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Emission and Cytotoxicity of Surgical Smoke: Cholesta-3,5-Diene Released from Pyrolysis of Prostate Tissue

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Abstract: Respiratory and lung irritants can be a by-product of the surgical pyrolysis of human tissues. Seven prostate tissues were collected during the transurethral resection of a prostate (TURP). Tissue samples, pyrolyzed in a pyrolysis sampling system, were collected and analyzed for the characterization of aerosols in the surgical smoke. In the pyrolyzed particulate matter (PM) from the TURP, Cholestra-3,5-diene was identified as the most dominant component along with 9-methylanthracene, hentriacontane, and dotriacontane based on the mass fragment structure determined using gas chromatography-mass spectrometry (GC-MS). As a molecular marker, Cholesta-3,5-diene can be associated with a cytotoxic in primary human oral keratinocytes (HOK). In this research, the presence of Cholestra-3,5-diene is reported for the first time as a by-product of surgical pyrolysis.

Keywords: organic compounds; surgical aerosol; surgical carbonaceous material; toxicity; surgical smoke

1. Introduction

During surgical tissue pyrolysis, noticeable quantities of surgical smoke (i.e., both particulate and gaseous matter) can be generated that negatively affect the surgical operators [1,2]. Electrocautery has become an integral tool in the operating room since it was first applied almost one hundred years ago [3]. With pyrolysis using energy-based technologies, surgical smoke can be generated when target cancer cells are thermally treated. This causes the membrane to scatter cellular contents into the irrigation solution and/or the indoor environment of an operating room. Surgical smoke can contain toxic gases including carbon monoxide (CO), particulate matters, such as deoxyribonucleic acid (DNA) constituents, carcinogens, and acrylonitrile [4]. Exposure to these smoke aerosols can pose significant

risks to human health during the operation. The chemical compositions and aerodynamic sizes of particles vary significantly, depending on the type of procedure, the pathology of the target tissue, and the scope of surgery [5]. A number of gas phase components (e.g., butyrolactone, vinyl acetylene, 1,3-butadiene, propylene, and isobutylene) have been identified from the transurethral resection of a prostate (TURP) [2]. Nonetheless, relatively little is known about the chemical composition of particulate matter (PM) in the surgical smoke released via the pyrolysis of prostate tissue. In this study, PM samples collected from the pyrolysis of prostate tissues were examined to determine the molecular marker(s) from the TURP and to investigate their cytotoxicity potential.

2. Measurements of the Organic Composition

2.1. Patients and Samples

A total of seven prostate tissues were collected during the TURP for the treatment of prostate hyperplasia. The overall patient age was 70 years old. The samples had an average mass of 4.48 ± 1.94 g (±standard deviation) and were stored in a freezer until pyrolysis in the laboratory combustion sampling system. The Institutional Review Board of the Korean Academy of Medical Sciences approved all procedures (e.g., sample transfer out of the operating room). Operations were performed under spinal epidural or anesthesia. The procedures were performed in the same operating theater with a positive pressure ventilation system.

2.2. Pyrolysis of Samples

The smoke aerosols released during the pyrolysis of human tissue samples were characterized as shown in Figure 1. Briefly, each prostate tissue was pyrolyzed on a pyrolysis plate inside a laboratory hood. Smoke PM resulting from pyrolysis of prostates was collected on a prebaked (450 °C) quartz filter (Pallflex, Pall Corp., Port Washington, NY, USA) and Teflon filter (PTFE, Pall Corp., Port Washington, NY, USA) using a middle volume PM sampler with a sampling flow rate of 92 L per minute (lpm) (cyclone; URG-2000-30EP/filter pack; URG-2000-30FG, URG Corp., Chapel Hill, NC, USA) and four low volume PM samplers with a flow rate of 16.7 lpm (URG-2000-30EHS/filter pack; URG-2000-30FG, URG Corp., Chapel Hill, NC, USA). The PM sampling was performed using iso-kinetic sampling performance during PM collection to capture particles that passed through a defined area for a defined time without disturbing their paths. All rotameters and orifices were calibrated using a Dry Test Meter (MesaLabs, Lakewood, CO, USA) before and after sample collections. The sampling time was 120 s per sample.

