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REACTIVE OXY-RADICALS FROM CIGARETTE SMOKE AND THEIR PHYSIOLOGICAL EFFECTS

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INTRODUCTION

Cigarette smoke contains high concentrations of radicals both in the gas phase and in tar.[1-5] For example, there are more than 10^{16} free radicals/puff in cigarette smoke. In this paper, we wish to discuss the nature of the radicals in tar and in gas phase smoke, and to suggest radical-mediated mechanisms for some of the physiological effects of smoke.

THE TAR FREE RADICAL

The free radical in cigarette tar originally had been suggested to be an odd electron delocalized over a polynuclear aromatic hydrocarbon (PAH) molecule,[6] probably a PAH-cation radical $PAH^{+\cdot}$. We have recently shown that there are in fact at least four paramagnetic species in tar, three of which are present at low concentrations: (A) a phosphorous-type radical; (B) a graphite-like radical; and (C) a radical with paramagnetic properities like those of the charge transfer (CT) complex between iodine and a PAH. The fourth paramagnetic species, radical D, which is by far the predominant paramagnetic species in tar, is not a monoradical but instead is a complex between quinone (Q) and hydroquinone (QH_2) groups in a polymeric matrix. This species is an excimer with a paramagnetic excited state.[5]

This structure for the tar radical may explain the co-carcinogenic properties of tar. Synthetic Q/QH_2 polymers are known to be able to catalyze the autoxidation of hydrocarbons to oxygenated species.[7a] Interestingly, the fraction of cigarette smoke containing hydroxy aromatic compounds is known to be the most potent co-carcinogenic fraction of smoke, with the most powerful single co-carcinogen being catechol.[7b] Thus, we suggest that the hydroxy aromatic polymer, radical D, acts as a

co-oxidation catalyst, causing the oxidation of PAH in tar to carcinogenic phenols and quinones,[5] as shown in equation 1.

$$\text{PAH} \xrightarrow[O_2]{Q/QH_2 \text{ paramagnetic polymer}} \text{PAH phenols, diols, and quinones} \quad (1)$$

This suggestion, for the first time, provides a connection between the free radical content of tar and some of its physiological effects.

THE GAS PHASE SMOKE FREE RADICAL

The radicals in cigarette tar are long lived; in contrast, the radicals in gas-phase cigarette smoke are much shorter lived[1,2,4] and can only be observed using electron spin resonance (esr) spin-trapping techniques.[8] Figure 1A shows the esr spectrum obtained when the smoke from about 1/5 of a single 1R1 standard research cigarette is passed through a Cambridge filter, down a 180 cm Pyrex tube,[9] and bubbled into a 0.1 M solution of the spin trap α-phenyl-N-tert-butyl nitrone (PBN) in benzene. The spectrum that is obtained is primarily due to an alkoxyl (RO·) adduct of PBN.[10] (Table I shows the hyperfine splitting constants for these radicals.) A similar spectrum is obtained if the smoke is held in a syringe for 10 minutes before being bubbled into the spin trap solution.

These long lifetimes for alkoxyl radicals in gas phase smoke are difficult to reconcile with the high reactivity and short lifetimes observed for alkoxyl radicals in pure chemical systems.[11] Carbon-centered radicals are known to be produced in the flame;[12] these radicals would become converted to peroxyl radicals as O_2 enters the smokestream through the cigarette paper,[13] forming peroxyl (ROO·) radicals. Tertiary peroxyl radicals do have unusually long lifetimes;[14] however, in cigarette smoke, all peroxyl radicals would rapidly react with NO (which is present at very high concentrations) and be converted to short-lived alkoxyl radicals.[10] Thus, it does not appear likely that the radicals produced in the flame could have the long lifetimes that we observe.

To account for these unexpectedly long apparent lifetimes for oxy-radicals, we propose that radicals are continuously produced in aging cigarette smoke and that a steady state in radicals exists in smoke. How might a steady state of reactive oxy-radicals be produced in gas phase cigarette smoke?

