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UVA Light-Induced Toxic Effects of 1-Hydroxypyrene on Human Jurkat T-Cells

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Polycyclic aromatic hydrocarbons (PAHs) and their derivatives constitute one of the largest groups of chemical carcinogens and mutagens. These environmental pollutants are primarily a class of stable organic molecules made up of carbon and hydrogen atoms entering the nature through incomplete burning of organic materials (Connell et al. 1997; Dipple 1985). Sources of PAHs include household heating, coal-fired power plants, automobile, cigarette smoke, and emissions from waste incineration plants. PAHs with polar substituents such as hydroxy and amino groups are found in natural waters and 1-hydroxypyrene (1-HOP) is widely used as a biomarker for studying PAH exposure (Dor et al. 1999; Jongeneelen 1994). Mechanistic studies reveal that DNA can be damaged if the DNA is concurrently exposed to PAH and light (Yu 2002). Light irradiation can also increase the toxicity of PAHs significantly by forming toxic species (Arfsten et al. 1996).

Previous investigations and research in progress have defined several important aspects of the photo-induced toxicity of PAHs, showing it is phototoxic to a variety of microorganisms, cell lines, animals, and humans (Huang et al. 1997; Hwang et al. 2001; Landrum et al. 1987; Mekeyan et al. 1994). In this investigation, the phototoxicity of 1-HOP is tested on a human immune cell line, Jurkat T-cells, using two methods: 1) single cell gel electrophoresis (Comet assay) to determine the damage to the cell nuclear DNA and 2) cell viability by fluorescein diacetate uptake to determine cytotoxicity.

T-cells are the main cellular elements for cell-mediated immune responses. PAHs, like any other environmental toxins, may be present in the T-cells. Therefore, we use an established human T-cell line to mimic the *in vivo* paradigm. This is also a first attempt to understand the *in vitro* mechanism involved in eliciting a typical T-cell-mediated response to 1-HOP.

MATERIALS AND METHODS

1-Hydroxypyrene (98%) was purchased from Sigma-Aldrich (Milwaukee, WI) and used without further purification. Stock solutions (10 mM) were prepared in

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HPLC grade methanol and stored in refrigerator. Media and chemicals for cell cultures were purchased from Gibco (Grand Island, NY). Fetal bovine serum (FBS) was obtained from Hyclone Laboratories (Utah). The human Jurkat T-cells were obtained from American Type Culture Collection (Rockville, MD; Jurkat TIB152). The cells were cultured in a humidified atmosphere with 5% CO_2 at 37°C for 24 h. RPMI 1640 medium was used for these non-adherent cells. Media were made complete by adding 10% FBS and 1% Fungizone (pen/strep).

After cells grew to the expected concentration of no less than 1×10^5 cells/mL, the cells were harvested and centrifuged at 2200 ×g for 5 min. The supernatant was discarded and the pellet was washed twice with 1× phosphate buffer saline (PBS). Finally the pellet was resuspended in 1× PBS to reach the cell concentration of 5×10^5 cells/mL. The whole process was conducted at 4°C. The above cell suspension was placed in a 96-well plate with 100 μL in each well. Two nine-well sets were used in each 96-well plate for each 1-HOP concentration. One set was covered with aluminum foil as dark control, and the other was irradiated by UVA light using a type B 100 W UVA lamp from UVP Inc. (Upland, CA). The lamp emits a UVA light band near 365 nm with an irradiance of 170 J/cm^2 per h at a distance of 6 cm. Among the nine wells of each set, six wells were used for cell viability assays and the remaining three for the Comet assay. The concentrations of 1-HOP at 0.2, 1.0, 5.0, 25, and 100 μM were obtained through series dilution of the 10 mM 1-HOP stock solution with 1× PBS. Later the 1-HOP concentrations were adjusted to 0.1, 0.25, 0.75, 2, and 5 μM because cell death was observed for concentrations greater than 5 μM under UVA irradiation.

