Cigarette Smoke Radicals and the Role of Free Radicals in Chemical Carcinogenicity

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This article consists of two parts: a brief overview of the ways in which free radicals can be involved in chemical carcinogenesis, and a review of cigarette smoke chemistry. Carcinogenesis is generally agreed to involve at least three stages: initiation, promotion, and progression. It is suggested that radicals sometimes are involved in the initiation step, either in the oxidative activation of a procarcinogen (such as benzo(a)pyrene) to its carcinogenic form or in the binding of the carcinogenic species to DNA, or both. The fraction of initiation events that involve radicals, as opposed to two-electron steps, is not known, but radicals probably are involved in a substantial number, although probably not a majority, of cancer initiation reactions. Promotion always involves radicals, at least to some extent. Progression probably does not normally involve radicals. The second part of this article reviews the molecular mechanisms involved in cigarette-induced tumors, particularly by aqueous cigarette tar (ACT) extracts and by a model of these solutions, aged solutions of catechol. ACT solutions as well as aged solutions of catechol contain a quinone–hydroquinone–semiquinone system that can reduce oxygen to produce superoxide and hence hydrogen peroxide and the hydroxyl radical. Both the cigarette tar radical and the catechol-derivated radical can penetrate viable cells, bind to DNA, and cause nicks. — Environ Health Perspect 105(Suppl 4):875–882 (1997)

Key words: adduct, cigarette, DNA, electron transfer, initiation, oxidation, promotion, radical, smoke, superoxide

Introduction

This review is divided into two parts. In the first, I suggest an overview and organizational scheme for the various ways in which free radicals may be involved in chemical carcinogenesis. In the second, I review some of the recent research on cigarette chemistry and biology done both in our laboratory and in others.

Mechanisms By Which Radicals Can Be Involved in Carcinogenicity

The involvement of radicals in chemical carcinogenesis seemed evident for many years, based on a number of generalities. First, since the activation of many procarcinogens involves an oxidation step, and since many oxidations involve radicals, it seemed likely that radicals would be involved in carcinogenesis, at least some for some carcinogens (1,2). Second, a number of radical scavengers and/or antioxidants protect cells or animals from tumor formation, although individual antioxidants seem to protect against different carcinogens without a general pattern being clear (2–6). Third, a proinflammatory state, which can predispose tissue to tumor development, involves higher concentrations of endogenous radicals such as superoxide (7–10). Fourth, antioxidant defenses appear to be altered in procarcinogenic states (11–14). Fifth, it is clear that radical activity in the pretumor state is increased regardless of whether the activity is measured by increased amounts of lipid (15,16) or DNA oxidation products (17–19).

In recent years, the literature on the involvement of radicals in carcinogenesis has grown enormously. That can be seen quite strikingly in the comparison of the 1986 review by Kensler (7) and that by Frenkel (8) in 1992, which is about twice the length and includes more than three times the number of references.

Carcinogenesis and Radicals

Cancer is a multistep process, which generally is identified as three stages: initiation, promotion, and progression. The activation of many types of initiators can involve radicals. For example, the oxidation of polycyclic aromatic hydrocarbons (PAHs) to an electrophilic derivative that can attack and bind to DNA may involve radicals. Promotion almost certainly involves the production of higher levels of endogenous radicals such as superoxide. Once a tumor is established, progression may be controlled by genetic factors that probably only minimally involve the pathological reactions of radicals.

The various mechanisms for radical involvement are summarized in Table 1. In mechanism 1, radicals are involved in neither initiation nor promotion. Cases of this type certainly occur; the activation of a PAH such as benzo(a)pyrene (B(a)P) via a nonradical, P450-dependent process is an example, as is the chemistry involved in the binding of aflatoxin to DNA, although even this process can involve radicals (20). It is not known what fraction of all cancers is due to these and other nonradical tumorigenesis processes, but it generally is believed that a considerable fraction of cancers probably fits this category.

Table 1. Mechanisms in which radicals are involved in either the initiation or promotion stages of carcinogenesis.

| Mechanism no. | Initiation2 | Promotion2 | Example |
|---------------|------------|------------|---------|
| 1             | No         | No         | P450/B(a)P |
| 2             | Yes        | No         | PGS/B(a)P |
| 3             | No         | Yes        | ?       |
| 4             | Yes        | Yes        | iso-P450/B(a)P phenols |
| 5             | Radicals can be involved in mechanisms that do not involve binding of the carcinogen, e.g., superoxide-dependent DNA nicks. |

PGS, prostaglandin synthase. 2Adapted from Pryor (2). 3Radicals are involved in carcinogen activation. 4Radicals involved in DNA binding of carcinogen.
Mechanism 2, in which initiation involves radicals but promotion does not, may be quite rare, since radical involvement in promotion is unquestionably important and common. Examples of radical-mediated activation of a procarcinogen include the prostaglandin synthase-dependent cooxidation of a PAH (21–26), one-electron oxidation of PAHs to their cation-radicals that bind to DNA (27–33), and reactions involving radical attack on carcinogens (34). Other examples can involve the reaction of products produced by radical-mediated processes (such as malonaldehyde or 4-hydroxynonenal from lipid peroxidation), either with procarcinogens to activate them or directly with DNA (35–39).