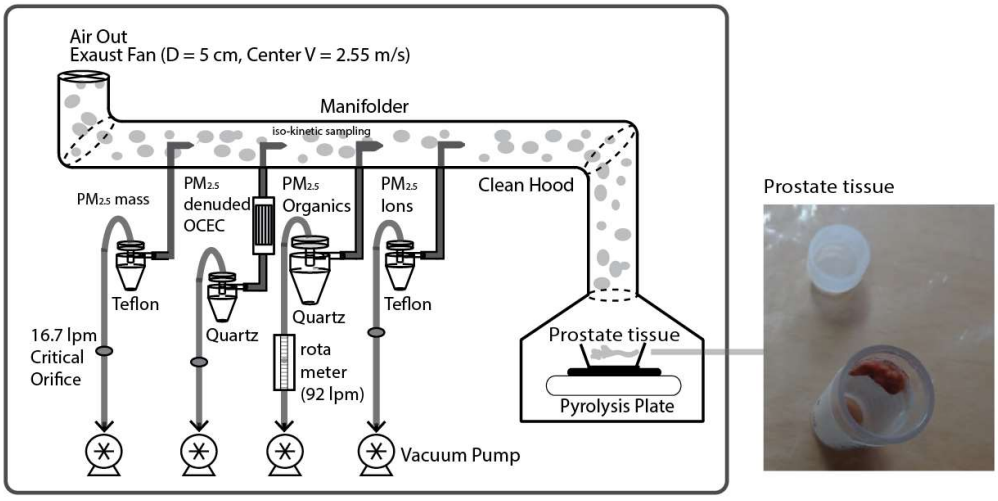

Figure 1. Schematic diagram of the pyrolysis sampling system.

2.3. Analysis of Organic Compounds

For the identification of detailed organic molecular markers, thermal desorption (TD) (Unity 2, Markes International Ltd., Llantrisant, UK) coupled with gas chromatography-mass spectrometry (GCMS) (Agilent Tech., Santa Clara, CA, USA) was utilized for the analysis of organic compounds from the TURP. Ten isotopically labeled internal standards (e.g., pyrene-d10, benz[a]anthracene-d12, coronene-d12, cholestane-d4, pentadecane-d32, eicosane-d42, tetracosane-d50, triacontane-d62, dotriacontane-d66, and hexatriacontane-d74) were spiked on sample punches (i.e., 3×1.0 cm^2) before loading into the TD. The glass thermal desorption sorbent tube with the sample was rapidly heated to 360 °C to desorb the organic markers from the sample. After the target analytes were collected in the cold trap (0 °C), the trap oven was heated rapidly again to 360 °C. This facilitated a splitless operation with a high-resolution capillary gas chromatography (GC) (Table S1). Final concentrations of organic molecular markers were blank-corrected using three field blanks (i.e., identical sampling activities without sampling suction flow). The details of this analytical method can be found elsewhere [6]. The coefficient of determination in the standards, percent of recovery, duplication, and method of detection limit for the target organic compounds are shown in Table S2. The emission rates for each organic compound were calculated based on the total mass reductions before and after pyrolysis within each sample.

2.4. Organic Carbon (OC) and Elemental Carbon (EC) Analysis

A thermo-optical carbon analyzer (Sunset Lab. Inc., Portland, OR, USA) recognized by the National Institute for Occupational Safety and Health (NIOSH) Method 5040 was used to analyze the pyrolyzed PM samples [7]. The samples were collected after removing the volatile organic carbon (VOC) using a carbon strip organic denuder. Duplication analysis and sucrose checks were performed during the analysis.