For cell viability assay, six wells of the UVA-irradiated cell suspensions of each set were added with 100 μL of Fluorescein Diacetate (FDA, 10 ng/mL) and the plates were incubated at 37°C for 35 min. The plates were read using a Fluoroscan Ascent FL (Thermo Labsystems) with filters set at emission wavelength of 485 nm and excitation of 538 nm.

For the Comet assay, the rest of the three wells of the UVA-irradiated cell suspensions of each set were transferred to 1 mL vials and centrifuged at 2200 ×g for 5 min. The pellets were washed twice with cold 1× PBS (4°C) and resuspended in media to obtain a cell concentration of 1×10^5 cells/mL. In a 2 mL tube, 20 μL of the cell suspension and 200 μL of melted agarose were mixed, after which 75 μL of it was pipetted onto a pre-warmed slide. The slides were placed in a refrigerator at 4°C for 10-20 min before placed in chilled lysis buffer for 45 min. Slides were then removed from the buffer and washed twice for 5 min with tris-borate-EDTA buffer (TBE) before placed in a horizontal gel electrophoresis box and ran at 25 V for 10 min. After electrophoresis, slides were placed in 70% ethanol for 10 min, removed, tapped to remove excess ethanol and placed in a 1% NaOH solution (100 mL) containing 0.1 μM EDTA for 45 min. Slides were air-dried for 2.5 h before stained with SYBR Green and allowed to set for 4 h. The slides were read using DNA Damage Analysis Software (Loates

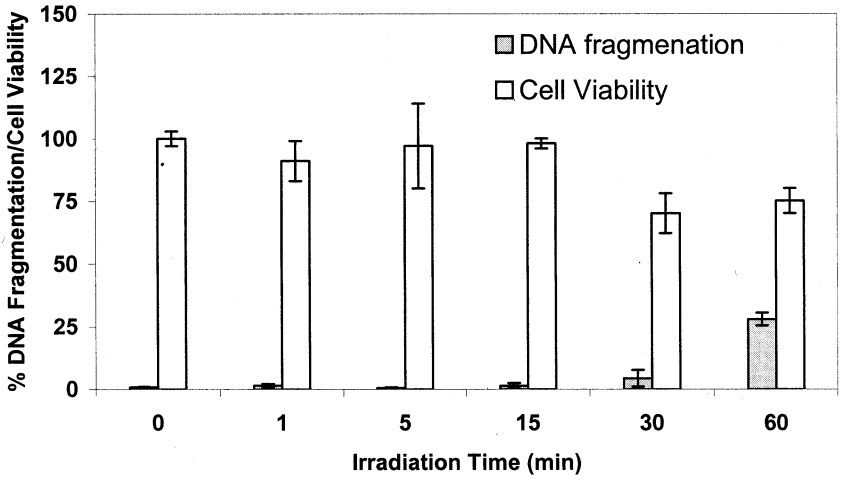

Figure 1. Percent of DNA fragmentation and percent of viable cells caused by UVA (170 J/cm^2/h) irradiation on the human Jurkat T-cells. The amount of DNA damage was determined by Comet assay and was repeated twice using three wells.

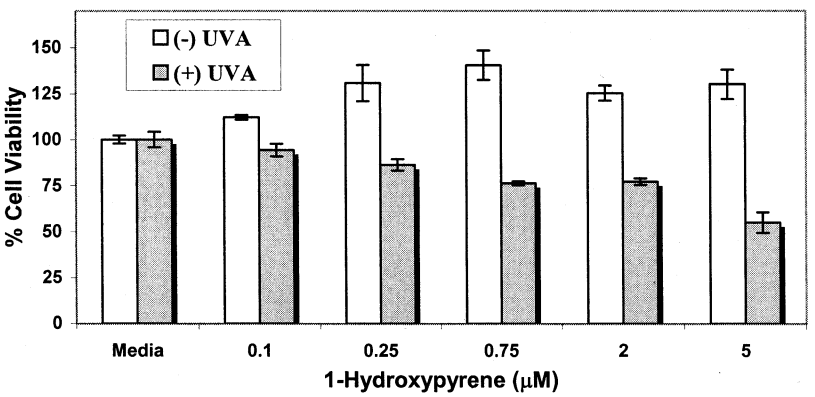

Figure 2. Cell viability count of human Jurkat T-cells after treatment with 1-hydroxypyrene with or without UVA irradiation (42 J/cm^2 or 15 min of irradiation). Total cell numbers were around 10,000 cells/well. Experiments were repeated twice using six wells.