The general perception is that carcinogens bind to DNA by even-electron (nonradical) pathway, and, to a certain extent, publications that argue the importance of a one-electron pathway for DNA binding are swimming upstream with the current against them. However, it is hard to determine whether this is because nature predominantly uses even-electron pathways for attacks on DNA, or because it is more or less a historical artifact, since the B(4)P-diol-epoxide pathway was elucidated very early, in studies by NIH authors (40–43), so that the known even-electron pathway dominated the thinking of many workers. I have always believed that the latter is true, at least in part.

Mechanism 3, in which radicals are involved in promotion but not initiation, may be common; but, as is true for mechanism 1, it is impossible to know what fraction of tumors results from this pathway. However, it is commonly accepted that the activation of procarcinogens to their carcinogenic state does not always involve radicals, whereas promotion most often involves a long-term, proinflammatory state with high radical fluxes in the affected tissues. Thus, it is likely that all initiation events—no matter whether radicals were involved or not—are followed by a radical-involving promotional state if a tumor is to result.

In mechanism 4, radicals are involved in both initiation and promotion, and probably are almost co-terminus with the mechanism in which radicals are involved in initiation alone.

In addition to these mechanisms, there is mechanism 5, in which radical-mediated damage is done to DNA without the formation of adducts (44–46). This mechanism may apply, for example, in high-energy radiation-initiated tumors.

**Other Mechanisms**

In addition to these somewhat obvious mechanisms by which radicals could be involved in carcinogenesis, there is a classification of "other mechanisms" that are more roundabout. Because this is a vague, catch-all category, many mechanistic types can fit it, and the examples given here do not include all the possibilities.

An example of the indirect effects radical reactions can have on cancer is the incompletely understood effects of oxidative stress on oncogenes. For example, an epidermal cell transfactant that overexpresses Cu/Zn superoxide dismutase (SOD) is more sensitive to oxidative stress, as monitored by increased c-fos message (11). Tumor cells may often be low in SOD activity (12).

Another example of these complex mechanisms involves the effects of β-carotene on gap junction regulation. β-Carotene strongly upregulates gap-junction communication (47), thus producing an antitumorigenic effect. Because β-carotene is often called an antioxidant, this effect might be ascribed to radical scavenging of tumor-promoting radicals (47).

**Cancer and the Diet**

The involvement of radical reactions in chemical carcinogenesis has recently been in the news, as it has become increasingly clear that the dietary intake of antioxidant nutrients such as vitamin E, vitamin C, and β-carotene can profoundly affect tumor susceptibilities of populations (4,48–52). These concerns also have emphasized the need for a biomarker for the oxidative stress that can lead to cancer. Were such a marker identified, it could be used as an end point in dietary trials, making them shorter and less expensive to perform (53–57).

The ways in which antioxidants might interfere with tumorigenesis are outlined in Figure 1. As can be seen, there are many possibilities.

**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 ≥ 0.1 μm, is used.) Both the tar and gas-phase smoke are very rich sources of radicals.

A smoker inhales gas-phase smoke (so called mainstream smoke) as well as particulates that penetrate whatever filter is being used. Both of these phases are highly oxidizing, putting an oxidative stress on the entire organism as well as on the lungs (58–60). For example, smokers have lower concentrations of vitamin C in their blood plasma and vitamin E in their lung lavage than do nonsmokers (13,14,61,62). Some biomarkers of smoking are reduced by antioxidant vitamins (63). Some antioxidant enzymes (e.g., SOD) increase whereas others decrease in activity in the red blood cells of guinea pigs exposed to cigarette smoke (64). Cigarette smoke initiates lipid peroxidation in rat tracheal explants (65) as well as in other systems (66–72). Breath ethane, a measure of the oxidation of n–3 polyunsaturated fatty acids, is elevated in smokers (62,73).

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**Figure 1.** A schematic presentation of ways in which radicals may be involved, and antioxidants may block, tumorigenesis. Abbreviations: E, vitamin E; C, vitamin C; GSH, glutathione.
Gas-phase Smoke Radicals

Gas-phase smoke contains more than $10^{14}$ low-molecular-weight carbon- and oxygen-centered radicals per puff (74). In addition, smoke contains up to 500 ppm nitric oxide (NO), which slowly undergoes oxidation to nitrogen dioxide (NO2) (75), and both these gases are, of course, radicals. The radicals in gas-phase smoke are too short lived to be detectable by direct electron spin resonance (ESR), but they can be studied by the indirect spin trap method (74,76,77).

The small organic radicals in gas-phase smoke are not produced in the flame: flame radicals are too short lived to pass through the cigarette. Rather, these organic radicals are produced in a steady state by the addition of nitrogen dioxide to isoprene and other similar compounds in the smoke to make carbon-centered radicals that react with oxygen to make oxiradicals (74,76,77). Because gas-phase smoke contains carbon- and oxygen-centered radicals and such high concentrations of nitric oxide (75), alkyl peroxy nitrite and peroxy nitrate esters also can be produced (78-82). Figure 2 shows a schematic outline of the isolation of gas-phase smoke and tar and the radicals that each phase produces.

