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Cigarette Smoking and DNA Damage

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Abbreviations

ACT, aqueous cigarette tar extracts
DMPO, 5,5-dimethyl-1-pyrroline-N-oxide
ESR, electron spin resonance

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I. Abstract

Cigarette tar contains over 5000 different compounds, including nicotine, polycyclic aromatic hydrocarbons, nitrosamines, phenols and polyphenols. Since many of these tar constituents are water-soluble, aqueous cigarette tar extract (ACT) solutions are complex mixtures with hydroquinone and catechol as major components that can reduce oxygen to produce superoxide and hence hydrogen peroxide and the hydroxyl radical. Cigarette tar radical and the catechol-derived radical can penetrate viable cells, bind to DNA, and might play an important role in causing DNA nicks and lung tumors. This report explores the possible role of different components of cigarette smoke in the development of pulmonary/cardiovascular disease(s), concentrating mainly on the role of cigarette smoke in producing DNA damage.

II. Introduction

Cigarette smoking is associated with an excess of roughly 400,000 deaths annually from cardiovascular disease in the United States alone. There is a clear relationship between the degree and duration of exposure to cigarettes and the incidence of cardiovascular events. Furthermore, a decline in risk follows cessation of smoking, although residual effects may persist for a decade. However, the relative importance of the mechanisms that mediate this cardiovascular hazard is poorly understood [1]. Moreover, cigarette smoking has been linked to cancer of the lung, colon, and esophagus; about 20% of all cancer cases in 1990 in the United States are attributed to cigarette smoking. In addition to threatening the health of smokers, tobacco smoke may also be deleterious to nonsmokers who are exposed to environmental tobacco smoke [2]. Cigarette smoke is known to contain a large number of oxidants, and it has been hypothesized that many of the adverse effects of smoking may result from oxidative damage to critical biological substances [3].

III. Cigarette Smoke Chemistry

Cigarette smoke is operationally divided into gas-phase smoke and particulate matter (or tar). Tar is the material retained on a filter, whereas gas-phase smoke passes through the filter. (Typically, a Cambridge filter [University of Kentucky, Lexington, KY], which retains 99.9% of the particles larger than 0.1 micron). Both the tar and gas-phase smoke are very rich sources of radicals [3].

A- Gas-phase Smoke Radicals

There are more than 10^{15} organic radicals per puff in gas-phase cigarette smoke [3]. In addition, smoke contains up to 500 ppm nitric oxide ($^{\bullet}NO$), which slowly undergoes oxidation to nitrogen dioxide ($^{\bullet}NO_2$), and both these gases are, of course, radicals. In contrast to the stable radicals in cigarette tar, the organic radicals in gas-phase cigarette smoke are reactive carbon- and oxygen-centered radicals that typically have life times of less than 1 second. These radicals are too short lived to be observed by direct ESR, but they can be studied by the ESR spin trap method [4]. Because gas-phase smoke contains carbon-and oxygen-centered radicals and high concentrations of nitric oxide, alkyl peroxynitrite and peroxynitrate esters also can be produced [5]. Figure 1 shows a schematic outline of the isolation of gas-phase smoke and tar and the radicals that each phase produces [3].

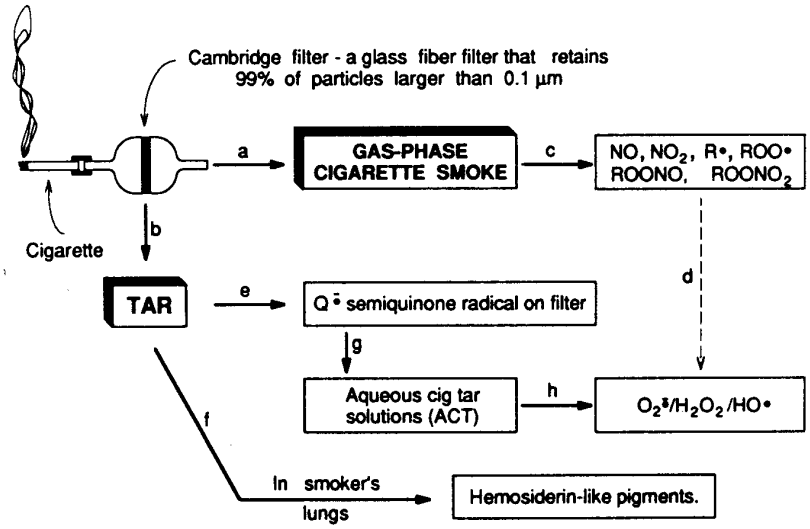

Figure1 Shows the separation of cigarette smoke into (a) gas-phase and (b) particulate matter (or tar) by the use of a Cambridge filter. (c) Gas-phase smoke contains both carbon-centered and oxygen-centered radicals that are produced from NO/NO_2 reactions with reactive compounds in smoke. Tar contains a semiquinone (e), This semiquinone can be extracted into aqueous cigarette tar solutions (ACT), as shown in (g), and these solutions yield superoxide and hydrogen peroxide. In the presence of iron, these solutions produce the hydroxyl radical, which can be spin trapped (h). Adapted from [3].

