Cigarette smoke and adverse health effects: An overview of research trends and future needs

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A large volume of data has accumulated on the issues of tobacco and health worldwide. The relationship between tobacco use and health stems initially from clinical observations about lung cancer, the first disease definitively linked to tobacco use. Almost 35 years ago, the Office of the Surgeon General of the United States Health Service reviewed over 7000 research papers on the topic of smoking and health, and publicly recognized the role of smoking in various diseases, including lung cancer. Since then, numerous studies have been published that substantiate the strong association of tobacco use with a

variety of adverse human health effects, most prominently with cancer and cardiovascular diseases. Cigarette smoking is regarded as a major risk factor in the development of lung cancer, which is the main cause of cancer deaths in men and women in the United States and the world. Major advances have been made by applying modern genetic technologies to examine the relationship between exposure to tobacco smoke and the development of diseases in human populations. The present review summarizes the major research areas of the past decade, important advances, future research needs and federal funding trends.

Key Words: Atherosclerosis; Cancer; Smoking; Tobacco

EPIDEMIOLOGY OF TOBACCO-RELATED DISEASE

As part of the Global Burden of Disease Study carried out by the Harvard University School of Public Health in 1997 (4), it was projected that mortality and morbidity from tobacco use will increase by almost threefold worldwide in 20 to 25 years. Similar predictions have been made by the Oxford University Center headed by Sir Richard Doll, who was one of the first researchers to link cigarette smoking with lung cancer in the 1950s (5,6). Cancer, cardiovascular diseases and chronic obstructive pulmonary disease continue to be the main health problems associated with cigarette smoking. An extensive database has accumulated, which has consistently documented a relationship between smoking and these specific diseases. The strength of the association is further demonstrated by measuring the RR and the presence of a dose-response relationship (ie, direct relationship between the intensity of exposure to cigarette smoke and the risk of disease). According to a 2004 Centers for Disease Control and Prevention report (3), approximately 2600 people die of cardiovascular disease in the United States every day, which translates into one death every 33 s. Furthermore, the likelihood of dying from heart disease increases fourfold as a result of smoking. The cost of heart disease and stroke in terms of health care expenses and lost productivity was estimated at US\$351 billion in the United States alone in 2003.

An analysis by European health experts (7) determined that in developed countries as a whole, tobacco is responsible for 24% of all male deaths and 7% of all female deaths; these

A repository for the collection, analysis, validation and dissemination of all smoking and health-related data was established by the World Health Organization. The data received from various member countries were compiled into a book entitled *Tobacco or Health: A Global Status Report*, 1997 (1). This report showed smoking prevalence and other tobacco use-related data from various countries and presented an analysis. It is estimated that there are approximately 1.1 billion smokers worldwide, of which 900 million are men and 200 million are women. The sex ratio of men to women is 2:1 for developed nations and 7:1 for developing nations. Smoking prevalence in men and women averages 42% and 24%, respectively, for developed countries, and 48% and 7%, respectively, for less developed countries. In comparison, approximately 47 million people smoke cigarettes in the United States (2), and smoking prevalence in the United States is estimated at 28% and 23% for men and women, respectively. The Surgeon General's report in 2004 concluded that in the United States, cigarette smoking has caused 12 million deaths since 1964, at a cost to the nation of approximately US\$157.7 billion each year (3). There has been a significant decline in the consumption of cigarettes in the United States since 1964. The production of cigarettes continues at a steady pace mainly to meet export demands, which continue to rise due to increasing tobacco use in the rest of the world, especially in far eastern and southeastern Asia. On the basis of consumption and disease incidence trends, it is predicted that there will be an epidemic of tobacco-related diseases in various countries of the world in the next 20 to 30 years.

