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UVA Photoirradiation of Methylated Benzo[a]pyrene and Benzo[e]pyrene leading to Induction of Lipid Peroxidation

Diógenes Herreño Sáenz[1*], Qingsu Xia[2], and Peter P. Fu[2]

[1]Department of Pharmacology and Toxicology, School of Medicine, University of Puerto Rico, P.O. Box 365067, San Juan, Puerto Rico 00936-5067
[2]National Center for Toxicological Research, Jefferson, AR 72079, USA
*Correspondence to Dr. Diógenes Herreño Sáenz; Email: dherreno@rcm.upr.edu

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Abstract: Polycyclic aromatic hydrocarbons (PAHs) are widespread genotoxic environmental pollutants and potentially pose a health risk to humans. Although the biological and toxicological activities, including metabolism, mutagenicity and carcinogenicity of PAHs have been thoroughly studied, their phototoxicity and photo-induced biological activities have not been well examined. In this research, we studied the photoirradiation of isomeric methylbenzo[a]pyrene (MBaP) and methylbenzo[e]pyrene (MBeP) by UVA light in the presence of a lipid, methyl linoleate, and evaluated the potential of these compounds to induce lipid peroxidation. The compounds chosen for study included BaP, 3-MBaP, 4-MBaP, 6-MBaP, 7-MBaP, 10-MBaP, BeP, 4-MBeP, and 9-MBeP. The results indicate that upon photoirradiation by UVA at 7 and 21 J/cm^2, these compounds induced lipid peroxidation. The levels of the induced lipid peroxidation were similar among BaP and the isomeric MBaPs, and among the BeP and MBePs, with the BaP group higher than the BeP group. There was also a co-relation between the UV A light dose and the level of lipid peroxidation induced. Lipid peroxide formation was inhibited by NaN_3 (singlet oxygen and free radical scavenger) and was enhanced by the presence of deuterium oxide (D_2O) (extends singlet oxygen lifetime). These results suggest that photoirradiation of MBaPs and MBePs by UVA light generates reactive oxygen species (ROS), which induce lipid peroxidation.

Introduction

It has long been established that many polycyclic aromatic hydrocarbons (PAHs) are carcinogenic environmental contaminants [1-5]. To determine structure-carcinogenicity relationships, a large number of PAHs of different structures have been tested for carcinogenicity [1-7]. Investigations concerning how substituents affect the tumorigenicity and metabolism of PAHs have provided insight into mechanisms by which these compounds induce tumors [1, 2, 6, 7]. In this regard, methyl and halogen substituents have long been employed to elucidate structure-activity relationships and to probe the geometric regions of PAHs that are involved in metabolic activation [1, 6, 7]. However, while people expose to the environmental PAHs on the skin are unavoidably exposed to sunlight, it is not known whether contact of PAHs with concomitant exposure to sunlight would result in any deleterious effects [8]. Consequently we are interested in determining whether or not photoirradiation of environmental PAHs and their methylated and halogenated derivatives by UVA light in the presence of a lipid, methyl linoleate, can induce lipid peroxidation.

We have previously reported the study of photoirradiation of a series of halo-PAHs with UVA light and found that most of them induce lipid peroxidation [9, 11]. As a continuation of our research, the present study was designed to photoirradiate a series of methylated benzo[a]pyrene (MBaP) and methylated benzo[e]pyrene (MBeP), and to investigate their potential to induce lipid peroxidation. The structures of the compounds used in this study, including BaP, 3-MBaP, 4-MBaP, 6-MBaP, 7-MBaP, 10-MBaP, BeP, 4-MBeP, and 9-MBeP, are shown in Figure 1.