B- Tar Radicals

Cigarette tar contains remarkably high concentrations of radicals (*ca*. 10^{17} spins/gram), which are sufficiently stable to be directly observable by electron spin resonance [3]. Aqueous extracts of cigarette tar (ACT) contain a low-molecular-weight quinone-hydroquinone-semi-

quinone system (Q-QH2-$^{\bullet}$QH). Figure 2 shows the ESR signal observed for the tar radical; it is a broad singlet with a g value of 2.0035. This signal has been assigned to the semiquinone radical [6].

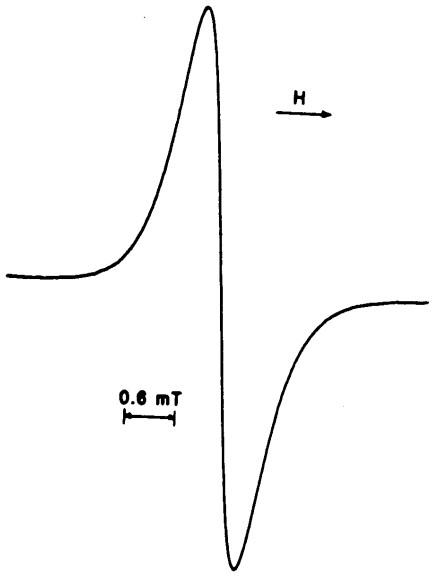

Figure 2 The ESR spectra of the tar radical from 1R1 Research cigarettes. The tar trapped on a Cambridge filter was extracted with 20 ml benzene. The signal shown is due to the spin component termed 'radical D'. This radical is a broad singlet with g =2.0035 that can be assigned to a semiquinone system. Adapted from [3].

Since ACT contain the quinone radical system, these ACT can reduce oxygen to form superoxide (equation 1). Superoxide, in turn, can dismutate to form hydrogen peroxide (equation 2).

$$Q^{\bullet-} + O_2 \rightarrow Q + O_2^{\bullet-} \quad (1)$$

$$2O_2^{\bullet-} + 2H^+ \rightarrow O_2 + H_2O_2 \quad (2)$$

Thus, these ACT solutions consume oxygen and produce a series of activated oxygen species that can cause biological damage [7]. Furthermore, both cellular fluids and cigarette tar itself contain metal ions (such as iron) that can catalyze the production of hydroxyl radicals from hydrogen peroxide via the Fenton reaction (equation 3). Thus, the reducing capability of cigarette tar gives superoxide, which ultimately leads to the production of the hydroxyl radical; a potent oxidant.

$$H_2O_2 + Fe(II) \rightarrow HO^{\bullet} + HO^{-} + Fe(III) \qquad (3)$$

When aqueous extracts of cigarette tar are allowed to react with spin traps, spin adduct spectra are obtained that show the presence of the hydroxyl radical. The spin adduct ESR spectra obtained using DMPO are shown in figure 3 [7].

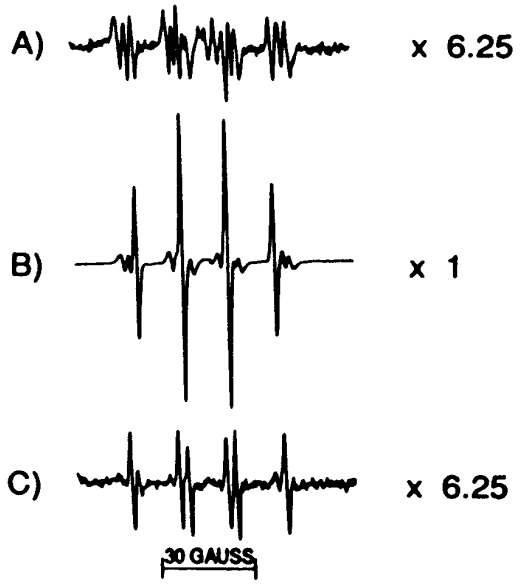

Figure3. The ESR spectra of the spin adducts obtained when aqueous extracts of cigarette tar are allowed to react with 0.04 M 5,5-dimethyl-l-pyrroline-N-oxide (DMPO). Panel (A) shows a complex spectrum that can be resolved as the signals of 3 spin adducts assigned to the hydroxyl radical, an alkyl radical, and the carbon dioxide radical anion. In Panel (B) the spectrum of the hydroxyl radical is intensified in the presence of EDTA. Spectrum (C) shows that a weaker alkyl radical signal is obtained if the solution is passed through a column containing Chelex-100 before mixing with DMPO. Adapted from [3].

