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UVA Photoirradiation of Oxygenated Benz[*a*]anthracene and 3-Methylcholanthene - Generation of Singlet Oxygen and Induction of Lipid Peroxidation

Jun-Jie Yin[1], Qingsu Xia[2], Shu-Hui Cherng[2], I-Wah Tang[2], Peter P. Fu[2], Ge Lin[3], Hongtao Yu[4] and Diógenes Herreño Sáenz[5*]

[1]Center for Food Safety and Applied Nutrition, U.S. Food and Drug Administration, College Park, MD 20740, USA
[2]National Center for Toxicological Research, Jefferson, AR 72079, USA
[3]Department of Pharmacology, The Chinese University of Hong Kong, Shatin, Hong Kong, SAR
[4]Department of Chemistry, Jackson State University, Jackson, MS 39217, USA
[5]Department of Pharmacology and Toxicology, School of Medicine, University of Puerto Rico, P.O. Box 365067, San Juan, Puerto Rico 00936-5067
*Corresponding 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 activity have not been well examined. We have long been interested in phototoxicity of PAHs and their derivatives induced by irradiation with UV light. In this paper we report the photoirradiation of a series of oxygenated benz[*a*]anthracene (BA) and 3-methylcholanthene (3-MC) by UVA light in the presence of a lipid, methyl linoleate. The studied PAHs include 2-hydroxy-BA (2-OH-BA), 3-hydroxy-BA (3-OH-BA), 5-hydroxymethyl-BA (5-CH_2OH-BA), 7-hydroxymethyl-BA (7-CH_2OH-BA), 12-hydroxymethyl-BA (12-CH_2OH-BA), 7-hydroxymethyl-12-methyl-BA (7-CH_2OH-12-MBA), 5-formyl-BA (5-CHO-BA), BA 5,6-*cis*-dihydrodiol (BA 5,6-*cis*-diol), 1-hydroxy-3-methylcholanthene (1-OH-3-MC), 1-keto-3-methylcholanthene (1-keto-3-MC), and 3-MC 1,2-diol. The results indicate that upon photoirradiation by UVA at 7 and 21 J/cm^2, respectively all these compounds induced lipid peroxidation and exhibited a relationship between the dose of the light and the level of lipid peroxidation induced. To determine whether or not photoirradiation of these compounds by UVA light produces ROS, an ESR spin-trap technique was employed to provide direct evidence. Photoirradiation of 3-keto-3-MC by UVA (at 389 nm) in the presence of 2,2,6,6-tetramethylpiperidine (TEMP), a specific probe for singlet oxygen, resulted in the formation of TEMPO, indicating that singlet oxygen was generated. These overall results suggest that UVA photoirradiation of oxygenated BA and 3-methylcholanthrene generates singlet oxygen, one of the reactive oxygen species (ROS), which induce lipid peroxidation.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of carcinogenic environmental contaminants [1-3]. Since PAHs require metabolic activation in order to exert biological activities, including carcinogenicity [2], study of the mechanisms by which PAHs induce tumors in experimental animals has been one of the most extensively investigated areas of chemical carcinogenesis for the past several decades [1-3]. Three metabolic activation pathways *in vivo* have been determined, metabolism into *bay*-region diolepoxides [1], radical-cation intermediates [4], and ortho-quinones [5]. All these pathways result in binding of the ultimate metabolites with cellular DNA to form DNA adducts leading to cancer formation.

Following light irradiation, PAHs are also phototoxic [6], and are known to induce skin cancer in experimental animals [7-9]. PAHs also play an important

role on human skin cancer since exposure of skin to terrestrial light is inevitable [10-13]. For example, it is known that coal tar topically applied on the skin followed by UV light irradiation for psoriasis treatment has an increased risk of developing cutaneous cancer [14]. Another example is that roofers and highway asphalt workers have a high risk to be exposed to both PAHs and light [15]. 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.