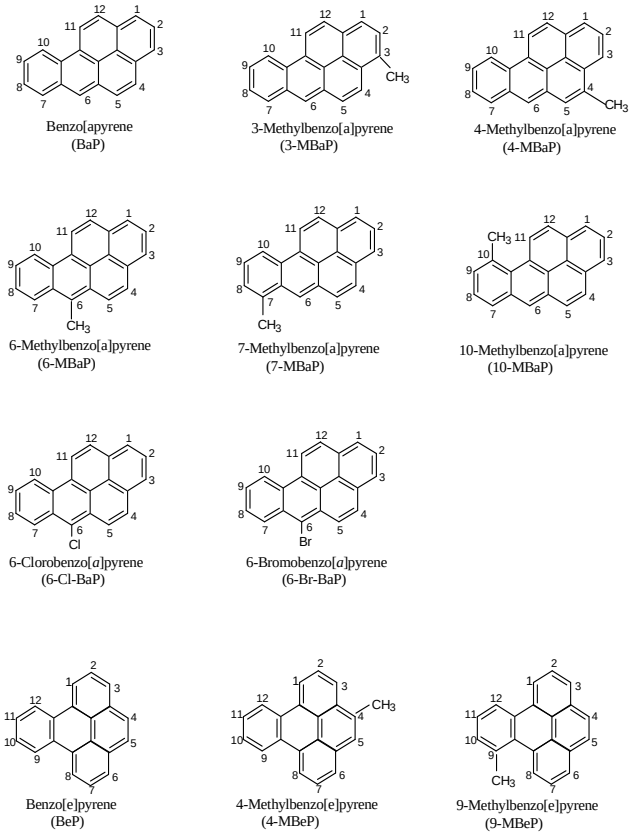

Figure 1: Structures of benzo[a]pyrene (BaP), methylated benzo[a]pyrene (MBaP), benzo[e]pyrene (BeP), and methylated benzo[e]pyrene (MBeP) used in this study.

Materials and Methods

Materials

Benzo[*a*]pyrene (BaP) and benzo[e]pyrene (BeP) were purchased from Aldrich Chemical Co. (Milwaukee, WI). 3-MBaP, 4-MBaP, 6-MBaP, 7-MBaP, 10-MBaP, 4-MBeP, and 9-MBeP were obtained from NCI Chemical Repository. All other reagents were obtained through commercial sources and were the highest quality available. All solvents used were HPLC grade.

Light Source

The UVA light box was custom made with a 4-lamp unit using UVA lamps (National Biologics). The irradiance of light was determined using an Optronics OL754 Spectroradiometer (Optronics Laboratories, Orlando, FL), and the light dose was routinely measured using a Solar Light PMA-2110 UVA detector (Solar Light Inc., Philadelphia, PA). The maximum emission of the UVA is between 340 – 355 nm. The light intensities at wavelengths below 320 nm (UVB light) and above 400 nm (visible light) are about two orders of magnitude lower than the maximum at 340-355 nm.

Photoirradiation of Methylated BaP, BeP, MBaPs, and MBePs with UVA Light in the Presence of Methyl Linoleate

Experiments were conducted with a solution of 100 mM methyl linoleate and 1.0 mM substrate in methanol. Samples were placed in a UV-transparent cuvette and irradiated with 0, 7, and 21 J/cm2 of UVA light. After irradiation, the methyl linoleate hydroperoxide products were separated by HPLC using a Prodigy 5 m ODS column (4.6 x 250 mm, Phenomenex, Torrance, CA) eluted isocratically with 10% water in methanol (v/v) at 1 mL/min. The extent of lipid peroxides formed by the photo-irradiation of methyl linoleate in the absence and presence of a PAH (BaP, BeP, MBaP, or MBeP) was determined by calculating the amount of methyl linoleate-hydroperoxides detected in resolved HPLC peak areas detected at 235 nm [12-15].

Effect of NaN_3 and D_2O on Peroxidation of Methyl Lineolate Initiated by Photoirradiation of 4-MBeP

The identification of the ROS species involved in the lipid peroxidation was determined with 4-MBeP. This compound and methyl lineoate were irradiated with UVA light in the presence of NaN_3, which is a singlet oxygen and free radical scavenger, or deuterium oxide (D_2O) with which singlet oxygen has an enhanced lifetime, under experimental conditions as described above. The concentration of NaN_3 was 50 mM, and the amount of deuterium oxide and water was 15% in methanol.