2.5. Cytotoxicity on Primary Human Oral Keratinocytes (HOK)

To find the relationship between a molecular marker of the pyrolyzed PM from the TURP and cytotoxicity, human oral keratinocytes (HOK) (ScienCell Research Lab., Carlsbad, CA, USA) were placed in Dulbecco's modified Eagle's medium (Life Tech., New York, NY, USA). The medium contained 10% fetal bovine serum (FBS) (Life Tech., New York, NY, USA). The cells were grown in a humidified incubator at 37 °C in 5% CO_2. The cells were seeded at a density of 1×10^5 cells per well in 96 well plates and were left to attach to the well overnight by incubation. To determine the dose-dependent effects of cholesta-3,5-diene on the cells, each cultured cell was treated with 0.1, 1, 10, and 100 μg/mL cholesta-3,5-diene incubated for 24 h at 37 °C. The cells were re-incubated for an additional 4 h in 20 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Life Tech., New York, NY, USA). The supernatant was eliminated, and the MTT crystals were dissolved in 200 μL/well di-methyl sulfoxide. Finally, the optical density was measured at least three times at 570 nm using a spectrometer.

3. Results and Discussion

3.1. Concentrations of Organic Compounds

The precision of the sampling and analysis measurement for OC and EC was determined by duplicate and sucrose analyses. The detailed methodological analysis has previously been presented [7]. The overall average organic carbon (OC) and elemental carbon (EC) concentrations for the measurements were 1206 and 91 μgC/m^3, respectively (Table S3). The analytical carbonaceous evolution peaks from the thermal distributions presented a significantly lower temperature mode (Figure S1). Table S4 presents the average OC and EC concentrations in pyrolyzed $PM_{2.5}$ from the TURP. The overall average OC concentration was 506,720 ± 1721 (average ± standard error) ng/mg in

$PM_{2.5}$, which was about 50% of the total $PM_{2.5}$ mass. The EC concentration was 39,920 ± 2581 ng/mg in $PM_{2.5}$. The ratio of EC to OC was 0.08.

A total of 46 non-polar organic compounds were quantitatively analyzed by gas chromatography mass spectrometry - thermal desorption (GCMS-TD) (Table S3). Fourteen polycyclic aromatic hydrocarbon (PAH) compounds were analyzed in this study including naphthalene, acenaphthylene, acenapthene, fluorine, phenanthrene, anthracene, fluoranthene, pyrene, methylfluoranthene, 9-Methylanthracene, benzo (GHI) fluoranthene, chrysene, coronene, and dibenzo(ae)pyrene. The presence of these PAHs in ambient air has been ascribed to emissions from various combustion sources such as gasoline, diesel engines, coal combustion, and fuel oil combustion [8]. 9-Methylanthracene is a highly specific PAH (172.2 ng/mg in $PM_{2.5}$) found in pyrolyzed $PM_{2.5}$ from the TURP. Dibenzo(ae)pyrene (77.6 ng/mg in $PM_{2.5}$) and coronene (21.4 ng/mg in $PM_{2.5}$) were identified as the major PAHs by the mass fragment structure determined using GCMS-TD.

A total of 31 n-alkanes ranging from tridecane ($C_{13}H_{28}$) to nonatriacontane ($C_{39}H_{80}$) were also observed. Dotriacontane ($C_{32}H_{66}$) was the most abundant compound in the n-alkanes at an average total concentration of 640.5 ng/mg. Although no specific patterns were observed for even and odd numbers of carbon-containing alkanes, the concentrations of hentriacontane ($C_{31}H_{64}$) and tritriacontane ($C_{33}H_{68}$) were 610.8 and 607.1 ng/mg in $PM_{2.5}$, respectively. The overall average concentration of n-alkanes was about 6.6 times higher than the average concentration of PAH.

It is interesting to note that relatively high concentrations of Cholestra-3,5-diene ($C_{27}H_{44}$, CAS Number 747-90-0) were observed in all of the pyrolyzed $PM_{2.5}$ samples from the TURP. This clearly demonstrates that the primary pyrolyzed organic tracer, Cholestra-3,5-diene, was enriched in pyrolyzed $PM_{2.5}$. The overall average Cholestra-3,5-diene concentration was 2551 ± 12 ng/mg in $PM_{2.5}$, which is about 15 times higher than the average concentration of alkanes. This enrichment suggests that surgical smoke emissions can produce different organic molecular markers compared to those from the known ambient source studies [9–11]. The molecular marker is expected to have a significant impact on human health in the operating room.