We have been interested in determining whether or not photoirradiation of environmental PAHs and their metabolites by UVA light in the presence of a lipid, methyl linoleate, can induce lipid peroxidation [16-21]. As a continuation of our research, in this study, we investigate the photoirradiation of a series of oxygenated benz[*a*]anthracene (BA) and 3-methylcholanthrene (3-MC) with UVA light in the presence of methyl linoleate. The structures and abbreviations of the compounds used in this study are given in Figure 1, which include 2-hydroxy-BA (2-OH-BA), 3-hydroxy-BA (3-OH-BA), 5-hydroxymethyl-BA (5-CH_2OH-BA), 7-hydroxymethyl-BA (7-CH_2OH-BA), 12-hydroxymethyl-BA (12-CH_2OH-BA), 7-hydroxymethyl-12-methyl-BA (7-CH_2OH-12-MBA), 5-formyl-BA (5-CHO-BA), BA 5,6-*cis*-dihydrodiol (BA 5,6-*cis*-diol), 1-hydroxy-3-methylcholanthene (1-OH-3-MC), 1-keto-3-methylcholanthene (1-keto-3-MC), and 3-methylcholanthene 1,2-*cis*-dihydrodiol (3-MC 1,2-*cis*-diol). It was found that all the photoirradiation resulted in lipid peroxide (methyl linoleate hydroperoxides) formation. For electron spin resonance (ESR) study, photoirradiation of 3-keto-3-MC by UVA (at 389 nm) in the presence of 2,2,6,6-tetramethylpiperidine (TEMP), a specific probe for singlet oxygen, resulted in the formation of TEMPO, indicating that singlet oxygen was generated.

Materials and Methods

Materials

2,2,6,6-Tetramethyl-piperidine (TEMP), 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ), 7,12-dimethylbenz[*a*]anthracene (DMBA), 5-methylbenz[*a*]anthracene, 7-methylbenz[*a*]anthracene, 12-methylbenz[*a*]anthracene, and 3-MC were purchased from the Sigma Chemical Co. (St. Louis, MO). 2-OH-BA, 3-OH-BA, and BA 5,6-*cis*-diol were purchased from NCI Chemical Repository. 7-CH_2OH-12-MBA was prepared by reaction of DMBA with lead tetraacetate, separation of the resulting 7-acetoxymethyl-12-methylbenz[*a*]anthracene by column chromatography and followed by hydrolysis as previously described [17, 22]. 5-CH_2OH-BA, 7-CH_2OH-BA, and 12-CH_2OH-BA were similarly synthesized started from 5-methylbenz[*a*]anthracene, 7-methylbenz[*a*]anthracene, and 12-methylbenz[*a*]anthracene, respectively. 5-CHO-BA was synthesized by oxidation of 5-hydroxymethyl-BA with DDQ in refluxing benzene for 1 hr. 3-MC 1,2-*cis*-diol was prepared by dehydrogenation of 3-methylcholanthene with DDQ to afford 1,2-dehydro-3-MC followed by reaction with OsO_4 in pyridine at room temperature for 6 days [23]. 1-keto-3-MC was synthesized by the reaction of 3-MC with DDQ in water/acetic acid and 1-OH-3-MC by reduction of 1-keto-3-MC with sodium borohydride in methanol [23, 24]. All the compounds used for study had purity >98% as determined by HPLC analysis. 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, Twinsburg, OH). 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 Oxygenated BA and 3-MC 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/cm^2 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 substrate (Figure 1) was determined by calculating the amount of methyl linoleate-hydroperoxides detected in resolved HPLC peak areas detected at 235 nm as previously described [25-28].

ESR Spectral Measurements of Singlet Oxygen

Singlet oxygen generated from the photoirradiation of 1-keto-3-MC with UVA light was detected by the ERS spin trapping detection method using the spin trap TEMP as described by Rinalducci et al. [29]. 1-Keto-3-MC was dissolved in 80% acetonitrile in water. Two 50 μL quartz capillaries were used. The UVA light was provided by a Schoeffel 1000 W Xenon lamp coupled with a Schoeffel grating monochromator. The excitation light had a maximum centered at 389 nm. All experiments were performed in duplicate. The data were obtained with error of less than 10%.

Conventional ESR spectra were obtained with a Varian E-109 X-band spectrometer. ESR signals were recorded with 15 mW incident microwave and 100 kHz field modulation of 1G. The scan width was 100 G for TEMP experiments. All measurements were performed at room temperature.