Tar Radicals

In sharp contrast with the gas-phase radicals, the radical in tar is a long-lived semiquinone that can be studied directly by ESR on the filter or in organic solvents or aqueous extracts (83-85). Aqueous extracts of cigarette tar (ACT) contain a low-molecular-weight quinone–hydroquinone–semi-quinone system (Q-QH2–QH2). The cigarette tar semiquinone radical, as is typical of such radicals, can reduce oxygen to produce superoxide, and hence hydrogen peroxide and the hydroxyl radical. Unlike the tar radical itself, the superoxide and hydroxyl radicals are too reactive and short lived to be observable by direct ESR; but they can be detected by the use of spin trap 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) (78,86,87).

Figure 3 shows the ESR spectra obtained when tar on a Cambridge filter is placed in the cavity of an ESR spectrometer after the smoke from a single cigarette has been pulled through the filter, and after an aged solution of catechol is filtered. Both spectra can be identified as being due to a semiquinone (78,92).

The tar radical, a relatively stable semiquinone, is not reactive. However, ACT solutions produce superoxide, hydrogen peroxide, and the hydroxyl radical, and thus become potent oxidants. These ACT solutions can initiate lipid peroxidation, oxidize proteins such as the antiprotease, α-1-protease inhibitor (88–90), and nick DNA (82,87). The quinone–hydroquinone–semi-quinone system can penetrate viable mammalian cells, bind to, and nick cellular DNA (2,86,87,91,92). The nicks produced by the tar radical require multistep repair, suggesting a process that could be error prone (93). These ACT solutions also interfere with mitochondrial electron transport (94).

The ESR and DNA binding and nicking behavior of the tar radical is very closely modeled by the semiquinone system present in aged solutions of catechol (85,95,96). Figure 4 shows the similarity in the ESR spectra obtained for solutions of ACT and aged catechol (92). Thus, the tar radical is a low-molecular-weight semiquinone radical, which exists in equilibrium with its quinone and hydroquinone derivatives. This radical system can bind to DNA, produce superoxide, hydrogen peroxide, and the hydroxyl radical in the vicinity of the DNA, and nick DNA (2,78).

Figure 5 shows that the tar semiquinone radical signal becomes associated with DNA when ACT solutions are incubated with calf thymus DNA (82). Since the radical-producing system in ACT solutions is a quinone–hydroquinone–semiquinone system of low molecular weight, it can pass through cell membranes, diffuse to the
nucleus, and bind to DNA. Figure 6 shows the ESR spectra obtained when the DNA from rat alveolar macrophages that have been incubated either with ACT or aged catechol solutions are trapped and washed on polycarbonate filters (82). These ACT and aged catechol solutions bind to and then nick DNA. A plot of the amount of tar or of catechol used versus DNA nicks shows saturation behavior, suggesting that there might be certain sites in the DNA that are particularly prone to bind the tar radical; once these sites are saturated, binding decreases (78,82,92). Analyses of these binding data using Lineweaver–Burke or Edie–Hofstee plots, or by computer curve fitting, give very similar binding constants and indicate that 0.24 cigarettes produce an ACT solution with the DNA nicking potency equivalent to a solution containing 254-μg/ml catechol (82). This amount of catechol actually would be that contained in 0.3 cigarette (82).

Catalase, but not SOD, provides significant protection against ACT- and aged catechol-induced DNA nicks (82).

Deferoxamine provides little protection, suggesting that the iron required to convert hydrogen peroxide to the hydroxyl radical is tightly associated either with the ACT radical or with DNA or both (82). Glutathione also protects significantly, as shown in Figure 7.

Figure 8 depicts the binding of the ACT (or catechol) radical to DNA, the reduction of oxygen by the semiquinone radical to produce superoxide, the dismutation of superoxide to give hydrogen peroxide, and the reduction of hydrogen peroxide by iron ions (probably associated with DNA, the radical, or both) to the hydroxyl radical.

Randerath and associates (97–106) and others (107,108) have used the phosphor­ ous postlabeling method to demonstrate that cigarette smoke and tar lead to the formation of DNA adducts both in vitro and in vivo. Radicals need not be involved in the binding of cigarette tar components, presumably PAH derivatives, to DNA. However, it is striking that glutathione appears to protect DNA very similarly against adduct formation, as observed by Randerath and associates (97) and the DNA nicks that we observe (Figure 7) (82).

Recently, we fractionated ACT solutions into 120 fractions and compared the strength of the ESR signal of the tar radical in each fraction with the ability of these

Figure 5. The electron spin resonance spectra of material remaining on polycarbonate filters after filtering the material from the following incubations: (A) ACT plus calf thymus DNA; (B) ACT solutions alone; (C) DNA alone (82).

Figure 6. The electron spin resonance (ESR) spectra of the material obtained when the DNA from rat alveolar macrophages (RAM) that have been incubated either with (A) ACT or (B) aged catechol solutions and then isolated on polycarbonate filters (82). No ESR signal is obtained if the RAM are not exposed to ACT or catechol (C).

Figure 7. Protection against ACT-induced DNA nicks in rat alveolar macrophages provided by glutathione (82).

Figure 8. Graphic representation showing 1) the binding of the ACT (or catechol) radical to DNA, 2) the reduction of oxygen by the semiquinone radical to produce superoxide, 3) the dismutation of superoxide to give hydrogen peroxide, and 4) the reduction of hydrogen peroxide by iron ions (probably associated with DNA, the radical, or both) to the hydroxyl radical or a ferryl species that nicks DNA (78,82,92).
fractions to take up and reduce oxygen to hydrogen peroxide and to nick DNA. If the cigarette tar radical is the species that reduces oxygen, ultimately producing hydrogen peroxide, and if this cigarette tar radical system is responsible for nicking DNA, then all three of these characteristics of the ACT fractions should be proportional.