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figures rise to over 40% in men in some countries of central and eastern Europe and to 17% in women in the United States. The average decreased life span of smokers is approximately eight years. Among United Kingdom doctors followed for 40 years, overall death rates in middle age were approximately three times higher among physicians who smoked cigarettes than in nonsmokers. In those United Kingdom physicians who stopped smoking, even in middle age, a substantial improvement in life expectancy was noticed. These same experts found that worldwide, smoking kills three million people each year and this figure is increasing. They predict that in most countries, the worst is yet to come, because by the time the young smokers of today reach middle or old age, there will be approximately 10 million deaths per year from tobacco use. Approximately 500 million individuals alive today can expect to be killed by tobacco and 250 million of these deaths will occur in the middle age group. Tobacco is already the biggest cause of adult death in developed countries. Over the next few decades tobacco is expected to become the biggest cause of adult death in the world. For men in developed countries, the full effects of smoking can already be seen. Tobacco causes one-third of all male deaths in the middle age group (plus one-fifth in the old age group) and is the cause of approximately one-half of all male cancer deaths in the middle age group (plus one-third in the old age group). Of those who start smoking in their teenage years and continue smoking, approximately one-half will be killed by tobacco. One-half of these deaths will be in middle-aged individuals (35 to 69 years of age) and each will lose an average of 20 to 25 years of nonsmoker life expectancy. In contrast, the total mortality is decreasing rapidly and cancer mortality is decreasing slowly in nonsmokers in many countries. Throughout Europe in the 1990s, tobacco smoking caused three-quarters of a million deaths in the middle age group. In the Member States of the European Union in the 1990s, there were over one-quarter of a million deaths in the middle age group directly caused by tobacco smoking, which included 219,700 deaths in men and 31,900 in women. There were many more deaths caused by tobacco at older ages. In countries of central and eastern Europe, including the former Union of Soviet Socialist Republics, there were 441,200 deaths in middle-aged men and 42,100 deaths in women. Several epidemiological studies examining the factors responsible for the interindividual differences in the susceptibility to tobacco-related cancers and cardiovascular diseases are being performed in the United States, Europe and Japan. Although still not common practice, many of the newer studies are employing molecular genetic assays in conjunction with epidemiology to identify genotypes susceptible to disease development and select suitable biomarkers of tobacco smoke exposure.

The frequency of investigations in the area of cigarette smoke composition and chemistry decreased during the last decade. Nonetheless, there are ample data to suggest that cigarette smoke is a highly complex mixture that contains approximately 4800 different compounds (8). Approximately 100 of these compounds are known carcinogens, cocarcinogens and/or mutagens. The complex mixture also contains gases such as ozone, formaldehyde, ammonia, carbon monoxide, toluene and benzene, and about 10^{10} particles of different sizes in each mL of mainstream smoke. In addition, a number of other toxic, mutagenic, tumour promoter and/or cocarcinogenic substances have been identified in both mainstream and sidestream cigarette smoke over the years. Many chemical and biological assays of smoke condensates have also documented

the presence of potent inhibitors of carcinogenesis in smoke. Such a complex chemical composition of smoke has made it difficult to determine the active constituent(s) responsible for the tobacco-related health risks of smoking and has led to studies of individual constituents of smoke such as polycyclic aromatic hydrocarbons (PAH), nitrosamines and nicotine. Thus, over the years, various individual groups of smoke constituents have been the focus of research at different times. For example, studies of PAH were in vogue during the 1970s and 1980s, followed by nitrosamines in the 1990s. Tobacco alkaloids have long been studied because of their pharmacological activity and have attracted increased attention because of their suspected role in addiction, smoking behaviour and cessation. However, it is also being realized now that the health effects of this complex mixture are likely to result from a combined effect of these chemicals through multiple mechanisms rather than as result of the effects of a single smoke constituent. The mixture contains compounds belonging to almost every class of chemicals that are toxic and protective, agonist and antagonist, carcinogenic and anticarcinogenic, and exists in the gaseous as well as the particulate phase. Extensive studies on the chemical constituents of tobacco smoke and their relationship to disease were published by Hoffmann and Hoffmann of the American Health Foundation (8). Newer studies have largely focused on the comparative chemistry of mainstream and sidestream smoke. Interest in the free radical chemistry of smoke has resurfaced due to the realization that smoke-induced oxidative injury may play an important role in the etiology of a variety of tobacco-related diseases. Pioneering studies on the free radical chemistry of tobacco smoke, performed in the laboratory of William Pryor at the Louisiana State University (9), identified short- and long-lived radicals in mainstream and sidestream cigarette smoke, and implicated them in various smoking-associated disease etiologies.

TOBACCO-RELATED CARDIOVASCULAR DISEASE

Cardiovascular diseases, and atherosclerosis in particular, are the leading causes of death in industrial societies. The predominant underlying cause of coronary artery disease (CAD) is atherogenesis, which also causes atherosclerotic aortic and peripheral vascular diseases. Cigarette smoking, independently and synergistically with other risk factors such as hypertension and hypercholesterolemia, contributes to the development and promotion of the atherosclerotic process. Various studies have shown that the risk of developing CAD increases with the number of cigarettes smoked per day, total number of smoking years and the age of initiation, thus indicating a dose-related response. In contrast, cessation of smoking is reported to reduce mortality and morbidity from atherosclerotic vascular disease.

The mechanisms through which smoking influences the development and progression of atherosclerosis are poorly understood at present, but recent studies point to an adverse effect of smoking on endothelial and smooth muscle cell functions as well as thrombotic disturbances produced by tobacco smoke (10,11). With the use of modern ultrasonographic techniques, three independent studies performed in the United States, Europe and Australia have demonstrated that both active and passive smokers exhibit impaired endothelium-dependent vasoregulation (12-14). Some degree of recovery of endothelial function in ex-passive

smokers who have stayed away from smoke-contaminated environments further supported a secondary role of smoke in endothelial dysfunction (15).