IV- Damage to DNA caused by cigarette Tar and ACT

ACT solutions produce superoxide, hydrogen peroxide, and the hydroxyl radical, and thus become potent oxidants. These ACT solutions can initiate lipid peroxidation, oxidize proteins, and nick DNA [8]. The quinone-hydroquinone-semi-quinone system can penetrate viable mammalian cells, bind to, and nick cellular DNA (8). The nicks produced by the tar radical require multi-step repair, suggesting a process that could be error prone [9]. These ACT solutions also interfere with mitochondrial electron transport [10].

Pryor and Stone (1998) showed in an interesting study the role of the tar radical in ACT extracts in the DNA damage that is caused by cigarette smoke [11]. In their paper, they reported

the fractionation of ACT solutions using Sephadex chromatography. The initial fractionation yielded 78 fractions that were analyzed by EPR and UV spectroscopy and assayed for O_2 consumption as a measure of H_2O_2 production. On the basis of similar UV spectra, the 78 fractions from the Sephadex column were combined into eight major fractions. Then, they have assayed these eight fractions for DNA nicking in rat thymocytes and then analyzed them by GC/MS and EPR spectroscopy. The fractions that contain the tar radical also produce superoxide, H_2O_2, and hydroxyl radicals; these fractions caused the predominant and most significant amounts of DNA nicking (Table 1) [11].

Table 1. Data for the eight combined ACT fractions from Sephadex G-25 chromatography

ACT fraction	eluent (mL)	dry weight (mg)	DNA damage, Q_d	H_2O_2 (μM)[a]	radicals detected by EPR[b]	components detected by GC/MS
I	0-56	5.76	2.4	1.8	none	
II	58-62	4.56	4.1	12	$R^{\bullet}$	nicotine
III	64-88	1.80	20.4	24	None	nicotine
IV	90-94	2.40	16.4	22	None	H_2Q, catechol
V	96-98	0.32	114	144	HO·, *o*-Q^-, *p*-Q^-, O_2^-	H_2Q, catechol
VI	100-106	0.14	88.7	82	·OH, *o*-Q^-, O_2^-	catechol
VII	108-114	0.18	16.0	14	None	
VIII	116-158	0.50	19.9	16	None	
total		15.66	272	313		

[a] H_2O_2 measurement is based on O_2 uptake. The 2.0-mL final volume of these solutions contained tar fractions at a concentration of 8 g/mL. [b] $R^{\bullet}$, an unidentified radical; $HO^{\bullet}$, the hydroxyl radical; $O_2^{\bullet-}$, superoxide; *o*-Q^-, *o*-benzosemiquinone; *p*-Q^-, *p*-benzosemiquinone; H_2Q, hydroquinone. Radicals were identified based upon literature values for splitting patterns, intensities, and hyperfine splitting constants (hfsc).

Of the eight combined fractions analyzed by direct EPR, only fractions II, V, and VI gave EPR signals. Fractions V and VI contained less than 3% of the total mass of the tar in these aqueous cigarette tar (ACT) extract solutions, yet these two fractions accounted for 70% of the total DNA damage caused by all of the ACT fractions (Table 1). Direct EPR measurements reveal the presence of *o*- and *p*-benzenesemiquinone and catechol radicals in fractions V and VI respectively (Figure 4). Fraction II contained 30% of the total mass of the ACT extract and caused just 1.5% of the total DNA damage. Fraction II contains a mixture of nicotine and

another major constituent that is unidentified (figure 4) [11]. These data provide clear evidence that the cigarette tar semiquinone radical is critically involved in causing DNA damage.