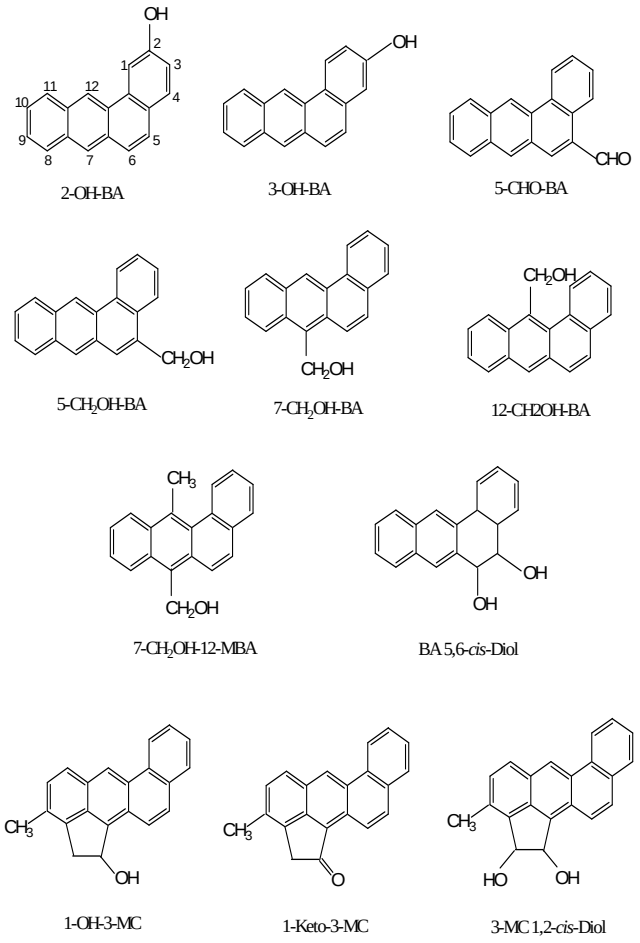

Figure 1: Structures of oxygenated benz[*a*]anthracene (BA) and 3-methylcholanthene (3-MC) used in this study.

Results

UVA Photoirradiation of Oxygenated BA and 3-MC in the Presence of Methyl Linoleate

Eleven oxygenated BA and 3-MC were each photoirradiated with UVA in the presence of a lipid, methyl linoleate. As shown in Figure 1, these PAHs all possess a BA aromatic conjugated system but bear one or more different functional groups. Each of the PAHs received two light doses, 7 and 21 J/cm^2, respectively for radiation. As the results summarized in Table 1, all the PAHs induced lipid peroxidation and exhibited a light dose response. The levels of lipid peroxidation induced by the oxygenated BA, including 2-OH-BA, 3-OH-BA, 5-CHO-BA, and BA 5,-6-*cis*-diol are shown in Figure 2. The levels of lipid peroxidation induced by oxygenated methylated BA are shown Figure 3, and those by oxygenated 3-MC are shown in Figure 4, respectively.

Among these PAHs, 3-MC 1,2- *cis*-diol exhibited the highest induction of lipid peroxidation, followed by 1-keto-3-MC, 1-OH-3-MC, 3-OH-BA, and 2-OH-BA. The PAHs that exhibited the lowest induction of lipid peroxidation are 7-CH_2OH-BA and 12-CH_2OH-BA.

Table 1: UVA light photoirradiation of oxygenated BA and 3-MC[a,b]

Compound	*0 J/cm^2*	*7 J/cm^2*	*21 J/cm^2*
Methyl linoleate (ML)	178 ± 15	267 ± 25	452 ± 59
2-Hydroxy-BA (2-OH-BA)	187 ± 13	2597 ± 153[a]	4283 ± 469[a]
3-Hydroxy-BA (3-OH-BA)	164 ± 15	2458 ± 190	4558 ± 412
5-Formyl-BA (5-CHO-BA)	191 ± 10	2434 ± 206	2919 ± 351
5-Hydroxymethyl-BA (5-CH_2OH-BA)	205 ± 21	2012 ± 189	2990 ± 311
7-Hydroxymethyl-BA (7-CH_2OH-BA)	177 ± 19	1956 ± 171	1777 ± 169
12-Hydroxymethyl-BA (12-CH_2OH-BA)	185 ± 12	1654 ± 150	1651 ± 193
7-Hydroxymethyl-12-methyl-BA (7-CH_2OH-12-MBA)	161 ± 17	1121 ± 132	2419 ± 270
Benz[*a*]anthracene 5,6-*cis*-Diol (BA 5,6-*cis*-Diol)	159 ± 15	920 ± 135	2120 ± 208
1-Hydroxy-3-MC (1-OH-3-MC)	201 ± 22	3370 ± 305	5160 ± 397
1-Keto-3-MC (1-Keto-3-MC)	210 ± 18	3316 ± 321	6181 ± 462
3-MC 1,2-*cis*-dihydrodiol (3-MC 1,2-*cis*-diol)	196 ± 26	4543 ± 489	10424 ± 580