Evidence has been presented that tobacco-related impairment of endothelial function may be related to its adverse effects on endothelial nitric oxide (NO) synthase (16,17). An association between a genetic polymorphism of the endothelial NO synthase gene and the predisposition of smokers to CAD was reported (18,19). Additionally, studies report that smoke interferes with L-arginine and NO metabolism, resulting in reduced NO formation (20). Upregulation of the expression of endothelial cell adhesion molecules (CAMs) such as vascular CAM-1 and intercellular CAM-1 by smoke condensates, and stimulation of leukocyte and endothelium attachment by exposure to cigarette smoke was demonstrated (21). Cigarette smoke extract has been shown to induce expression of CAMs (22). However, the expression of a specific adhesion molecule is determined *in vivo* and the relationship between various events is poorly understood.

Exposure to tobacco smoke is known to increase oxidative stress in the body by various mechanisms, including depletion of plasma antioxidants such as vitamin C. At least two studies have been performed to determine the role of oxidative stress in increasing leukocyte-endothelial interactions that precede the development of atherosclerosis in smokers. One study showed that a high intake of vitamin C by smokers significantly reduced the adhesiveness of their monocytes to endothelial cells (23). However, in a second study, sera from young smokers was collected before and after a single oral supplementation with vitamin C and L-arginine (a substrate for NO production). The sera were tested for promotion of the adherence of human monocytes to human umbilical vein endothelial cell monolayers. It was shown that while oral L-arginine caused reduction in such leukocyte adherence, no reduction was seen with vitamin C supplementation (24). This suggested that the NO levels may be important in smoking-induced leukocyte-endothelial interactions, at least during the early stages. Neither NO nor any other markers of oxidative stress were measured in either of these studies.

The levels of 8-hydroxydeoxyguanosine, an oxidized DNA product, and F2-isoprostane, an oxidative arachidonic acid product, were found to be elevated in passive smokers (25,26). Oxidation of low-density lipoprotein (LDL), which is a gold standard risk factor of the atherosclerotic process, was also found to be elevated in smokers, as determined by the presence of increased levels of autoantibodies against oxidized LDL. It was further demonstrated that dietary supplementation with a lipid-soluble antioxidant, α -tocopherol, significantly reduced plasma levels of oxidized LDL autoantibodies (27). Similarly, intake of a mixture of antioxidants was found to increase the resistance of smoker LDL to oxidative modification (28) and reduce the plasma levels of 8-hydroxydeoxyguanosine in passive smokers (25). These studies have thus identified newer, more specific markers of oxidative stress that can be used as biomarkers of oxidant injury and used for the development of dietary and/or pharmacological interventions against disease development.

Relatively few studies related to cardiovascular effects of cigarette smoke have been performed in rodent models. Such animal studies are, however, needed to delineate the role of different mechanisms in promoting atherosclerotic disease and for developing appropriate interventions.

TOBACCO-RELATED CANCERS

Tobacco carcinogenesis has remained a focus of research during the past 10 years, and various epidemiological and experimental studies have not only confirmed the major role of tobacco smoke exposure in lung and bladder cancers, but have also reported on its association with cancers of various other sites, such as the oral cavity, esophagus, colon, pancreas, breast, larynx and kidney. It is also associated with leukemia, especially acute myeloid leukemia.

In addition to the highly recognized role of cigarette smoking in lung cancer, it has been implicated in many other chronic diseases, including chronic bronchitis and pulmonary emphysema. In the United States, the reduction in smoking has resulted in a decline in death due to lung cancer in men since the mid 1980s. However, the incidence of lung cancer in women has surpassed that of breast cancer and continues to rise; it will likely be the focus of future studies (29,30). Both active and passive smoking are implicated in this increase, and several studies of smoking behaviour and disease incidence in women suggest greater susceptibility of women to tobacco carcinogens (31). It is believed that 80% to 90% of all respiratory cancers are related to active smoking.

Because of the antiestrogenic protective effects of smoking, the role of smoking in breast cancer is controversial. However, recent studies suggest that both active and passive smoking may have a role in the occurrence of breast cancer. One example is a study that found an OR of 4.5 for breast cancer among women who were exposed to passive smoke before 12 years of age and an OR of 7.5 for active smokers. Women who were first exposed to passive smoke after 12 years of age had a lower, although still elevated, OR (32).