Results

Photoirradiation of MBaPs, MBePs, BaP, and BeP in the Presence of Methyl Linoleate

BaP, 3-MBaP, 4-MBaP, 6-MBaP, 7-MBaP, 10-MBaP, BeP, 4-MBeP, and 9-MBeP were selected for the study of photoirradiation with UVA in the presence of a lipid, methyl linoleate (Figure 1). Each of the PAHs received two light doses, 7 and 21 J/cm^2, respectively. All the PAHs tested resulted in lipid peroxidation and exhibited a dose response (Table 1). As shown in Table 1 and Figure 2, the levels of the induced lipid peroxidation are similar among BaP and its 3-MBaP, 4-MBaP, 6-MBaP, 7-MBaP, and 10-MBaP isomers, and among BeP and its 4-MBeP and 9-MBeP isomers. In general, the levels of induced lipid peroxidation by BeP and MBePs are lower than those induced by BaP and MBaPs ($P<0.05$).

Mechanistic study on Peroxidation of Methyl Linoleate Initiated by Photoirradiation of 4-MBeP

To determine the involvement of free radical intermediates in the peroxidation of methyl linoleate initiated by photoirradiation of PAHs, 4-MBeP was selected for photoirradiation by UVA light in the presence of a free radical scavenger or enhancer. Besides as a free radical scavenger, NaN_3 also effectively reacts with singlet

oxygen ($^1O_2$) and hydroxyl radical [16, 17] preventing their reactions with other molecules (*e.g.* methyl lineolate). As a result NaN_3 alone cannot be relied upon to determine whether singlet oxygen is involved in peroxidation. Since singlet oxygen has a longer half-life in deuterium water (D_2O), use of both NaN_3 and D_2O should provide a reliable approach for determining whether singlet oxygen is involved in peroxidation.

Table 1: Induction lipid peroxidation by benzo[a]pyrene, benzo[e]pyrene and their methylated derivatives with concomitant exposure to UVA light irradiation[a]

Compound	*0 J/cm²*	*7 J/cm²*	*21 J/cm²*
Methyl linoleate (ML)	238	334	453
Benzo[*a*]pyrene (BaP)	262	3294±318[a]	5526±337[a]
3-MBaP	255	2908±217[a]	4938±417[a]
4-MBaP	230	3128±288[a]	5338±218[a]
6-MBaP	251	2881±391[a]	5482±352[a]
7-MBaP	233	3030±511[a]	5111±416[a]
10-MBaP	208	3222±406[a]	5352±438[a]
Benzo[e]pyrene (BeP)	266	1725±207[a]	2367±275[a]
4-MBeP	252	1667±138[a]	2343±319[a]
9-MBeP	232	1891±113[a]	2767±333[a]
Benzo[*a*]pyrene (BaP)[b]	100	2467±217[a]	5819±425[a]
6-Cl-BaP[b]	153	2404±29[a]	4840±137[a]
6-Br-BaP[b]	135	2639±224[a]	5201±291[a]
Anthracene (A)[b]	138	1350±66[a]	2459±197[a]
Phenanthrene (Ph)[b]	144	32 ±13[a]	803±37[a]
Benz[*a*]anthracene(BA)[b]	141	2719±133[a]	3917±403[a]
5-MBA[b]	142	1582±58[a]	1885± 236[a]

[a]Average of three Experiments/standard deviation
[b]Data from our previously published study [11]

