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Urban Air Carcinogens and their Effects on Health

John F. Lechner, Ph.D.

Inhalation Toxicology Research Institute

P.O. Box 5890

Albuquerque, NM 87185

Phone (505) 845-1121

Fax (505) 845-1229

Although tobacco smoking is the primary cause of lung cancer (1), exposure to other agents, such as radon, asbestos fibers, particulates, and metals, can also increase the risk for developing this disease (2). Exposures to these latter carcinogenic agents have been traditionally correlated with occupational encounters (3). However, it has also been suggested that non-occupational exposures to non-tobacco airborne carcinogens increases the risk of developing lung cancer (4). Foremost of the non-tobacco airborne carcinogens is radon; perhaps as many as 10% of the 170,000 lung cancers that arise each year in the United States may be related to inhalation of radon in homes and office buildings (5). Other airborne carcinogens may also be relevant, especially in metropolitan regions with extreme smog. The data supporting this latter supposition are more controversial than is the case for radon. However, lung cancer is most common in urban environs and the incidence directly correlates with the size of the city (3,4). In addition, several, but not all formal epidemiological studies also suggest a positive correlation between lung cancer incidence and the intensity of air pollution exposure (4). There is further support for a role of air pollution; as of 1993, 4.4% of all of the bronchogenic adenocarcinoma cancer cases among Mexicans living in industrialized cities are under 40 years of age (6). In addition, more than half these individuals never smoked. In all other comparable epidemiological studies (reviewed in reference 6), less than 15% of lung cancer cases occurred in never-smokers. Thus, it is plausible that chronic inhalation of automobile combustion products, factory emissions, and/or radon is at least partially responsible for the higher incidence of lung cancer exemplified by the never-smoking urban residents.

Urban Air Carcinogens

Tumor-promoting agents, inherent susceptibility, and nutritional status are all important cancer risk factors. However, the magnitude of exposure to a chemical or physical carcinogen is the principal risk determinant (2,7). Unfortunately, to a large extent, only general information on the character of airborne carcinogens is available, and rarely are the concentrations of these various agents known. Thus, direct dose-response interpretations between the concentrations of airborne carcinogens and lung cancer incidence among urban dwellers who never smoked is not possible. Nonetheless, several primary sources of airborne carcinogens are suspected, and their

potential importance can be inferred. One, secondhand tobacco smoke, is unlikely to be of sufficient importance, because its estimated carcinogenic potency in urban air is too low (8). Radon exposure, on the other hand, can be a plausible risk factor. Iinhalation of radon may be a specific risk for inhabitants of Mexico City, because the average indoor radon concentration in many homes exceeds the U.S. Environmental Protection Agency level that mandates remedial action (9). Further, coal combustion releases small amounts of radon. Metropolitan areas, such as those in China, that heavily rely on coal for heat and energy, may experience substantial increases in airborne radioactivity when weather conditions that support excessive smog accumulations (3).

Smog is a major health problem for most urban centers; however, most of the concern is not associated with cancer. Nonetheless, in addition to being a possible source of radon, smog can also be a source of respirable particulate carcinogens and metals. Respirable particulates, and particularly amphibole fibers, are well established lung carcinogens (10). Organic extracts of particulates collected on filters in highly polluted urban centers have been shown to be genotoxic to cultured hamster epithelial cells (11). Nevertheless, the risk for airborne particulates is more commonly associated with acute mortality than cancer (12). With respect to respirable fibers, the maximal concentrations of these fibers in urban air is on the order of 0.001 fibers per ml. Based on this number, conservative exposure-response estimates, which tend to overestimate risk, predict an annual lung cancer risk from fibers for 1,000,000 individuals exposed for 20 years to urban air to be less than 0.02 cases (10).

Soot, lead, and in some cities arsenic, are also present in urban air. Soot is derived principally from diesel exhaust, which is prevalent in most urban centers. Airborne soot particles themselves may be carcinogenic. This supposition stems from recent rat exposure experiments (13). These rats received life-span exposures to carbon black particles that had respirable qualities similar to soot, but were practically devoid of soluble organic matter. Yet at comparable exposures, carbon black particles produced a lung cancer incidence that was nearly identical to those caused by diesel exhaust, which also contains organic extractable carcinogens in the form of polycyclic organic matter (POM). On the other hand, the metals in smog are of little risk for lung cancer. Although lead is frequently prevalent, it not considered as a human carcinogen (12). In contrast, occupational arsenic exposure has been associated with excess rates

of lung cancer among smelter workers. However, the increased risk for lung cancer among urban dwellers is only minimally discernable within a 1 Km radius of arsenic smelters (14).