3.2. Emission Rate of Organic Compounds

Cholestra-3,5-diene (CAS Number 747-90-0) and other organic molecular markers were present in all pyrolyzed samples from the TURP. The results, with data for other PM molecular markers, are listed in Table 1. The data in Table 1 were calculated from the absolute mass emission rates (ERs) based on the total mass reductions before and after pyrolysis within each sample (Table S5). Cholestra-3,5-diene (ER = 79.67 μg/s) was the major compound that was analyzed in all smoke PM samples from prostate tissues. While Cholestra-3,5-diene in tissue lipid [12] has been minimally recorded, to the best of our knowledge, no records of publication for smoke samples can be found. Cholestra-3,5-diene could not be detected using the normal solvent extraction approach due to the small number of samples. Consequently, Cholestra-3,5-diene was the major molecular marker of the pyrolyzed sample from the TURP, characterized at m/z 368 as the major mass fragment (Figure 2 and Figure S2). Cholestra-3,5-diene has not been found as an organic component in the PM of ambient aerosols. However, it can be the major molecular marker in an operating room, acting as a respiratory and lung irritant to the operators. In addition, 9-methylanthracene (ER = 5.38 μg/s), hentriacontane (ER = 19.08 μg/s), and dotriacontane (ER = 20.00 μg/s) are also suspected to be major molecular components of the TURP.

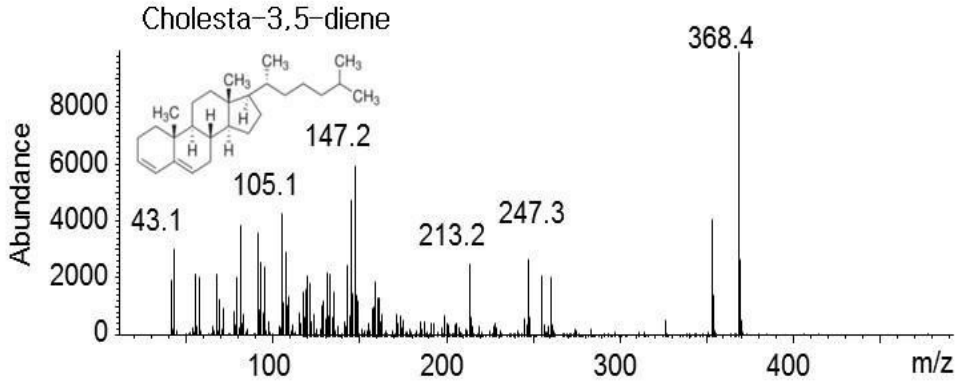

Figure 2. Mass fragments of Cholesta-3,5-diene in the surgical smoke by gas chromatography mass spectrometry—thermal desorption (GC/MS-TD) (99% match with NIST 2008 MS library).

Table 1. Emission rate of organic compounds in pyrolyzed transurethral resection of a prostate (TURP)-smoke by GCMS-TD.