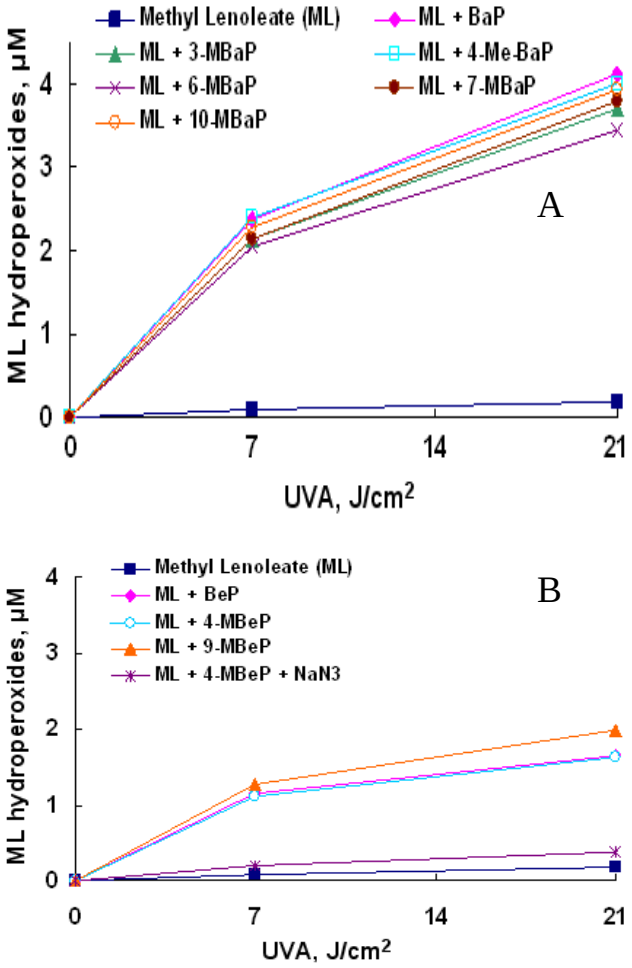

Figure 2: Peroxidation of methyl linoleate initiated by photoirradiation (with 0, 7, and 21 J/cm2 of UVA light) of BaP, 3-MBaP, 4-MBaP, 6-MBaP, 7-MBaP, and 10-MBaP (Upper Panel) and BeP, 4-MBeP, and 9-MBeP and photoirradiation of 4-MBeP in the presence of NaN_3, (Lower Panel).

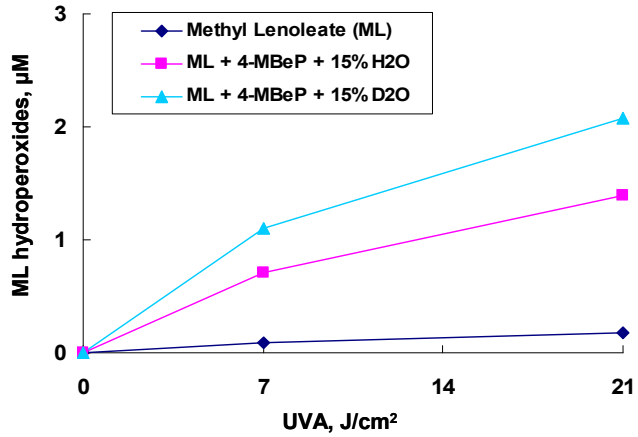

Figure 3: Enhancement effect of D_2O on the peroxidation of methyl linoleate initiated by photoirradiation of 4-MBeP with UVA light at light doses of 7 and 21 J/cm^2.

Lipid peroxidation induced by photoirradiation of 4-MBeP with UVA was significantly inhibited by NaN_3 (Figure 2, Lower Panel). Lipid peroxidation was increased by 22% when H_2O was replaced by D_2O ($p < 0.05$) (Figure 3). These results suggest that peroxidation of methyl linoleate initiated by photoirradiation of 4-MBeP is mediated by free radicals and that singlet oxygen is involved in peroxidation (Figure 2, Lower Panel, and Figure 3).