Other than fibers, soot particles, and metals, the constituents of typical smog is a complex mixture of ozone, nitrogen oxides, sulfur dioxides, aldehydes, POM, each in varying concentrations (4, 15, 16). Ozone and nitrogen oxides are not considered to be carcinogens (4,17,18). Sulfur oxides are also not carcinogenic, although H_2SO_4 (and other) acid mists may be weakly carcinogenic for lung tissue (19). Formaldehyde and other aldehydes arise through combustion of automotive fuels, particularly those that contain methanol. (Thus, they should be prevalent in Mexico City smog, since methanol is present in the gasoline.) Formaldehyde and some other aldehydes are animal carcinogens. In addition, formaldehyde is considered as a suspect human carcinogen, although direct epidemiological evidence for this supposition is lacking (20).

Epidemiological data suggest that diesel exhaust is a weak human carcinogen, whose potency is dependent on both dose and duration of exposure (21). Doot from diesel exhaust also contains significant amounts of soluble POM (21). POM is also produced by coal combustion and pyrolysis, and combustion of gasoline, wood, and plastics (15). Within the constellation of airborne POM are several polyaromatic hydrocarbon (PHA) species, and many of these have been shown to be both mutagenic in Ames assays and carcinogenic in animal models (15). In addition, PHA adducts have been detected on DNA from blood cells collected from non-smokers that were exposed to polluted air in Northern Bohemia (22), suggesting that in highly polluted areas these compounds are inhaled in quantities sufficient enough to be cause genotoxicity.

Molecular Epidemiology

As noted by Speizer and Samet in their recent review on air pollution and lung cancer (4), definitive evaluation of the possible role of air pollution in the genesis of lung cancer cannot be derived from epidemiological studies alone. One promising adjunct approach in defining the risk potential of air pollution is the developing science of molecular epidemiology. This is a relatively recent strategy that relates the molecular characteristics of genetic alterations found in tumor cells with the mode of action of specific carcinogens (23). Two different types of gene

targets are being used for the molecular analyses. One is the chromosome X-linked gene hypoxanthine guanine phosphoribosyl transferase (hprt). Because the hprt gene is 33 kb in length it is a relatively large target for mutational alterations (24). Under normal conditions, an hprt mutation does not confer any disadvantage to the cell. However, cells possessing a hprt mutation can be easily separated from their normal counterparts. This latter feature markedly facilitates determining the molecular nature of the mutation by direct sequencing. Finally, many investigators have now studied the mutation spectra of this gene as a function of carcinogen exposure (24). Thus, there is a large body of knowledge that relates mutation patterns to carcinogen classes.

An equally common experimental strategy is to evaluate the mutation spectrum in cancer genes. The advantage to this strategy is that these mutations are greatly amplified, because most cells in the tumor possess the same mutation. There are two general classes of cancer genes. The first is termed protooncogene. More than 60 protooncogenes are known and most are part of the mechanisms that control cell division. Protooncogenes can be altered by a mutation or rearrangement; when they are abnormal, they are referred to as oncogenes. When a protooncogene is converted to an oncogene, because of mutation, the pathways that promote cell division are overstimulated, and the cells divide uncontrollably (25). Normal cells also contain a second form of cancer gene termed tumor suppressor. More than 10 tumor suppressor genes have been identified to date, and more are anticipated (26). Their protein products function as natural neutralizers protooncogene activities. Mutation, and thus loss of the normal function of these genes, equates with the loss of cellular growth rate breaking activity.