Compound [1]	Average	Std Error [2]	Compound	Average	Std Error [2]
$PM_{2.5}$ Mass	31,230	5342	Cholestra-3,5-diene	79.673	12.146
Organic Carbon	15,825	2736	Eicosane	0.973	0.592
Elemental Carbon	1247	185	Heneicosane	1.198	0.327
Naphthalene	0.528	0.037	Docosane	1.207	0.261
Acenaphthylene	0.124	0.069	Tricosane	2.132	0.418
Acenapthene	0.347	0.140	Tetracosane	5.710	1.668
Fluorene	0.059	0.038	Pentacosane	4.914	0.765
Phenanthrene	0.528	0.214	Hexacosane	5.957	1.165
Anthracene	0.581	0.148	Heptacosane	7.713	1.593
Fluoranthene	0.168	0.074	Octacosane	6.405	1.898
Pyrene	0.244	0.065	iso-nonacosane	2.598	0.493
Methylfluoranthene	0.072	0.031	Nonacosane	8.952	2.816
9-Methylanthracene	5.379	2.296	anteiso-triacontane	3.217	2.426
Benzo(GHI)fluoranthene	0.413	0.098	Triacontane	9.925	4.174
Chrysene	0.124	0.024	iso-hentriacontane	0.971	0.640
Coronene	0.669	0.135	Hentriacontane	19.076	7.746
Dibenzo(ae)pyrene	2.423	0.564	anteiso-dotriacontane	1.663	0.574
17A(H)-21B(H)-Hopane	0.283	0.089	Dotriacontane	20.003	10.924
Tridecane	1.555	0.708	Tritriacontane	18.961	12.540
Tetradecane	3.404	0.973	Tetratriacontane	11.257	5.322
Pentadecane	8.013	2.014	Pentatriacontane	7.926	3.026
Hexadecane	0.555	0.276	Hexatriacontane	4.807	1.188
Heptadecane	1.053	0.283	Heptatriacontane	2.884	0.867
Octadecane	0.888	0.237	Octatriacontane	3.173	0.991
Nonadecane	0.895	0.269	Nonatriacontane	1.633	1.246

[1] unit: μg/s in pyrolysis. [2] standard error.

3.3. Cytotoxicity of Cholesta-3,5-Diene on Primary HOK

The detailed cell survival study using calcein green AM for staining live cells and ethidium homodimer-1 (Life Tech., New York, NY, USA) for staining dead cells can be found elsewhere [13]. In this study, to find the cytotoxicity against Cholesta-3,5-diene, HOK was stimulated on chamber slides with Cholesta-3,5-diene (10 and 100 μg/mL), with calcein green AM and ethidium homodimer-1.

The cells were finally analyzed using fluorescence microscopy (Eclipse TE200, Nikon Inc., New York, NY, USA). To assess the cytotoxic effects of Cholesta-3,5-diene on primary HOK, the cells were treated with various concentrations of Cholesta-3,5-diene for 24 h. As shown in Figure 3, cytotoxicity increased dose-dependently in primary HOK treated with Cholesta-3,5-diene (0.1–100 μg/mL). To confirm the cytotoxicity of Cholesta-3,5-diene in primary HOK, a cell survival assay was visualized from both live cells by calcein green AM and dead cells by ethidium homodimer-1 (Figure 3). Primary HOK with 10 and 100 μg/mL Cholesta-3,5-diene were indicated (stained green by cytosolic esterases) from the membrane permeable calcein AM in living cells. It was extremely important to

analyze a significant number of dead cells (stained red by ethidium homodimer-1) in the primary HOK from Cholesta-3,5-diene. These results certainly demonstrate that Cholesta-3,5-diene can be strongly associated with a cytotoxic effect in primary HOK.