Indeed, Figure 9 shows that this is the case. This is the strongest evidence we have discovered so far that it is the tar radical that is responsible for binding to and nicking DNA (96).

Figure 10 suggests possible ways for the cigarette tar radical, O_2^•−, to produce DNA damage. Mechanisms A and B show adduct formation and mechanism C shows DNA nicking. In Figure 10A the tar radical is proposed to interact with a procarcinogen (such as a PAH) to produce an activated PAH, PAH•*, which can bind to DNA; this activated species, PAH•*, might for example be a cation-radical or a phenoxyl radical of the PAH. In (B), the tar radical is proposed to interact with DNA to activate a base (DNA•*) that can then bind a carcinogen.

REFERENCES

1. Ames BN, Hollstein MC, Cathcart R. Lipid peroxidation and oxidative damage to DNA. In: Lipid Peroxide in Biology and Medicine (Yagi K, ed). New York: Academic Press, 1981.
2. Pryor WA. Cigarette smoke and the involvement of free radical reactions in chemical carcinogenesis. Br J Cancer 55(Suppl 8):19–23 (1987).
3. Wattenberg LW. Inhibition of carcinogenic and toxic effects of polycyclic hydrocarbons by phenolic antioxidants and ethoxyquin. J Natl Cancer Inst 48:1425–1430 (1972).
4. Wattenberg LW. Inhibition of carcinogenesis by minor dietary constituents. Cancer Res 52:2085S–2091S (1992).
5. Wattenberg LW. Inhibition of carcinogenesis by minor nutrient constituents of the diet. Proc Nutr Soc 49:173–183 (1990).
6. Pryor WA. Cancer and free radicals. In: Antimutagenesis and Anticarcinogenesis Mechanisms (Shankel D, Hartman P, Kada T, Hollaender A, eds). New York: Plenum Press, 1986:45–59.
7. Kensler TW, Taffe BG. Free radicals in tumor promotion. Adv Free Radic Biol Med 2:347–387 (1986).
8. Frenkel K. Carcinogen-mediated oxidant formation and oxidative DNA damage. Pharmacol Ther 53:127–166 (1992).
9. Witz G. Active oxygen species as factors in multistage carcinogenesis. Proc Soc Exp Biol Med 198:675–682 (1991).
10. Kamp DW, Graceffa P, Pryor WA, Weitzman SA. The role of free radicals in asbestos-induced diseases. Free Radic Biol Med 12:293–315 (1992).

11. Cerutti P, Ghosh R, Oya Y, Amstad P. The role of the cellular antioxidant defense in oxidant carcinogenesis. Environ Health Perspect 102:123–130 (1994).

12. Sun Y. Free radicals, antioxidant enzymes, and carcinogenesis. Free Radic Biol Med 6:583–590 (1988).

13. Pacht EB, Kaseki H, Mohammed JR, Cornwell DG, Davis WB. Deficiency of vitamin E in the alveolar fluid of cigarette smokers: influence on alveolar macrophage cytotoxicity. J Clin Invest 77:789–796 (1986).

14. Würzel H, Yeh CC, Gáirola C, Chow CK. Oxidative damage and antioxidant status in the lungs and bronchoalveolar lavage fluid of rats exposed chronically to cigarette smoke. J Biochem Toxicol 10:11–17 (1995).

15. Diplock AT, Rice-Evans CA, Burdon RH. Is there a significant role for lipid peroxidation in the causation of malignancy and for antioxidants in cancer prevention? Cancer Res 7(Suppl 5):1567–1568 (1994).

16. Boyd NF, McGuire V. The possible role of lipid peroxidation in breast cancer risk. Free Radic Biol Med 10:185–190 (1991).

17. deRojas-Walker T, Tamir S, Ji H, Wishnik JS, Tannenbaum SR. Nitric oxide induces oxidative damage in addition to deamination in macrophage DNA. Chem Res Toxicol 8:473–477 (1995).

18. Green AP, Murphy JA. Reactions of ovaly radicals with DNA. Free Radic Biol Med 18:1033–1077 (1995).

19. Wood ML, Dizdaroglu M, Gajewski E, Essigmann JM. Mechanistic studies of ionizing radiation and oxidative mutagenesis: genetic effects of a single 8-hydroxyguanine (7-hydro-8-oxoguanine) residue inserted at a unique site in a viral genome. Biochemistry 29:7024–7032 (1990).

20. Battista JR, Marnett LJ. Prostaglandin H synthase-dependent epoxidation of aflatoxin B1. Carcinogenesis 6:1227–1229 (1985).

21. Pnuess-Schwartz D, Nimesheim A, Marnett LJ. Peroxyl radical and cytochrome P-450-dependent metabolic activation of (+)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene in mouse skin in vitro and in vivo. Cancer Res 49:1732–1737 (1989).

22. Labue R, Marnett LJ. Reaction of hematin with allylic fatty acid hydroperoxides: identification of products and implications for pathways of hydroperoxide dependent epoxidation of 7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene. Biochemistry 27:7060–7070 (1988).