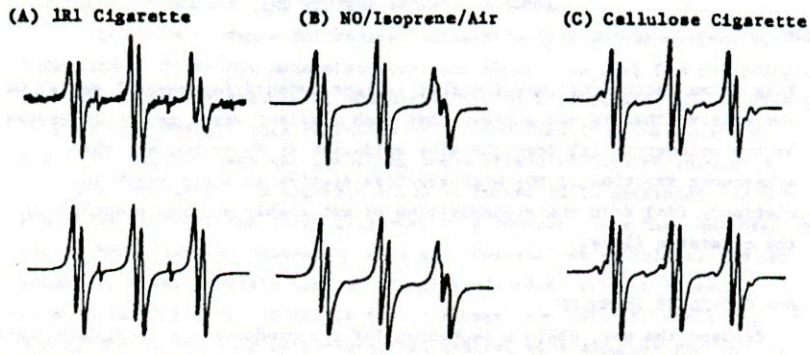


Figure 1. Experimental (above) and simulated (below) electron-spin resonance (esr) spectra. The experimental spectra were obtained by spin trapping at room temperature from gas streams bubbled at a flow rate of 500 mL/min through 4 ml of a 0.1 M solution of PBN in benzene. After bubbling was complete, 0.5 mL of the sample was transferred to a cylindrical esr tube, purged with nitrogen for 10 minutes, and stored at -78°C until the sample was analyzed. Spectra were obtained using an IBM Model 100D esr spectrometer with an ASPECT 2000 computer system. The simulations were done with the ASPECT computer using the measured hyperfine splitting constants and assuming 100% Lorentzian line shapes.
(A) The spectrum obtained from a 1R1 cigarette "smoked" for 180 sec; about 0.3 g of tobacco was consumed in this time.
(B) The spectrum obtained when a mixture of NO (460 ppm) and isoprene (3900 ppm) in air was bubbled through the PBN solution for 180 sec.
(C) The spectrum obtained when a cellulose cigarette was smoked for 26 sec; about 0.8 g of cellulose was consumed.

Table I. Hyperfine Splitting Constants for the Spin Adducts of PBN Obtained from (A) the Smoke from 1/5 of a 1R1 Research Cigarette, (B) from a Mixture of 460 ppm Nitric Oxide, Oxygen, and 3900 ppm Isoprene, and (C) from a "cigarette" made from pure cellulose.[a]

Trapped Radical Identification	a_N/a_H (in mT)[b]		
	1R1 Cigarette	$NO/O_2/C_5H_8$	Cellulose "Cigarette"
$RO^{\cdot}$	1.37/0.19 (65%)	1.38/0.21 (42%)	1.35/0.19 (80%)
$R^{\cdot}$	1.43/0.21 (31%)	1.42/0.21 (55%)	1.42/0.32 (8%)
$R^{\cdot}(?)$[c]	-	-	1.48/0.51 (8%)
PBNOx	0.79 (4%)	0.79 (3%)	0.80 (4%)

(a) Unpublished data of W.A. Pryor, M. Tamura, and D.F. Church.
(b) The % distribution of the radicals and the hfsc are the data that were used to produce the simulated spectra shown in Figure 1.
(c) The unassigned adduct may be due to $CO_2^{\cdot-}$.

This is an intriguing question that we have pursued for several years; we now believe that we can suggest that such a steady state can be generated by two pathways: (i) from the slow oxidation of NO to NO_2 and the subsequent reaction of NO_2 with reactive species in smoke (such as olefins); (ii) from the decomposition of metastable species produced in the cigarette flame.

NO_x CHEMISTRY IN SMOKE

Perhaps the most obvious mechanism for the production of a steady-state of radicals in gas phase smoke is that organic radicals somehow arise from the reactions of NO and NO_2 in smoke, since both these species are free radicals and are present at high levels in smoke. The standard 1R1 research cigarettes that we have used produce 10.6 μmoles NO/cigarette[15] and this NO could lead to organic radicals as shown in equations 2 and 3. Thus, we suggest that NO, which is known to be quite

$$NO \xrightarrow[\text{slow}]{O_2} NO_2 \xrightarrow[\text{species (such as isoprene)}]{\text{Reactive}} R\cdot \xrightarrow{O_2} ROO\cdot \qquad (2)$$

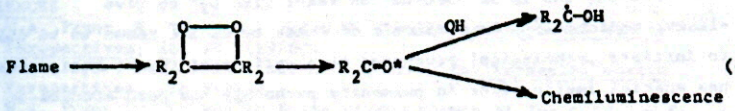
$$ROO\cdot + NO \longrightarrow RO\cdot + NO_2 \qquad (3)$$

unreactive, undergoes slow oxidation to NO_2, and NO_2 is known to be able to react with a variety of types of organic molecules to produce radicals.[16] For example, isoprene, which is present in smoke at concentrations up to 58.8 μmoles/cigarette,[17] can react with NO_2 to give carbon-centered radicals that would rapidly react with oxygen to become peroxidized. In this way, NO could serve as a reservoir of radicals that can slowly be converted to a steady-state concentration of much more reactive carbon- and oxygen-centered radicals. To test this suggestion, we have modeled the flowing cigarette smoke stream by mixing 3900 ppm isoprene and 460 ppm NO in air and bubbling this gas mixture into the spin trap solution. The spectrum obtained (Figure 1B) shows both alkoxyl and alkyl[18] adducts of PBN. Thus, the NO/O_2/isoprene model system gives mixtures of carbon-centered and alkoxyl radicals much like those from 1R1 cigarettes (see Table I).