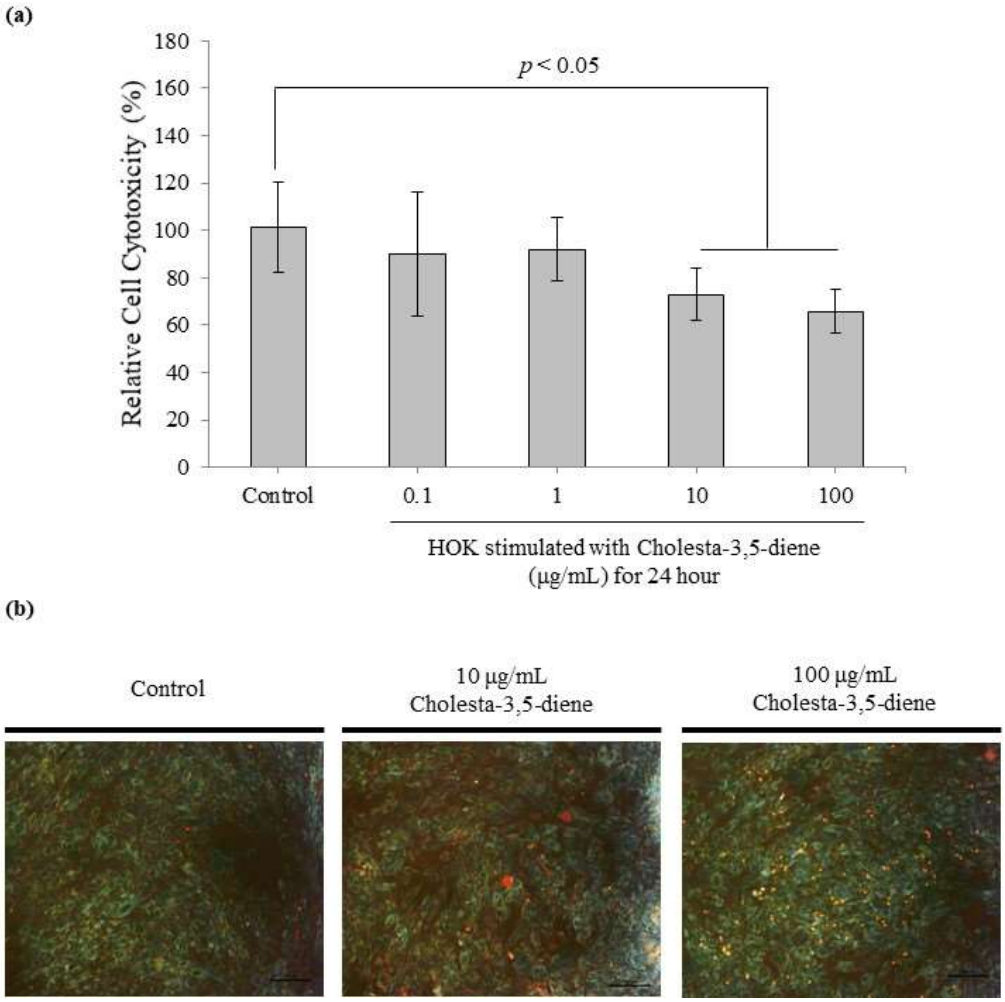

Figure 3. (**a**) Relative cell cytotoxicity and (**b**) image of cytotoxic effects in human oral keratinocytes (HOK).

4. Conclusions

Surgeons and operating room personnel risk potential adverse effects from surgical smoke. Seven TURP samples were pyrolyzed to examine the characteristics of the smoke aerosols. Cholestra-3,5-diene (emission rate of 79.67 μg/s), 9-methylanthracene, hentriacontane, and dotriacontane were identified as the major molecular components in all smoke PM samples collected from the pyrolysis of human prostate tissues. In addition, Cholesta-3,5-diene was found to have a cytotoxic effect in primary HOK. Therefore, surgical smoke can have adverse effects on human health. The results of this study offer valuable information regarding the health risks of operating room surgical smoke exposure.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4433/9/10/381/s1, Figure S1: Carbonaceous Thermal Distributions by a laboratory-based thermo-optical ECOC analyzer for the pyrolyzed prostate tissue samples, Figure S2: Mass fragments (**a**,**b**) of Cholesta-3,5-diene in smoke sample of patient 01 by GC/MS-TD and (**c**) NIST 2008 MS library research result, Table S1: Operational conditions of GCMS-TD system, Table S2: Coefficient of determination (r^2) of standards, percent of duplication, and method of detection limit for the target organic compounds in particulate matter, Table S3: Organic carbon (OC) and elemental carbon (EC) concentrations by a laboratory-based thermo-optical ECOC analyzer, Table S4: Concentration of Organic Compounds normalized to $PM_{2.5}$ mass in pyrolyzed TURP-smoke by GC/MS-TD, Table S5: Emission Rate of PM2.5 mass in pyrolyzed TURP-smoke.

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Conflicts of Interest: The authors declare no conflict of interest.

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