23. Dix TA, Marnett LJ. Metabolism of polycyclic aromatic hydrocarbon derivatives to ultimate carcinogens during lipid peroxidation. Science 221:77–79 (1983).

24. Eling T, Curtis J, Battista J, Marnett LJ. Oxidation of (+)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene by mouse keratinocytes: evidence for peroxyl radical and monooxygenase dependent metabolism. Carcinogenesis 7:1957–1963 (1986).

25. Ji C, Marnett LJ. Oxygen radical dependent epoxidation of (7S,8S)-dihydroxy-7,8-dihydrobenzo[a]pyrene in mouse skin in vivo. Stimulation by phorbol esters and inhibition by anti-inflammatory steroids. J Biol Chem 267:17842–17848 (1992).

26. Marnett LJ. Prostaglandin synthase-mediated metabolism of carcinogens and a potential role for peroxyl radicals as reactive intermediates. Environ Health Perspect 88:5–12 (1990).

27. Cavaliere EL, Rogan EG. The approach to understanding aromatic hydrocarbon carcinogenesis. The central role of radical cations in metabolic activation. Pharmacol Ther 55:183–199 (1992).

28. RamaKrishna NV, Gao F, Padmavathi NS, Cavaliere EL, Rogan EG, Cerny RL, Gross ML. Model adducts of benzo[a]pyrene to DNA and nucleosides formed from its radical cation and diol epoxide. Chem Res Toxicol 5:293–302 (1992).

29. Cremonesi P, Stack DE, Rogan EG, Cavaliere EL. Radical cations of benzo[a]pyrene and 6-substituted derivatives: synthesis and reaction with nucleophiles. J Org Chem 59:7683–7687 (1994).

30. RamaKrishna NVS, Li K-M, Rogan EG, Cavaliere EL, George M, Cerny RL, Gross ML. Adducts of 6-methylbenzo[a]pyrene formed by electrochemical oxidation in the presence of deoxyribonucleotides. Chem Res Toxicol 6:837–845 (1993).

31. RamaKrishna NVS, Padmavathi NS, Cavaliere EL, Rogan EG, Cerny RL, Gross ML. Synthesis and structure determination of the adducts formed by electrochemical oxidation of the potent carcinogenic dibenzo[a]pyrene in the presence of nucleosides. Chem Res Toxicol 6:554–560 (1993).

32. Todorovic R, Devanesan PD, Rogan EG, Cavaliere EL. 32P-postlabeling analysis of the DNA adducts of 6-fluorobenzo[a]pyrene and 6-methylbenzo[a]pyrene formed in vitro. Chem Res Toxicol 6:530–534 (1993).

33. Bodell WJ, Devanesan PD, Rogan EG, Cavaliere EL. 32P-postlabeling analysis of benzo[a]pyrene DNA adducts formed in vitro and in vivo. Chem Res Toxicol 2:312–315 (1989).

34. Augusto Q, Cavaliere EL, Rogan EG, RamaKrishna NVS, Kolar C. Formation of 8-methylguanine as a result of DNA alkylation by methyl radicals generated during horseradish peroxidase-catalyzed oxidation of methyldihydroquinazoline. J Biol Chem 365:22093–22096 (1990).

35. Chaudhary AK, Nokubo M, Reddy GR, Yeola SN, Morrow JD, Blair IA, Marnett LJ. Detection of endogenous malondialdehyde deoxyguanosine adducts in human liver. Science 265:1580–1582 (1994).

36. Goda Y, Marnett LJ. High-performance liquid chromatography with electrochemical detection for determination of the major malondialdehyde-guanine adduct. Chem Res Toxicol 8:520–524 (1995).

37. Estabrook H, Zollner H, Saur HJ. Hydroxylalkanals: cytotoxic products of lipid peroxidation. In: ISAT Atlas of Science; Biochemistry, Vol. 1. Philadelphia:ISI, 1988:311–317.

38. Ferro M, Mariniari UM, Poli G, Dianzani MU, Fauler G, Zollner H, Estabrook H. Metabolism of 4-hydroxynonenal by the rat hepatoma cell line MH: C9. Cell Biochem Funct 6:245–250 (1988).

39. Yagi K. Increased serum lipid peroxides initiate atherosclerosis. Bioessays 1:58–60 (1984).

40. Jerina DM, Yagi H, Lehr RE, Thakker DR, Schafer-Ridder M, Karle JI, Levin W, Wood AW, Chang RL, Conney AH. The bay-region theory of carcinogenesis by polycyclic aromatic hydrocarbons. In: Polycyclic Hydrocarbons and Cancer: Environment, Chemistry, and Metabolism (Gelboin HV, Ts’ POP, eds). New York:Academic Press, 1978:173–188.

41. Levin W, Wood AW, Wisloeki GC, Chang RL, Kapitulnik J, Mah HD, Yagi H, Jerina DM, Conney AH. Mutagenicity and carcinogenicity of benzo[a]pyrene and benzo[a]pyrene derivatives. In: Polycyclic Hydrocarbons and Cancer: Environment, Chemistry, and Metabolism (Gelboin HV, Ts’ POP, eds). New York:Academic Press, 1978:189–202.

42. Kapitulnik J, Wislocki GC, Levin W, Yagi H, Jerina DM, Conney AH. Tumorigenicity studies with diol-epoxides of benzo[a]pyrene which indicate that (a) trans-β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene is an ultimate carcinogen in newborn mice. Cancer Res 38:354–358 (1978).

