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Brief Communication

A COMPARISON OF THE FREE RADICAL CHEMISTRY OF TOBACCO-BURNING CIGARETTES AND CIGARETTES THAT ONLY HEAT TOBACCO

WILLIAM A. PRYOR,† DANIEL F. CHURCH,* MARK D. EVANS,* WILLIAM Y. RICE, JR.,‡ and JOHNNIE R. HAYES,‡

*Biodynamics Institute, 711 Choppin Hall, Louisiana State University, Baton Rouge, LA 70803-1800; †R.J. Reynolds Tobacco, Bowman Gray Technical Center, Winston-Salem, NC 27102

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Abstract—Cigarette smoke contains free radicals both in the particulate matter (tar) and in vapor-phase smoke. Vapor-phase smoke decreases the activity of α-1-proteinase inhibitor (α1PI) in vitro. The free radical content of the tar and vapor-phase smoke from a cigarette that heats rather than burns tobacco has been compared with data on a standard 1R4F cigarette. No radicals were detected in the tar from the new cigarette and radicals in its vapor-phase smoke are lower by more than 99% relative to the 1R4F standard cigarettes. The vapor-phase smoke from the new cigarette causes essentially no reduction of α1PI activity in vitro. These findings support our previously published mechanisms for the production of radicals in tar and in vapor-phase smoke.

Keywords—Cigarette, Vapor-phase smoke, Tar, Free radicals, Oxy-radicals, Electron spin resonance, Alpha-1-proteinase inhibitor

INTRODUCTION

We have been studying the structures and concentrations of free radicals in cigarette smoke with the goal of elucidating the connection between these radicals and the adverse health effects associated with smoking. Cigarette smoke radicals are of two distinct classes: 1) Long-lived radicals associated with the particulate phase (tar)—these radicals can be observed by direct electron spin resonance (ESR) spectroscopy; and 2) short-lived radicals associated with the vapor-phase that are strongly oxidizing radicals—these species cannot be seen by direct ESR and can only be studied by the ESR spin trapping technique.[1–4]

The long-lived tar-associated radical appears to be a semiquinone radical associated with quinones (Q) and hydroquinones (QH_2) in the tar.[1,2] These Q/QH_2 probably include catechol, hydroquinone, and other quinoid compounds that are either present in smoke or are generated by oxidation of polycyclic aromatic hydrocarbons (PAH) in the tobacco combustion process.

Vapor-phase cigarette smoke contains carbon- and oxygen-centered radicals with structures that predict lifetimes of fractions of a second; [2–4] however, spin trap experiments show that these radicals have *apparent* lifetimes in excess of 5 min.[4] To rationalize this paradox, a steady-state mechanism for continuous formation and destruction of these vapor-phase radicals was postulated.[3,4] In this mechanism, NO is slowly oxidized to nitrogen dioxide, which reacts with smoke constituents such as isoprene, butadiene, and acrolein. In support of this mechanism, we have shown that NO/isoprene mixtures in air, *without a flame*, lead to the same mixtures of spin-trapped radicals as are produced by a burning 1R1 cigarette.[2–5]

We also tested this mechanism by burning pure cellulose in an argon/oxgen mixture. To our surprise, this combustion process produces radicals also, although NOx chemistry cannot be involved.[5] However, the radicals produced when cellulose or wood is burned arise by very different mechanisms than do those produced when tobacco burns.[6,7]

In view of these surprising results with cellulose, we have studied the radical chemistry of the tar and smoke from a new cigarette that heats rather than burns tobacco.[8] This tobacco-heating cigarette (THC) heats a combination of tobacco and tobacco extract at about 250°C and produces an aerosol that contains much lower

†Author to whom correspondence should be addressed.

concentrations of many compounds than does smoke from burning a standard 1R4F cigarette (see Table 1). Notice that both Q/QH_2 compounds that could be in the tar phase (such as catechol) and vapor-phase components such as NOx are lower in the THC smoke.

Thus, the tar from the THC contains much lower concentrations of quinones and hydroquinones and the vapor phase contains much less NOx and isoprene relative to a 1R4F cigarette. Therefore, we predict that both the tar and vapor-phase smoke from the THC should have much lower radical content than does a 1R4F cigarette.

Smoke radicals appear to be involved in the inactivation of α1PI, the principal human antiproteinase,[9-13] and this process may be important in the etiology of emphysema. Both vapor-phase smoke radicals and an NO/air/isoprene model system (which mimics much of the radical and oxidant chemistry of smoke) directly inactivate α1PI in vitro.[9,10] Since THC smoke contains much less isoprene and nitrogen oxides (as well as other species), it would be predicted to inactivate α1PI less than does smoke from standard cigarettes.