[a]Data based on HPLC peak area of ML hydroperoxides measured at 235 nm.
[b]Average of two experiments/standard deviation

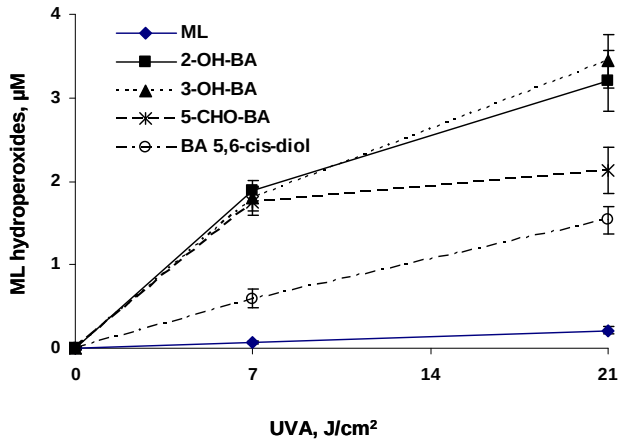

Figure 2: Peroxidation of methyl linoleate (ML) initiated by photoirradiation of 2-OH-BA, 3-OH-BA, 5-CHO-BA, and BA 5,6-cis-diol, respectively in the presence of ML by 0, 7, and 21 J/cm^2 of UVA light.

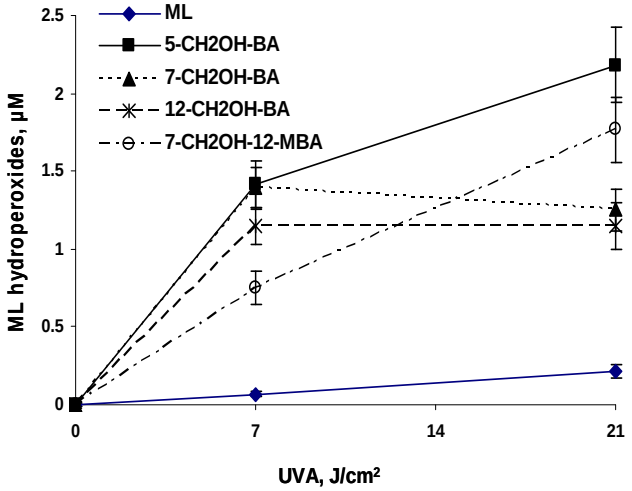

Figure 3: Peroxidation of methyl linoleate (ML) initiated by photoirradiation of 5-CH_2OH-BA, 7-CH_2OH-BA, 12-CH_2OH-BA, and 7-CH_2OH-12MBA, respectively in the presence of ML by 0, 7, and 21 J/cm^2 of UVA light.

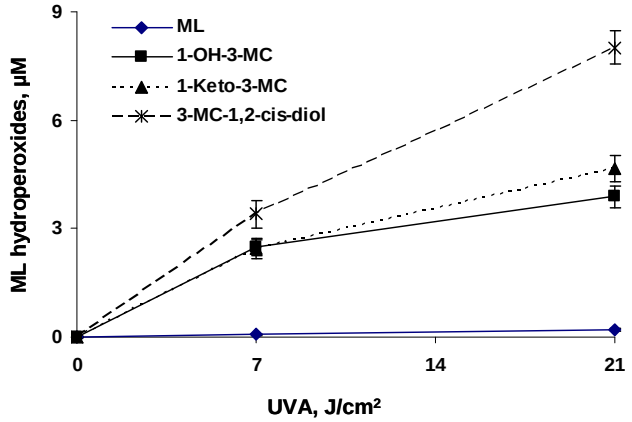

Figure 4: Peroxidation of methyl linoleate (ML) initiated by photoirradiation of 1-OH-3-MC, 1-keto-3-MC, and 3-MC 1,2-*cis*-diol, respectively in the presence of ML by 0, 7, and 21 J/cm^2 of UVA light.

ESR Spectral Measurements of Singlet Oxygen

Although singlet oxygen is highly unstable, Lion et al. [30, 31] successfully developed the use of the spin trap TEMP as a specific probe for detecting singlet oxygen formation. Upon reaction of singlet oxygen with TEMP, the resulting TEMPO, a stable nitroxide, can be detected by ESR spectroscopy [30, 31]. Consequently, we used the spin trap TEMP for our mechanistic study and 1-keto-3-MC was selected for photoirradiation.