43. Koreeda M, Moore PD, Wislocki GC, Levin W, Conney AH, Yagi H, Jerina DM. Binding of benzo[a]pyrene 7,8-diol-9,10-epoxides to DNA, RNA, and protein of mouse skin occurs with high stereoselectivity. Science 199:778–781 (1978).

44. Copeland ES, Borg DC, Cerutti P, Kaufman DG, Birnboim HC, Pryor WA. Free radicals in promotion—a Chemical Pathology Study Section workshop. Cancer Res 43:5631–5637 (1983).

45. Birnboim HC. Superoxide anion may trigger DNA strand breaks in human granulocytes by acting as a membrane target. In: Membrane in Cancer Cells, Vol 551 (Galleotti T, Cittadini A, Meri G, Scarpa A, eds). New York: The New York Academy of Sciences. New York:New York Academy of Sciences, 1988:83–84.

46. Birnboim HC. DNA strand breaks in human leukocytes induced by superoxide anion, hydrogen peroxide and tumor promoters are repaired slowly compared to breaks induced by ionizing radiation. Carcinogenesis 7:1511–1517 (1986).
47. Zhang LX, Cooney RV, Bertram JS. Carotenoids enhance gap junctional communication and inhibit lipid peroxidation in C3H10T1/2 cells: relationship to their cancer chemopreventive action. Carcinogenesis 12:2109–2114 (1991).

48. Li J-Y, Taylor PR, Li B, Dawsey S, Wang G-Q, Ershow AG, Guo W, Liu S-F, Yang CS, Shen Q et al. Nutrition intervention trials in Lixinian, China: multiple vitamin/mineral supplementation, cancer incidence, and disease-specific mortality among adults with phenogaphic dysplasia. J Natl Cancer Inst 85:1492–1498 (1993).

49. Blot WJ, Li J-Y, Taylor PR, Guo W, Dawsey S, Wang G-Q, Yang CS, Zheng S-F, Gail M, Li G-Y. Nutrition intervention trials in Lixinian, China: supplementation with specific vitamin/mineral combinations, cancer incidence, and disease-specific mortality in the general population. J Natl Cancer Inst 85:1483–1492 (1993).

50. Ziegler RG, Subar AF, Craft NE, Ursin G, Patterson BH, Graubard BI. Does β-carotene explain why reduced cancer risk is associated with vegetable and fruit intake? Cancer Res 52(Suppl):2060S–2066S (1992).

51. Valstar E. Nutrition and cancer: a review of the preventive and therapeutic abilities of single nutrients. J Nutr Med 4:179–198 (1994).

52. Diplock AT. Dietary supplementation with antioxidants. Is there a case for exceeding the recommended dietary allowance? Free Radic Biol Med 3:199–201 (1987).

53. Benner SE, Hong WK. Clinical chemoprevention: developing a cancer prevention strategy. J Natl Cancer Inst 85:1446–1447 (1993).

54. Pryor WA. Measurement of oxidative stress status in humans. Cancer Epidemiol Biomarkers Prev 2:289–292 (1993).

55. Pryor WA, Godder SS. Noninvasive measures of oxidative stress status in humans. Free Radic Biol Med 10:177–184 (1991).

56. Malins DC. The etiology of breast cancer: characteristic alterations in hydroxyl radical induced DNA base lesions during oncogenesis with potential for evaluating incidence risk. Cancer 71:3036–3043 (1993).

57. Loft S, Vistisen K, Ewertz M, Tjønneland A, Overvad K, Poulsen HE. Oxidative DNA damage estimated by 8-hydroxy-2-deoxyguanosine excretion in humans: influence of smoking, gender and body mass index. Carcinogenesis 13:2241–2247 (1992).

58. Church DF, Pryor WA. The oxidative stress placed on the lung by cigarette smoke. In: Lung Injury (Crystal RG, West JB, eds), New York: Raven Press, 1992;215–219.

59. Peon A, Sarkar GA. Inhalation of sidestream cigarette smoke accelerates development of arteriosclerotic plaques. Circulation 88:1820–1825 (1993).

60. Reznick AZ, Cross CE, Hu M, Suzuki YJ, Khwaja S, Safadi A, Motchnik PA, Packer L, Halliwell B. Modification of plasma proteins by cigarette smoke as measured by protein carboxyl formation. Biochem J 286:607–611 (1992).

61. Airriess GR, Changchit C, Chen L-C, Chow CK. Increased vitamin E levels in the lungs of guinea pigs exposed to mainstream or sidestream smoke. Nutr Res 8:653–661 (1988).

62. Allard JP, Royall D, Kurian R, Muggli R, Jeejeebhoy KN. Effects of β-carotene supplementation on lipid peroxidation in humans. Am J Clin Nutr 59:884–890 (1994).

63. Benner SE, Wargovich ML, Lippman SM, Fisher R, Velasco M, Winn RJ, Hong WK. Reduction in oral mucosa microneu- clei frequency following α-tocopherol treatment of oral leukoplaikia. Cancer Epidemiol Biomarkers Prev 3:73–76 (1994).

64. Mukherjee S, Woods L, Weston Z, Williams AB, Das SK. The effect of mainstream and sidestream cigarette smoke exposure on oxygen defense mechanisms of guinea pig erythrocytes. J Biochem Toxicol 8:119–125 (1993).