MATERIALS AND METHODS

Chemicals

Elastase (from porcine pancreas) and human α1PI were purchased from Calbiochem Corp. (San Diego, CA). The elastase substate (*N*-succinyl-L-alanyl-L-alanyl-L-alanine-*p*-nitroanilide) was obtained from Sigma (St. Louis, MO). α-Phenyl-*N*-*tert*-butyl nitrone (PBN), from Kodak (Rochester, NY), was recrystallized from hexane.

Cigarettes

The reference cigarette was the 1R4F, an air-diluted filtered cigarette that burns tobacco, which was obtained from the University of Kentucky, Tobacco and Health Research Institute, Lexington, Kentucky. The cigarette that only heats tobacco (THC) was obtained from R. J. Reynolds Tobacco Company, Winston-Salem, North Carolina. The 1R4F cigarettes (Tobacco and Health Res. Inst., Lexington, KY) were preconditioned at 76°F and 64% relative humidity for at least 24 h prior to use.[1-11] The THC were used from freshly opened packs as recommended by R. J. Reynolds (Private Communication from Dr. W. Y. Rice and ref. 8, P. 126).

Table 1. Selected chemical components in the smoke from a reference cigarette (1R4F) and a new cigarette that heats tobacco (THC)[5-13]

Compound	Amount		% Decrease for the THC
	1R4F	THC	
NOx (μg/cig)[a]	234	11	95
Acrolein (μg/cig)[a]	73	10	86
Benzo[a]pyrene (ng/cig)[a]	10.5	0.1	99
Benz[a]anthrecene (ng/cig)[a]	9.2	0.08	99
Hydroquinone (μg/cig)[a]	37	1.3	97
Catechol (μg/cig)[a]	38	1.6	96
Acrolein (μg/cig)[b]	170	7.6	96
1,3-Butadiene (μg/cig)[b]	66	3.4	95
Isoprene (μg/cig)[b]	477	0.2	99

[a]Data from R. J. Reynolds Tobacco Co., Reference 8.
[b]Data from Brunnemann, et al., Reference 16.

Cigarettes were lighted with a butane lighter. Smoke from the cigarettes was drawn through a Cambridge filter, and the vapor phase smoke was drawn into a hand-operated syringe, aged for 10–15 s, and then bubbled through 2 mL of the spin-trap solution [0.1 M PBN in benzene]; several cigarettes were smoked to obtain a total of 20 puffs. The PBN solutions were transferred to an ESR cell, deaired, and the spectrum recorded as previously reported.[2,3] Five spectra were accumulated and averaged. Peak heights were measured using the left line of the central pair of lines.

The Cambridge filter pad used to trap the mainstream-smoke particulate matter was twice extracted with benzene (25 mL total). The benzene was evaporated on a rotary evaporator and the residue redissolved in 2 mL of benzene. The resulting solution was transferred to an ESR cell and scanned as described.[1-3]

Alpha-1-proteinase inhibitor

An aqueous Chelex-100 treated 0.1 M sodium phosphate buffer (pH 7.4 at 37°C) containing 125 μg/mL human α1PI was bubbled with 12 puffs of vapor-phase smoke from two cigarettes. Six 35 ml puffs per cigarette were drawn over 2 s at 1-min intervals.[11] Each puff bubbled through the α1PI solution over 7 s as previously described.[10] Elastase activity was determined at pH 8.0 using the method of Bieth et al.[15] After incubation of a 1PI with the smoke solution for 24 hr at 37°C, the elastase inhibitory capacity (EIC) was determined by measuring the decrease in elastase activity toward *N*-succinyl-L-alanyl-L-alanyl-L-alanine-*p*-nitroanilide, a synthetic elastase substrate. Either native or vapor-phase smoke treated α1PI was preincubated with the elastase for 5 min at 25° before elastase activity was measured. The EIC of the native α1PI was defined to be 100%.