In the absence of 1-keto-3-MC, photoirradiation of TEMP with UVA light at 389 nm did not result in an ESR signal (Figure 5A). In the absence of TEMP, UVA light irradiation of 1-keto-3-MC also resulted in no ESR signals (Figure 5B). When 1-keto-3-MC and TEMP were concomitantly exposure to UVA light for 5 min, singlet oxygen was generated, as evidenced by an ESR spectral profile which is typical of TEMPO (Figure 5C) [28,29,32,33]. The intensity of these ESR signals progressively enhanced when photoirradiation time increased to 10, 20, 30, and 40 min, respectively (Figure 5D-G). These results provide direct evidence that photoirradiation of 1-keto-3-MC with UVA light generates singlet oxygen and that the quantity of singlet oxygen formed is dependent on the light dose.

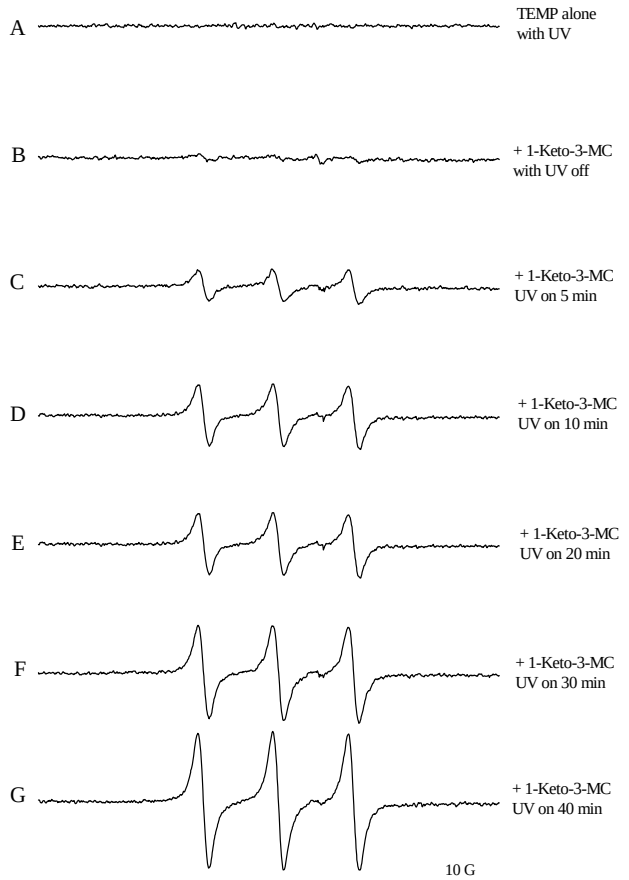

Figure 5: Time dependent of singlet oxygen formation of 1-keto-3-MC photosensitized with UV irradiation (389 nm); sample containing 2 mM TEMP spin trap and 1 mM 1-keto-3-MC in 80% CH_3CN.

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 oxygenated BA and 3-MC were selected for the study. It was found that when exposed to UVA light, all these PAHs initiate lipid peroxidation, forming the methyl linoleate hydroperoxides, but with different induction potency. As shown in Table 1 and Figures 2-4, the levels of lipid peroxidation by each tested compound are dose (light) dependent.

In this study, there is no correlation between the level of lipid peroxidation induction and the tumorigenic potency of the parent PAHs. For example, while 7-CH_2OH-12-MBA is carcinogenic [34], it induced lipid peroxidation less than 3-OH-BA which is not carcinogenic [34]. This finding is consistent with the results we previously reported on the UVA photoirradiation of PAHs and their derivatives [18-21].

The ESR spin trap study suggests that the photoinduced lipid peroxidation by PAHs is mediated by reactive oxygen species (ROS), specifically singlet oxygen, which is generated during the photoirradiation of PAHs. Lipid peroxidation produces aldehyde products that can bind covalently with cellular DNA, form DNA adducts, and induce tumors in experimental animals [27, 28]. Singlet oxygen itself can react with amino acid, proteins, lipids, and DNA resulting in cell damage and diseases [35], and can also damage DNA and proteins leading to aging, inflammation, cardiovascular diseases, cancer and other age-related diseases [36]. PAHs are potential environmental contaminants [1,34] and, as shown in our study, can induce lipid peroxidation. Since the majority of the PAHs and derivatives that we have studied 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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