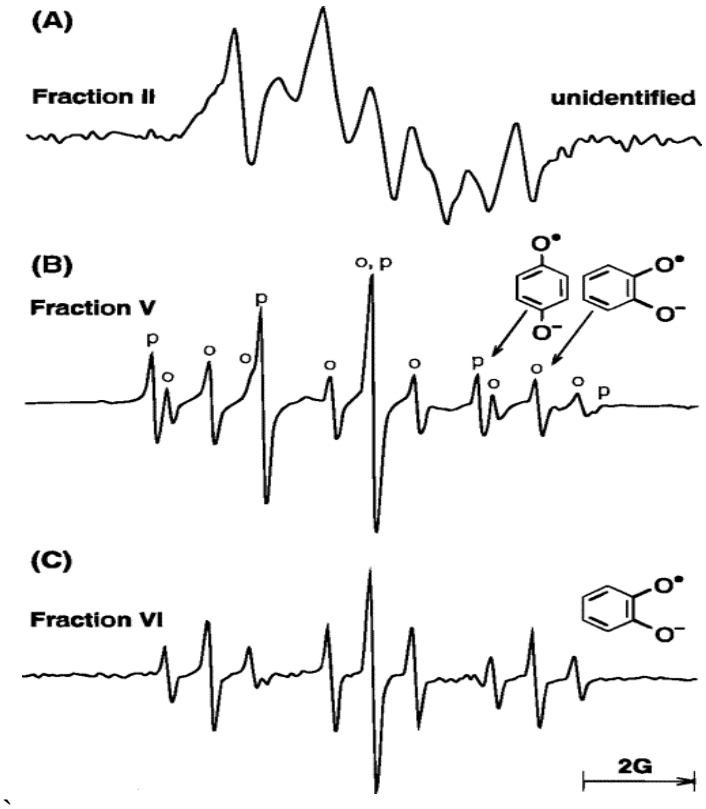

Figure 4 EPR spectra of the fractions of ACT
A, fraction II; B, fraction V; C, fraction VI. Note the presence of *o*- and *p*-benzenesemiquinone radicals in fractions V and catechol in fraction VI and a mixture of nicotine and another major constituent that is unidentified in fraction II.
Adapted from [11].

In recent years, it has become clear that free radicals are involved in many of the biological processes that occur when chemicals transform cells. Since cigarette smoking increases the concentrations of radicals in the lungs, it appears reasonable to assume that some of the tumorigenicity of smoke derives from the free radicals it contains or causes to be produced in the lung. The DNA strand breaks caused by ACT are the type of strand break that cannot be repaired in a single step, and the likelihood for mutation is increased by the possibility for error at each step of a multi-step repair process. A group of enzymes similar to that suggested for repairing breaks induced by ionizing radiation is probably necessary for repair of strand breaks induced by ACT. At least two possibilities for mutation exist in this repair scheme. The DNA ligase may join DNA strands across nucleotide gaps after excision of the 3' phosphate or the 5'

nucleoside; thus, deletion mutation might occur by ligation across tar-induced lesions where base release accompanies strand scission. Mutation might also result from base misincorporation during gap filling by a polymerase. Regardless of the detailed mechanism, it seems clear that the types of DNA nicks produced by cigarette smoke and ACT must lead to mutations; and these mutations may be related to the known ability of cigarette smoke to induce lung cancer [8,12]. Figure 5 suggests the possible mechanisms that might lead to DNA damage by the cigarette tar radicals.

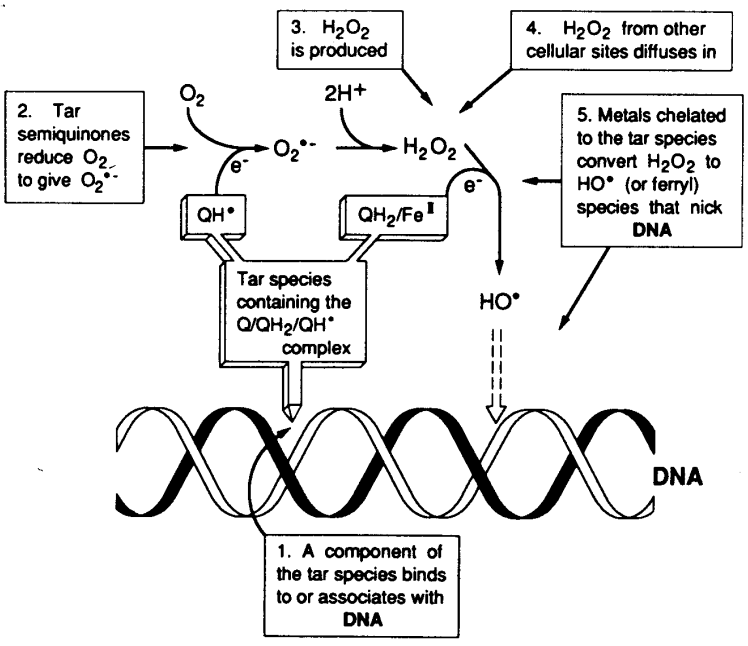

Figure 5 shows the binding of a tar species that contains a polyhydroxyaromatic component to DNA. The component associates with, or binds to, DNA, could be of the semiquinone -containing species, *step1*, which then reduces oxygen to form superoxide, *step* 2. Hydrogen peroxide is generated either from dismutation of the superoxide or by diffusion of H_20_2 from other loci in the cell, *step* 3. The hydrogen peroxide then is reduced to form the hydroxyl radical by metal ions bond by the tar complex, *step* 4. Finally, DNA nicking can occur . Adapted from [3].

V- Summary

This paper reviewed the different oxidants present in tobacco tar and smoke and the evidence for their involvement in cigarette-induced pathology and DNA damage. The oxidants consist of organic radicals of a variety of types and reactivities as well as species such as nitrogen dioxide, nitric oxide, hydrogen peroxide, peroxynitrite, and peroxynitrate.

VI- References

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