The p53 Tumor Suppressor Gene as a Target Gene for Molecular Epidemiology

The efficacy of using the p53 gene as a target for molecular epidemiology investigations was first shown show in investigations that related hepatocarcinomas with aflatoxin B_1 (AfB_1) exposure and skin cancers with ultraviolet irradiation. In both cases, mutation fingerprints of these carcinogens were left in the *p53* gene (23). It has been long established that AfB_1 forms guanine adducts which result in G to T transversions (27). Therefore, human liver tumors from individuals exposed to AfB_1 should also have predominately G to T mutations. This was

confirmed by sequencing the *p53* gene from these tumors. It was found that these tumors frequently had the same G to T (hot-spot) mutation in codon 249 (28). The hot-spot mutation is also more frequent in noncancerous liver tissue of AfB_1-exposed individuals (29). Hepatitis viruses B and C are also hepatocarcinogens, whose mechanisms of action are different from AfB_1. *p53* mutations also arise in hepatitis-caused liver cancer, but the codon 249 hot-spot mutation is rarely found (30). Skin cancer tumors are a second example of the efficacy of molecular epidemiology. Ultraviolet light exposure is the accepted cause of these tumors (31). Ultraviolet light primarily mutates DNA at pyrimidine dimers, and the p53 mutations in these tumors are predominately at CC and TT tracts (31).

For lung cancers, the mutated form of the p53 tumor suppressor gene is present in nearly 100% of small cell carcinomas and in more than 50% of non-small cell carcinomas (32). This level of mutation frequency among individual lung cancer tumors provides an attractive target for molecular epidemiology investigations of lung cancer. Of special relevance to the question of airborne carcinogen exposures is a recent study by Takeshima et al. (33). These investigators studied the mutation *p53* profile in lung cancer tumors resected from nonsmoking Japanese, one-half of the group were atomic bomb survivors. The mutation spectrum data could not differentiate between atomic bomb survivors and unexposed individuals. However, the smokers and never-smokers were clearly distinguished. Throughout the world, the specific mutations in *p53* in more than 400 lung cancers have been analyzed. The results (see Table 1) show that G to T transversions occur twice as frequently as any other class of base substitution. Tobacco smoke contains many electrophilic mutagen, such as benzo(a)pyrene [B(a)P]. The complex mixtures of carcinogenic POM adsorbed to respirable diesel soot are similar in that they also preferentially cause a G to T substitution (24) This is seen by comparing rows 'BPDE' (benzo(a)pyrene diolepoxide) and 'Smokers' in Table 1; BPDE is the metabolically activated form of B(a)P. This kind of mutation occurs either by mispairing of the adducted guanine with adenine or by preferential insertion of adenine opposite the noninstructive modified base (34).

The Table also shows data on the 17 Japanese never-smokers. Relative to the smokers, the percentages of G to T transversions is lower and G to A transitions is higher. These G to A mutations can arise through two mechanisms. The first is at a 5-methylCpG dinucleotide. In this case, the 5-methylcytosine residue is converted to a T by the loss of an amino group. The second

mechanism is through adduct formation on the G. When the DNA is replicated, this adducted G often appears as an A and is misbase-paired with a T (the A rule; 33). Aldehydes are one class of agents that causes G to A mutations predominantly at non CpG tracts (35).

The Oncogene K-ras as a Target Gene for Molecular Epidemiology:

Radon decay produces alpha particle radiation, and as noted above, exposure to radon may arise from inhalation of coal fire-derived smog, or in the atmospheres of homes built on soils with high concentrations of uranium and its radioactive progeny (36). However, it has been difficult to quantify a causative role for radon inhalation in the development of lung cancer among never smokers using epidemiological evidence. Thus, it would be invaluable to have a molecular epidemiological signature for alpha particles that could be used to corroborate the traditional epidemiological data. One candidate molecular signature for alpha particle-caused mutation is a high frequency of G to A transition mutations. G to A transversions were the predominant form of mutations found in plasmids exposed to ^{210}Po (37; also see 'α-particles' row in Table 1). In addition, this type of mutation was frequently found in the 12th codon of the *K-ras* oncogene of lung tumors of rats that inhaled plutonium; also see 'rats' row in the Table). Further, the hyperplastic lesions and adenomas of these animals exhibited the same *K-ras* mutation. The K-ras mutation is frequent in lung cancers, especially adenocarcinomas (38), and adenocarcinomas are the predominant histotype of lung tumors arising in never-smokers. Thus, it is plausible that the detection of G to A mutation in tumors resected from never smokers would reflect alpha particle exposure. Alternatively, in an interesting recent report, Bridges, et al., (39) noted that the number of blood-derived lymphocytes with hprt mutations directly correlated with domestic radon concentration. However, analyses of the molecular nature of these mutations have not been reported. If these hprt mutations are due to alpha particles, they should be predominately G to A transitions.