Associates, Inc.). Two sets of DNA damage parameters, percent of DNA fragmentation and Comet tail moment, were used. The percent of DNA fragmentation is defined as the percent of DNA in the Comet tail versus total DNA from both the Comet tail (DNA fragments) and Comet head (nucleus). The Comet tail moment is the product of the distance and normalized intensity integrated over the tail length for a damage measure combining the amount of

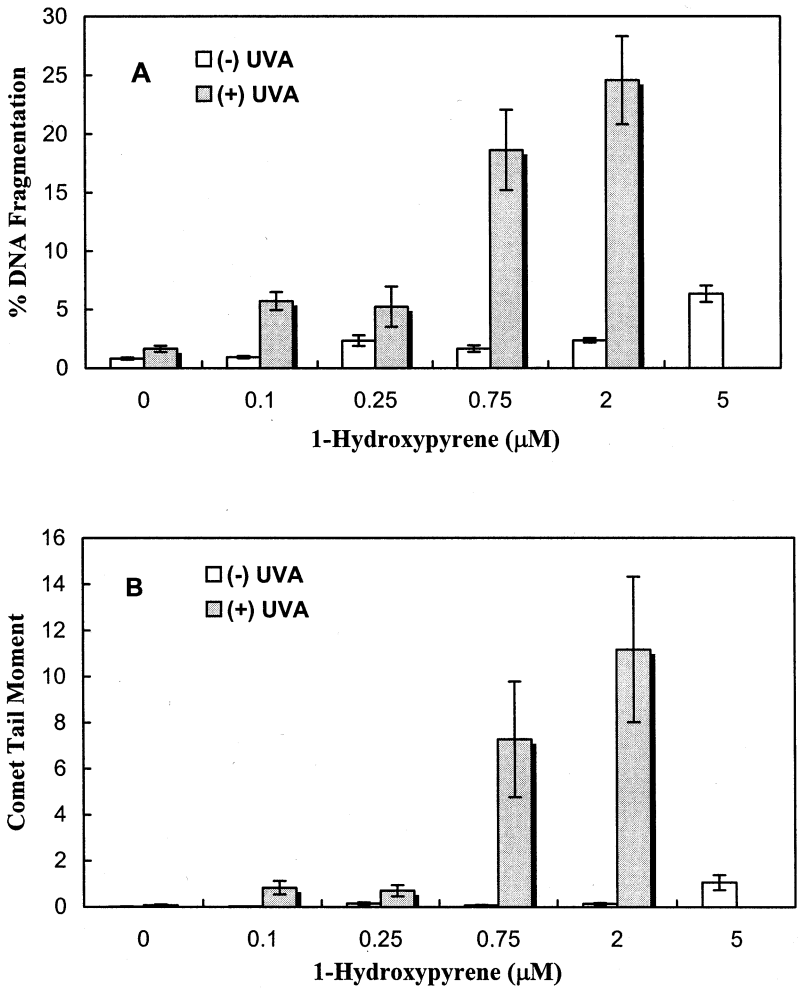

Figure 3. DNA damage measured by the percent of DNA fragmentation (A) and Comet tail moment (B) for human Jurkat T-cells in the presence of 1-hydroxypyrene upon UVA (15 min or 42 J/cm^2) irradiation. A total of 70 cells were scored and eight individual median scores were chosen for calculation of data on this graph.

DNA in the tail with the distance of migration. A total of 70 cells per sample were scored and eight individual median scores were chosen for calculating the percent of DNA fragmentation and tail moment.