CELLULOSE "CIGARETTE" AND EXCITED SPECIES IN SMOKE

We also have evidence for radical production by a second mechanism, independent of the NOx mechanism described above. To test for the presence of radicals in the absence of NOx chemistry, we have studied the burning of "cigarettes" made of pure cellulose,[19] burning them in a atmosphere of 20% O_2 in argon. When the resulting smoke stream is bubbled through a spin trap solution, the spectrum shown in Figure 1C is obtained. To our knowledge, this is the first time that free radicals have been detected by esr methods from the combustion of a material such as cellulose. To our surprise, these radicals also are long-lived. Just as with tobacco cigarettes, the major adduct is due to alkoxyl radicals; in addition, a strong signal due to a carbon-centered radical spin adduct[18] and an unassigned species[20] can be observed (see Table I).[21]

One hypothesis to rationalize the long-lived free radicals in cellulose smoke is that they arise from the reactions of metastable species. For example, excited species are known to be present in smoke, since both cigarette smoke and smoke from cellulose are known to give rise to very intense chemiluminescence.[22] Although there are many possible precursors for this chemiluminescence, it appears that the light emitting species is a carbonyl compound. Thus, the precursor for chemiluminescence could be a metastable species that decomposes to form excited carbonyl compounds; one possibility for such a species is a dioxetane.[23] If dioxetanes are present in smoke, they could slowly decompose to produce species that might react like alkoxyl radicals.[24] (See equation 4.) Interestingly, the chemiluminescence in smoke has a long lifetime.



(4)

Experiments of a different type also provide evidence for metastable species in smoke. The gas stream from either 1R1 cigarettes, cellulose cigarettes, or the NO/isoprene gas mixture can be bubbled into a Freon 11 solution of spin trap at -78°C. Alternatively, these smoke streams can be bubbled into Freon 11 at -78°C with no spin trap initially present and the spin trap can be added 30 minutes later. In both cases, a spin adduct

signal is observed immediately upon addition of the spin trap at -78°C. When the solution is slowly warmed, a marked increase in the signal intensity occurs as the temperature reaches -20 to -30°C. This behavior suggests that there are thermally labile species present in all three smoke streams that decompose to give radicals. Equation 5 suggests a possible explanation for the observation of metastable species in the smoke from 1R1 cigarettes or the NO/isoprene system. In both of these systems,

$$ROO\cdot + NO_x \longrightarrow ROONO_x \qquad (5)$$

alkylperoxy nitrites and nitrates could be formed; these species are known to be thermally unstable and to decompose to give radicals.[25] In the case of the cellulose smoke, as described above, the labile species could be some type of oxygenated metastable compound such as a dioxetane that decomposes to give excited carbonyl compounds.

ADDITIONAL RADICAL MECHANISMS—DESTRUCTION OF ANTIPROTEASE

The mechanisms of radical production described above all apply to gas phase radicals. However, when the smoke stream enters the lung additional radical production mechanisms become possible. Firstly, smoke enhances the activity of pulmonary alveolar macrophages (PAM), and these release $O_2^{\cdot}$ and thus H_2O_2. These two species react (by unknown mechanisms that appear to be metal dependent) to give the damaging HO· radical.[26] In addition, prostaglandin and leukotriene hydroperoxides are released, and these species can react with $O_2^{\cdot}$ to give alkoxyl radicals.[27] Oxy-radicals of these types are known to be able to initiate pathological processes of a variety of types; one process that has special implications in pulmonary pathology has been studied in some detail in recent years. Superoxide radicals from PAM initiate processes that are known to inactivate the human pulmonary antiprotease, suggesting the involvement of radicals in the etiology of emphysema.[28]

We have recently discovered other radical reactions that might initiate emphysema.[29] We have shown that NO_2 (from smoke) can react with pulmonary H_2O_2 (from PAM) to produce oxy-radicals that inactivate the human antiprotease. The antiprotease is protected by SOD and mannitol, implicating $O_2^{\cdot}$ and HO· as reactive species from the NO_2/H_2O_2 system.

$$NO_2 + H_2O_2 \longrightarrow HONO_2 + HO\cdot \quad (6)$$

$$NO_2 + H_2O_2 \longrightarrow NO_2^- + 2H^+ + O_2^{\cdot-} \quad (7)$$

SUMMARY AND PHYSIOLOGICAL IMPLICATIONS

All of these radical-producing processes could cause pulmonary damage. Alkoxyl radicals in smoke could directly attack reactive pulmonary target molecules such as unsaturated lipids or sulfur-containing enzymes and proteins.[30] In some cases these reactions produce toxic products (e.g., conjugated aldehydes from oxidized lipids[31]). Smoke also stimulates pulmonary macrophages to produce $O_2^{\cdot-}$ and H_2O_2. In addition, NO_2 can react with the hydrogen peroxide produced to release hydroxyl radicals and/or superoxide. Finally, the radicals in smoke (peroxyl, alkoxyl, NO_2, or the tar radical) could cause the oxidation of the polycylic aromatic hydrocarbons in smoke to carcinogenic species.[32-34]

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