RESULTS AND DISCUSSION

The 1R4F research cigarette is typical of the majority of cigarettes sold in the United States. In contrast, the tobacco-heating cigarette (THC) burns a carbon pellet

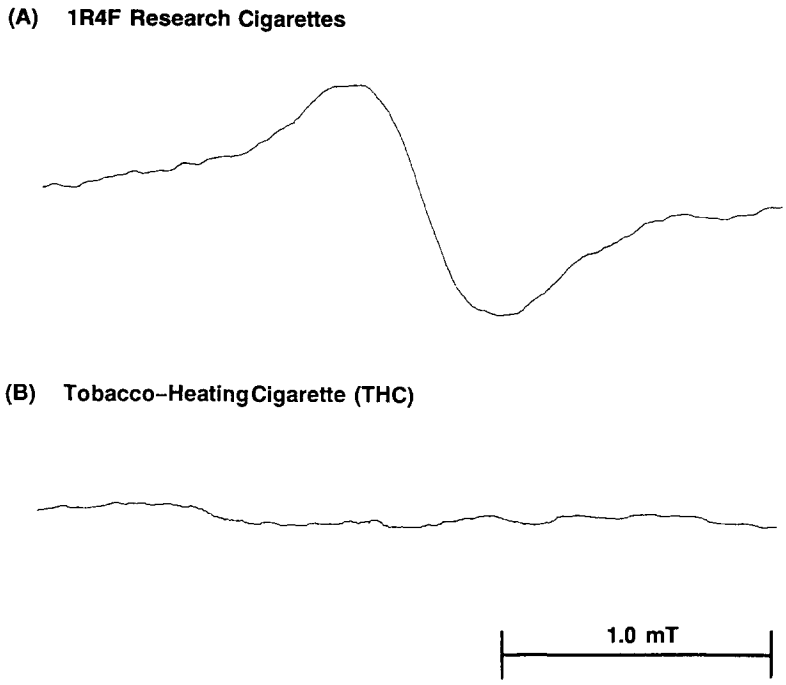

Fig. 1. Comparison of tar radical spectra observed for (A) 1R4F research cigarettes and (B) the tobacco-heating cigarette (THC). Smoke (total of 20 puffs) was passed through a Cambridge filter. The tar was then extracted with two 2–10 mL portions of benzene, the benzene extracts were combined, and the volume reduced to 1 mL for ESR analysis. The radical observed in the tar from 1R4F cigarettes has a g-value of 2.0035; as we have previously described (1), this species results from quinone and hydroquinone species bound into a polymeric matrix. Instrument settings: Modulation amplitude = 0.1 mT; time constant = 5000 ms; center field = 349.5 mT; sweep width = 2.0 mT; sweep time = 2000 s.

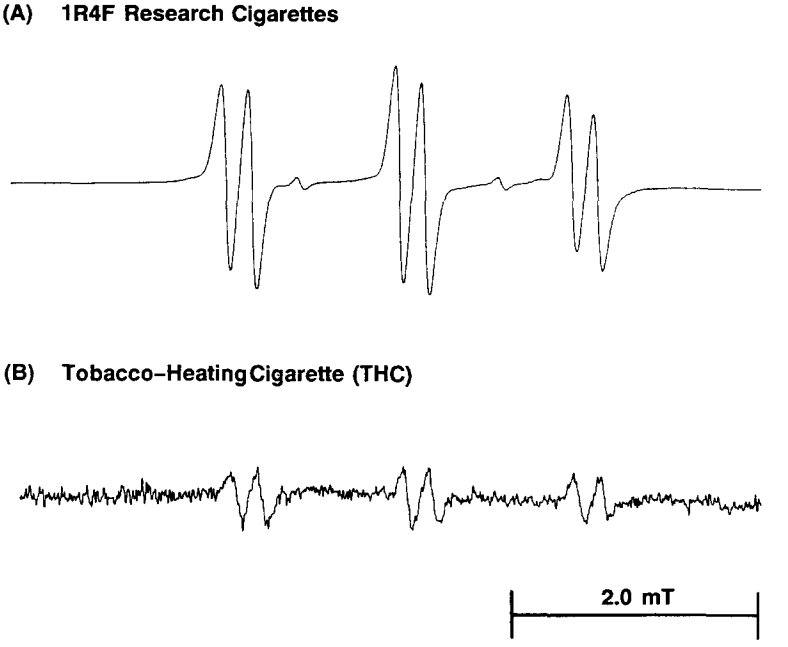

Fig. 2. Comparison of PBN spin adduct spectra observed for (A) 1R4F research cigarettes and (B) the tobacco-heating cigarette (THC). Smoke (total of 20 puffs) was bubbled through 2 mL of 0.1 M PBN in benzene. The solution was then transferred to a standard 4 mm ESR tube and degassed by nitrogen bubbling before ESR analysis. The principal species present in the smoke from either cigarette has hyperfine splitting constants of 0.2 mT (a_H) and 1.4 mT (a_N), values characteristic of an oxygen-centered radical. The ESR spectrometer gain used to obtain spectrum B is 83 times higher than used to obtain spectrum A. Instrument settings: Modulation amplitude = 0.05 mT; time constant = 100 ms (A) or 1000 ms (B); center field = 349.5 mT; sweep width = 6.0 mT; sweep time = 100 s (A) or 1000 s (B).