RESULTS AND DISCUSSION

UVA light irradiation alone causes damages to the cellular nuclear DNA only at

high doses as seen by the Comet assay conducted immediately after UVA irradiation. There is no appreciable effect on the cellular DNA with UVA irradiation of up to 15 min, or a dose of 42 J/cm^2 (Figure 1) on either the cell viability or on DNA fragmentation. However, at higher UVA doses (30 or 60 min of irradiation), about 25% of the cells died. A closer examination by Comet assay revealed that the cellular DNA was damaged by about 28% for 60 min of irradiation and there was only about 5% of DNA damage for 30 min of irradiation (Figure 1). Therefore, the irradiation time of 15 min or a UVA dose of 42 J/cm^2 was chosen for all the experiments in order to minimize the effect of UVA alone on the cells.

Treatment of human Jurkat T-cells with 1-HOP at concentrations of 0, 0.1, 0.25, 0.75, 2, and 5 μM does not inhibit cell growth (Figure 2). On the contrary, the cells seem to be able to grow to as much as 40% under these conditions. However, the reason for cell growth is not known at this point. Irradiation with UVA light (15 min) on the cells in the presence of 1-HOP causes the cells to die in a dose dependent manner (Figure 2). At the highest 1-HOP concentration used (5 μM), about 40% of the cells died due to the concurrent exposure to 1-HOP and UVA light.

Irradiation of the cells in the presence of 1-HOP with UVA light (15 min) also caused damages to the nuclear DNA of the T-cells (Figure 3A). The percent of DNA fragmentation is dose dependent on the concentration of 1-HOP from 0 to 2 μM, but cell death was observed at 5 μM of 1-HOP. At this 1-HOP concentration, the cellular DNA was so fragmented that there were not enough useful cells left for analysis. The same trend is also observed for the concentration dependence of the Comet tail moment (Figure 3B).

Previously, we have demonstrated that combination of 1-HOP and UVA light can cause single strand DNA cleavage on pure plasmid DNA and form covalent DNA adducts with calf thymus DNA (Dong et al. 2000b). The results presented here show that the combination of 1-HOP and UVA light can cause damages to the nuclear DNA of T-cells. In order for this to happen, 1-HOP molecules must be present in the proximity of the cell nuclear DNA. This implies that 1-HOP molecules must first enter the cell by penetrating the plasma membrane, followed by penetration into the cellular cytoplasm to interact with the nuclear DNA. Otherwise, no DNA damage should be seen due to the combination of 1-HOP and UVA. It is shown in our previous studies that light irradiation of 1-HOP can cause DNA single strand cleavage and form DNA covalent adduct through reactive intermediates such as singlet molecular oxygen and oxygen radicals if DNA molecules are mixed with 1-HOP (Dong et al. 2000b). The main photoproducts for 1-HOP photolysis are pyrene quinones such as 1,6- and 1,8-pyrenequinone (Zeng et al. 2000). It is also known that quinones are reactive oxygen species sensitizers (Feilberg and Nielsen, 2001). Therefore, it is likely that upon activation of 1-HOP in the cells by UVA light, free radicals and reactive

oxygen species are generated to cause DNA fragmentations shown by the Comet assay.

The DNA fragmentation and other damages can be shown by either acute or chronic toxicity to the cells. As acute toxicity, the DNA damage is too severe for the cells to recover and thus results in cell death. As chronic toxicity, the cells are able to recover from the damages, but may leave some of the damages not repaired. The damages to DNA, if not repaired, can lead to mutations and other genetic problems. It is shown in this study that if either the UVA or the 1-HOP dose is high, much of the Jurkat T-cells will be killed. On the other hand, if both doses are low, the cell nuclear DNA is damaged, but the cells themselves are still viable. All the damages, if not repaired, may cause potential genetic problems later. Comparing with the parent compound pyrene, the phototoxicity of 1-HOP to cells and its effect on DNA strand cleavage (Dong et al. 2000a; Dong et al. 2000b) is much greater. Since 1-HOP is the main metabolite of pyrene and other hydroxy-PAHs are the chief metabolites of the respective PAHs (Jongeneelen, 1994), contamination of these chemicals on humans is not avoidable. Therefore, the phototoxicity of hydroxy-PAHs, both acute and chronic, needs to be examined in many cell types in order to understand the combination effect of PAHs and UVA-light.

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