65. Chung A, Cherukupalli K. Cigarette smoke causes rapid lipid peroxidation of rat tracheal epithelium. Int J Exp Path 74:127–132 (1993).

66. Brown KM, Morrice PC, Duthie GG. Vitamin E supplementation suppresses indexes of lipid peroxidation and platelet counts in blood of smokers and nonsmokers but plasma lipoprotein concentrations remain unchanged. Am J Clin Nutr 60:383–387 (1994).

67. Gupta MP, Khanduja KL, Sharma RR. Effect of cigarette smoke inhalation on antioxidant enzymes and lipid peroxidation in the rat. Toxicol Lett 41:107–114 (1988).

68. Lapenna D, Mezzetti A, De Gioia S, Pierdomenico SD, Daniele F, Cucurullo F. Plasma copper and lipid peroxidation in cigarette smokers. Nucl Med Biol 19:849–852 (1995).

69. Petruzelli S, Hietanen E, Bartsch H, Caus M, Massi A, Angelletti CA, Saracci R, Giuntini C. Pulmonary lipid peroxidation in cigarette smokers and lung cancer patients. Chest 98:930–933 (1990).

70. Frei B, Forte TM, Ames BN, Cross CE. Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma. Protective effects of ascorbic acid. Biochem J 277:133–138 (1991).

71. Tamura M. Autoxidation of methyl linoleate initiated by gas-phase cigarette smoke. J Japan Soc Air Pollut 26:171–175 (1991).

72. Willey JC, Graftstrom RG, Moses CE, Jr, Osnander C, Sundqvist K, Harris CC. Biochemical and morphological effects of cigarette smoke condensate and its fractions on normal human bronchial epithelial cells in vitro. Cancer Res 47:2045–2049 (1987).

73. Wade CR, Van Rij AM. In vitro lipid peroxidation in man as measured by the exhalation of volatile hydrocarbons: the effect of cigarette smoke inhalation. Proc Univ Otago Med Sch 64:75–76 (1986).

74. Pryor WA, Prier DG, Church DF. Electron-spin resonance study of mainstream and sidestream cigarette smoke: nature of the free radicals in gas-phase smoke and in cigarette tar. Environ Health Perspect 47:345–355 (1983).

75. Cueto R, Pryor WA. Cigarette smoke chemistry: conversion of nitric oxide to nitrogen dioxide and reactions of nitrogen oxides with other smoke components as studied by Fourier transform infrared spectroscopy. ViB Spectros 7:97–111 (1994).

76. Pryor WA, Tamura M, Church DF. ESR spin trapping study of the radicals produced in NOx/olefin reactions: a mechanism for the production of the apparently long-lived radicals in gas-phase cigarette smoke. J Am Chem Soc 106:5073–5079 (1984).

77. Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect 64:111–126 (1985).

78. Pryor WA, Stone K. Oxidants in cigarette smoke: radicals, hydrogen peroxide, peroxynitrite, and peroxynitrite. In: Tobacco Smoking and Its Effects. Inhalation of Tobacco Associated Health Risks, Vol 686 (Diana J, Pryor WA, eds), Annals of the New York Academy of Sciences. New York: New York Academy of Sciences, 1993;12–28.

79. Pryor WA, Dooley MM, Church DF. Mechanisms of cigarette smoke toxicity: the inactivation of human α1-proteinase inhibitor by nitric oxide/isoprene mixtures in air. Chem Biol Interact 54:171–183 (1985).

80. Pryor WA, Dooley MM, Church DF. The inactivation of α1-proteinase inhibitor by gas-phase cigarette smoke: protection by antioxidants and reducing species. Chem Biol Interact 57:271–283 (1986).

81. Pryor WA. The free radical chemistry of cigarette smoke and the inactivation of α1-proteinase inhibitor. In: Pulmonary Emphysema and Proteolysis, 1986 (Taylor JC, Mittman C, eds), Orlando: Academic Press, 1987;369–392.

82. Stone K, Bermúdez E, Pryor WA. Aqueous extracts of cigarette tar containing the tar free radical cause DNA nicks in mamalian cells. Environ Health Perspect 102:173–178 (1994).

83. Pryor WA, Hales BJ, Premovic PI, Church DF. The radicals in cigarette tar: their nature and suggested physiological implications. Science 220:425–427 (1983).

84. Church DF, Pryor WA. An ESR study of the radicals in cigarette tar. In: IBM Instruments Application Note G565-9583. Danbury, CT:International Business Machines, 1985:1–4.