and passes the heated air through a hollow aluminum cylinder containing α-alumina beads and tobacco extract that is wrapped with a roll of tobacco. Heat from the burning carbon vaporizes volatile materials from the tobacco roll and the α-alumina beads to form the cigarette smoke.[8] Selected data on the composition of the smoke from these two cigarettes are shown in Table 1.

ESR analysis of the free radical content of the particulate matter and vapor phase smoke is presented in Figures 1 and 2 and summarized in Table 2. As previously reported for other tobacco-burning cigarettes,[1,2] the tar from 1R4F cigarettes produces ESR signals indicating long-lived radical species that can be detected by ESR. In contrast, this radical is not detected in the tar from the THC (see Fig. 1). We have suggested that one source of tar radicals in tobacco-burning cigarettes are semiquinones produced from hydroquinone/quinone couples held in the tar matrix.[1] Table 1 shows the hydroquinone concentration in the tar from the THC is 96% less than in tar from the 1R4F cigarette.

The gas-phase smoke from the 1R4F cigarette demonstrates the presence of radical species similar to those we have already reported.[3] The spin adduct spectra indicate that there are less than 1% as many radicals in the THC smoke as in 1R4F smoke (Fig. 2 and Table 2). The mechanism we have postulated for radical formation in gas-phase smoke requires the presence of nitrogen oxides and olefins such as isoprene, butadiene, and acrolein.[2,3] Table 1 shows that the amount of NOx in the THC smoke is reduced by more than 90% and the concentrations of isoprene, acrolein, and butadiene concentrations are reduced by 95, 96, and 99 + %, respectively.[4,14] Thus, the 99 + % reduction in the radical concentration in the gas-phase from the THC is consistent with the mechanism for gas-phase radical formation that we previously suggested.

A comparison of the EIC of α1PI exposed to gas-phase smoke is shown in Table 3. As can be seen, α1PI exposed to vapor-phase smoke from 1R4F cigarettes retains only 68% of its EIC, similar to values previously reported for other cigarettes.[8-10] The α1PI exposed to gas-phase smoke from the THC, on the other hand, retains 96% EIC, a value little, if any, different from that of air. The THC smoke contains lower concentrations of many species; nevertheless, the absence of α1PI inhibition by THC smoke is consistent with the virtual absence of radicals in the smoke.[4-11]

Table 2. ESR analysis of radical species in the particulate matter and gas phase for reference cigarette (1R4F) and new cigarette (THC)[a]

Cigarette	Tar Phase[c]	Gas Phase[d]
1R4F	83 ± 8	8255 ± 992
THC	nd[b]	26 ± 6

[a]Methods are described in the text. Date represent the mean ± standard deviation for at least three replications. Numbers given are relative peak heights.
[b]nd = not detected.
[c]By direct ESR.
[d]By ESR spin trapping.

Table 3. Comparison of elastase inhibitory capacity (EIC) for alpha-1-proteinase inhibitor (α1PI) exposed to smoke from the standard 1R4F cigarette and the new cigarette (THC).[a]

Cigarette	EIC (%)
1R4F	68 ± 6
THC	96 ± 1
Control (No smoke)	100

[a]Methods are described in the text. Data represent the mean ± the standard deviation of the mean for three independent experiments.

SUMMARY

Smoke from the cigarette that only heats tobacco contains no detectable tar radicals, and gas-phase radicals are greatly reduced compared with smoke from tobacco-burning cigarettes. These observations support our previously proposed mechanisms for radical production in the tar and gas-phase of cigarette smoke. Consistent with these observations is the virtual absence of in vitro α1PI inhibition by the smoke from the new cigarette.

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ABBREVIATIONS

α1PI—α-1-proteinase inhibitor
EIC—elastase inhibitory capacity
ESR—electron spin resonance
PAH—polycyclic aromatic hydrocarbons
PBN—α-phenyl-*N*-*tert*-butyl nitrone
THC—tobacco-heating cigarette
Q/QH_2—quinone-hydroquinone