Figure 2. Formation of NO radical from NPRO with UVA (●) and UVB (□). (a) Dose Dependence on NPRO. Irradiation was for 20 min at 4.61 W/m^2 (UVB) and for 15 min at 5.92 W/m^2 (UVB). (b) Dependence on irradiation time. NPRO, 01. mM.

radical scavenger (mannitol), scavengers of singlet oxygen (histidine and NaN_3) or an NO-radical scavenger(2-(4-carboxyphenyl)-4,4,5,5-tetramethylomodazoline-1-oxyl-3-oxide) (data not shown). Nitric oxide formation was observed in the NPRO solution irradiated with UVA and UVB was dependent both on the NPRO concentration (Figure 2a) and on the irradiaiton time (Figure 2g). The amounts of NO formed from NPRO+UVA was higher than that from NPRO+UVB. NO was reported to induced mutations in cultured human cell and in *S. typhimurium* [5]. The observations showin in Figure 2 suggest that reactive-oxygen species and nitric oxide are also involved in the photo-process of NPRO. Therefore, it is likely that NPRO works as a photosensitizer in these reactions: a photon-excited NPRO molecule may be converted to a mutagenic compound, and the photo-energy may be transferred to other molecules to produce radicals. The intensity of UVA used in the UVA irradiation experiments are comparable with those in sunlight. The results suggest an additional way in which UVA may be involved in the etiology of skin cancer.

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