85. Zang L-Y, Stone K, Pryor WA. Detection of free radicals in...
aqueous extracts of cigarette tar by electron spin resonance. Free Radic Biol Med 19:161–167 (1995).
86. Cosgrove JP, Borish ET, Church DF, Pryor WA. The metal-mediated formation of hydroxyl radical by aqueous extracts of cigarette tar. Biochem Biophys Res Commun 132:390–396 (1985).
87. Borish ET, Cosgrove JP, Church DF, Deutsch WA, Pryor WA. Cigarette tar causes single-strand breaks in DNA. Biochem Biophys Res Commun 133:780–786 (1985).
88. Evans MD, Church DF, Pryor WA. Aqueous cigarette tar extracts damage human alpha-1-proteinase inhibitor. Chem Biol Interact 79:151–164 (1991).
89. Evans MD, Pryor WA. Damage to human α-1-proteinase inhibitor by aqueous cigarette tar extracts and the formation of methionine sulfoxide. Chem Res Toxicol 5:654–660 (1992).
90. Evans MD, Pryor WA. An Invited Review: Cigarette smoking, emphysema and damage to alpha-1-proteinase inhibitor. Am J Physiol (Lung Cell Mol Physiol 10) 266:L593–L611 (1994).
91. Halliwell B, Borish ET, Pryor WA, Ames BN, Saul RL, McCord JM, Harman D. Oxygen radicals and human disease. Ann Intern Med 107:526–545 (1987).
92. Stone K, Bermúdez E, Zang L-Y, Carter KM, Queenan KE, Pryor WA. The ESR properties, DNA nicking and DNA association of aged solutions of catechol versus aqueous extracts of tar from cigarette smoke. Arch Biochem Biophys 319:196–203 (1995).
93. Borish ET, Pryor WA, Venugopal S, Deutsch WA. DNA synthesis is blocked by cigarette tar-induced DNA single-strand breaks. Carcinogenesis 8:1517–1520 (1987).
94. Pryor WA, Arbour NC, Upham B, Church DF. The inhibitory effect of extracts of cigarette tar on electron transport of mitochondria and submitochondrial particles. Free Radic Biol Med 12:365–372 (1992).
95. Stone K, Pryor WA. The effects of cigarette smoke free radicals. In: Lung Cancer: Principles and Practice (Pass HI, Mitchell JB, Johnson PH, Turriss AT, eds). Philadelphia:J.B. Lippincott, 1996;323–328.
96. Zang L-Y, Stone K, Bermúdez E, Pryor WA. Fractionation of aqueous cigarette tar extracts: fractions that contain the tar radical cause DNA damage. Free Radic Biol Med (in press).
97. Randerath E, Danna TF, Randerath K. DNA damage induced by cigarette smoke condensate in vitro as assayed by 32P-postlabeling. Comparison with cigarette smoke-associated DNA adduct profiles in vivo. Mutat Res 268:139–153 (1992).
98. Randerath K, Reddy R, Danna TF, Watson WP, Crane AE, Randerath E. Formation of ribonucleotides in DNA modified by oxidative damage in vitro and in vivo. Characterization by 32P-postlabeling. Mutat Res 275:355–366 (1992).
99. Randerath K, Yang PF, Danna TF, Reddy R, Watson WP, Randerath E. Bulky adducts detected by 32P-postlabeling in DNA modified by oxidative damage in vitro. Comparison with rat lung l-compounds. Mutat Res 250:135–144 (1991).
100. Randerath E, Miller RH, Mittal D, Avitsa TA, Dunford HA, Randerath K. Covalent DNA damage in tissues of cigarette smokers as determined by 32P-postlabeling assay. J Natl Cancer Inst 81:341–347 (1989).
101. Randerath E, Mittal D, Randerath K. Tissue distribution of covalent DNA damage in mice treated dermally with cigarette “tar”: preference for lung and heart DNA. Carcinogenesis 9:75–80 (1988).
102. Randerath E, Fowler-Danna T, Mittal D, Randerath K. Direct chemical reaction of unfraccionated cigarette smoke condensate (CSC) with DNA in vitro, adduct detection by 32P-postlabeling. Proc Annu Meet Am Assoc Cancer Res 29:120 (1988).
103. Everson RB, Randerath E, Santella RM, Cefalo RC, Avitsa TA, Randerath K. Detection of smoking-related covalent DNA adducts in human placenta. Science 231:54–57 (1986).
104. Randerath E, Avitsa TA, Reddy MV, Miller RH, Everson RB, Randerath K. Comparative 32P-analysis of cigarette smoke induced DNA damage in human tissues and mouse skin. Cancer Res 46:5869–5877 (1986).
105. Randerath K, Reddy MV, Avitsa TA, Miller RH, Everson RB, Randerath E. 32P-postlabeling test for smoking-related DNA adducts in animal and human tissues. In: Banbury Report 23: Mechanisms in Tobacco Carcinogenesis (Hoffmann D, Harris CC, eds). Cold Spring Harbor, NY:Cold Spring Harbor Laboratory Press, 1986;85–98.
106. Randerath K, Reddy MV, Gupta RC. 32P-labeling test for DNA damage. Proc Natl Acad Sci USA 78:6126–6129 (1981).
107. Gupta RC, Sopori ML, Gairola CG. Formation of cigarette smoke-induced DNA adducts in the rat lung and nasal mucosa. Cancer Res 49:1916–1920 (1989).
108. Bond JA, Chen BT, Griffith WC, Mauderly J. Inhaled cigarette smoke induces the formation of DNA adducts in lungs of rats. Toxicol Appl Pharmacol 99:161–172 (1989).
109. Borish ET, Winston GW, Deutsch WA, Church DF, Pryor WA. Redox cycling and cigarette tar-induced DNA damage. Biochemistry 26:4168 (1987).
110. Church DF, Pryor WA. The oxidative stress placed on the lung by cigarette smoke. In: The Lung, Vol 2 (Crystal RG, West JB, Barres P, Chernick NS, Weibel ER, eds). New York:Raven Press, 1991;1975–1979.