Discussion

As a continuation of our study on the induction of lipid peroxidation by photoirradiation of PAHs with UVA light in the presence of a lipid (methyl linoleate), a series of MBaPs and MBePs were selected for the study. It was found that when exposed to UVA light, all these MBaPs and MBePs initiate lipid peroxidation, forming the methyl linoleate hydroperoxides, but with different induction potency. The levels of lipid peroxidation by each tested compound were dose (light) dependent. Compared with the parent substrate, BaP or BeP, in general, the addition of a methyl group to the parent PAH did not significantly alter the level of induction of lipid peroxidation.

As shown in Table 1 and Figure 2, in general, BaP and its methylated derivatives (3-MBaP, 4-MBaP, 6-MBaP, 7-MBaP, and 10-MBaP) induce lipid peroxidation in a higher level than that from BeP and its methylated derivatives, 4-MBeP, and 9-MBeP. We have previously studied the photoirradiation of 6-chlorobenzo[*a*]pyrene (6-Cl-BaP) and 6-bromobenzo[*a*]pyrene (6-Br-BaP) with UVA light under similar experimental conditions [11]. The combined data of 6-MBaP, 6-Cl-BaP, and 6-Br-BaP indicate that all these 6-substituted BaP derivatives exhibit similar level of induction of lipid peroxidation (Figure 4). These results suggest that the substituent at C6 position of BaP does not significantly affect the induction of lipid peroxidation.

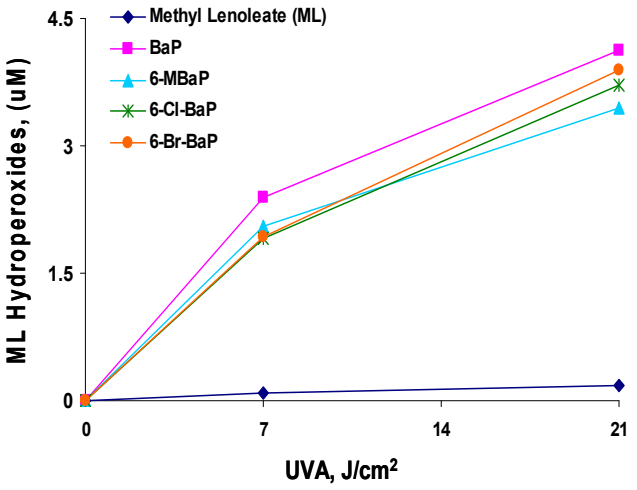

Figure 4: Peroxidation of methyl linoleate initiated by BaP, 6-MBaP, 6-Cl-BaP and 6-Br-BaP with, 0, 7, and 21 J/cm2 of UVA light. The results of 6-Cl-BaP and 6-Br-BaP are from our previously published study [11].

The tumorigenic potency of the isomeric MBaPs has been reported [2]. Comparison of the levels of lipid peroxidation induced by MBaPs (Table 1) with the tumorigenicity of MBaPs indicates that there is no correlation between the level of lipid peroxidation and the tumorigenic potency among BaP and MBaPs,.

We previously found that the photoinduced lipid peroxidation by PAHs is mediated by reactive oxygen species (ROS), forming singlet oxygen and superoxide [9, 10]. In this current study, we further demonstrate that photoinduced lipid peroxidation by MBaPs and MBePs is mediated by reactive oxygen species (ROS). Lipid peroxidation produces aldehydes that can bind covalently with cellular DNA, form DNA adducts, and induce tumors in experimental animals [18]. Singlet oxygen itself can react with amino acid, proteins, lipids, and DNA resulting in cell damage and diseases [19]. ROS can also damage DNA and proteins leading to aging, inflammation, cardiovascular diseases, cancer and other age-related diseases [20]. All the tested PAHs are potential environmental contaminants and, as shown in our study, can induce lipid peroxidation. Since the majority of the PAHs and their methylated and halogenated derivatives that we have studied [9-11 and present study] can induce lipid peroxidation upon UVA light exposure, it is of particular importance and significance to investigate human health risks posed to the combination of PAHs and light.

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