Because alpha particles are not adduct-forming chemicals, transitions of the G to A type most likely arise from misrepair of double strand breaks in the DNA. The consequences of the alpha radiation tract could be the formation of a base pair gap in one strand of the rejoined DNA. In the majority of cases, these gaps are filled with an A as dictated by the 'A rule' (33).

Alternatively, the high-LET radiation could result in extensive numbers of lipid peroxidation free radicals that could preferentially attach the C of a G:C base-pair, such that it behaves as a T and thus base-pairs with an A. Although both mechanisms are presently suppositions, the data strongly suggest that the frequent presence of a G to A mutation in the *K-ras* gene in the tumors of the never-smoked urban residents would indicate that inhaled radon plays a significant role in the development of the disease. On the other hand, activating *K-ras* mutations (codons 12, 13, and 61) are infrequent in lung tumors that arise in never-smokers (40). Thus, the absence of a *K-ras* mutation in the tumors from the never-smokers would suggest that neither radon nor carcinogenic POM were involved in the genesis of the tumors.

Another possible molecular signature for radon having a role in the genesis of a lung cancer in a never-smoker has been proposed from investigations on tumors obtained from uranium miners. The results by Vähäkangas et al. (41) and Taylor et al. (42) are presented in the Table as 'U miners I' and 'U miners II', respectively. The 'U miners I' results do not suggest a molecular signature for high-LET radiation. Unfortunately, from the perospective of molecular epidemiology, most of these radon-exposed miners also smoked. Thus, the data suggest that the tobacco smoke carcinogens, e.g., B(a)P, were more potent in causing the ultimate tumors. The second study, 'U miners II' suggests a hot-spot mutation analogous to that found for hepatocarcinomas caused by exposure to AfB_1. However, this particular mutation appears to be restricted to exposure scenarios that are orders of magnitude higher than would be encountered by urban residents

Role of Tumor Susceptibility Genes in Risk Assessment

Well known is the phenomenon of interindividual variation among smokers, i.e., only 20% of heavy (2pk/day for 40 years) smokers develop this disease. Although the absolute reason for this interindividual variation is unknown, for outbred animals and for human beings, it is now recognized that the intrinsic constellation of tumor susceptibility genes (TSGs) also plays a major role in defining the risk that a particular animal or human will develop cancer after exposure to tobacco carcinogens (43). These TSGs operate as interindividual polymorphic variants of "normal" genes or genetic loci that either positively or negatively change the probability of

cancer developing in each carcinogen-exposed individual. Interindividual variability of risk is also true for carcinogens contained within air pollution. Presently, the molecular nature of these polymorphic genetic alleles remain to be elucidated. However, once the responsible genes are identified, it will be possible to better refine the appropriate limits of exposure to airborne POM and radon.

Conclusions

The exceptionally high incidence of lung cancer cases among never-smokers living in highly industrialized Mexican cities offers a unique opportunity to use molecular epidemiology to test whether chronic inhalation of atmospheric pollutants increases the risk for this disease. Overall, the analysis of the genetic alterations in two cancer genes, and possibly the hprt locus should give new insight as to whether the urban never-smokers developed their cancers because of exposure to environmental pollutants.

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Table 1: Mutation Patterns of Various Carcinogens[1]

	G → A			A → G	G → T	G → C	A → C	A → T	Insertions & Deletions
	@Cpg	nonCpG	Total						
Smokers (32)	16	7	23	8	38	11	4	5	10
Never-smokers (33)	13	54	69	0	13	6	0	6	6
UV (24)			61	2	5	10	5	12	4
BPDE (24)			5	0	62	14	0	9	10
α-Particles (37)			60	0	9	10	0	1	20
U miners I (41)			13	0	38	12	0	12	25
U miners II (42)			16	3	62[2]	0	0	3	16
Rat *K-ras* (44)			90	0	10	0	0	0	NR[3]

[1] Reference in parentheses
[2] Hot Spot -- AGG → ATG for 53% of all mutants
[3] Not relevant; deletions and insertions would not be detected in this study.

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