In both men and women, cancers of the head and neck are also on the rise, and this has been attributed to increased use of smokeless tobacco products. Also, a synergistic interaction between cigarette smoking and radon exposure was confirmed in a large study that showed that lung cancer incidence due to an interaction between smoking and radon exposure exceeded incidence accounted for by additive effects and, therefore, indicated multiplicative effects (33).

Comparative toxicity studies have shown that in comparison with standard cigarettes, the new experimental cigarettes that heat tobacco have a relatively low toxicity (34). In comparing lung cancer risk in smokers of different types of cigarettes, Lee (35) determined in 2001 that the risk was 36% lower in individuals smoking filtered cigarettes than in those smoking unfiltered cigarettes, and the risk was 23% lower for smokers of low-tar cigarettes than smokers of high-tar cigarettes. The risk increased by 42% in hand-rolled cigarette smokers and by 75% in smokers using black tobacco.

One interesting observation relates to the nature of lung cancer, which has changed over the years with respect to the location and the types of lung tumours observed in smokers. In the past, the primary tumours observed among smokers were the centrally located squamous cell carcinomas of the airways. Now, the predominant lung tumours in smokers are peripheral adenocarcinomas and other non-small-cell lung cancers. This shift in tumour types has been attributed to changes in the composition of cigarettes and its effect on the smoking patterns of tobacco users over the past 30 years (8,36). Significant reductions in cigarette tar and nicotine and increased levels of nitrates in cigarettes have markedly altered the manner in which cigarettes are smoked. The number and

volume of puffs taken by smokers have increased from a single 35 mL puff/min with 1950s cigarettes to two to four 50 mL puffs/min of low-tar or low-nicotine cigarettes; the depth of inhalation has also increased. These changes in smoking patterns have promoted greater deposition of smoke constituents into the peripheral lungs, where adenocarcinomas develop.

Major advances are being made in the area of molecular epidemiology of tobacco-related cancers in human populations. Many recent epidemiological studies have focused on the differential susceptibility to tobacco-related cancers; they have employed polymerase chain reaction-based molecular assays that permit genotypic analysis of small human samples and supplement the information generated by enzymatic and immunological assays. These assays are increasingly being used in human and experimental studies to examine various gene-gene and gene-environment interactions. One area that has received considerable attention in recent years is the role of polymorphic enzymes in the development of diseases. It is now well recognized that genetic polymorphism strongly influences cancer susceptibility and incidence. The frequencies of mutated alleles of proto-oncogenes, tumour suppressor genes and xenobiotic biotransformation genes vary significantly among different populations and impact substantially on their susceptibility to cancer. Nearly every enzyme in the carcinogen metabolism pathways has been found to exist in multiple forms, many of which vary in binding affinity and/or turnover efficiency. Some are even entirely absent in individuals, thereby influencing their susceptibility to disease development.

The chemical complexity of tobacco smoke and the metabolic activation requirements for many of its carcinogenic constituents have drawn particular attention to genetic polymorphisms of biotransformation enzymes that metabolize tobacco smoke carcinogens. Thus, genes for various activating enzymes such as cytochrome P450 (CYP) proteins, and deactivating enzymes such as glutathione S-transferase (GST), N-acetyl transferase (NAT) and uridine diphosphate-glucose transferase have been the main target of many recent studies in the context of tobacco carcinogenesis. Also, pre-existing inherited mutations and/or mutation susceptibility of tumour suppressor genes such as *p53*, which are known to play a major role in determining cancer susceptibility, have been a subject of investigations in tobacco-related carcinogenesis (37,38).

Several human studies have suggested a strong interplay of various polymorphic CYP1A1, CYP1A2, CYP2E1, NAT1, NAT2, GSTM1 and GSTT1 enzymes in modulating the formation of DNA adducts, induction of mutations and chromosomal damage, and/or the incidence of cancers of various sites in different populations (39-47).

The *CYP1A1* gene has been extensively studied in Japanese populations. Two polymorphic variants that interact with smoking to modify lung cancer risk have been identified (47,48). Thus, a homozygous minor allele combined with smoking was found to increase lung cancer risk. Studies of the same gene in Western populations have, however, yielded negative or conflicting results (49), although an interaction of *CYP1A1* variants with the GST null genotype has been reported to significantly increase lung cancer risks in non-Japanese populations (50,51).

NATs are polymorphic conjugation enzymes (produced by the *NAT1* and *NAT2* genes) involved in the detoxification of aromatic amines by N-acetylation. Depending on the presence

or absence of a particular variant, individuals can be categorized as slow or fast acetylators, which in turn can influence the incidence of bladder cancer. It was shown that slow acetylator NAT2 is an important modifier of the amount of aromatic amine-DNA adduct formation even at a low dose of tobacco smoke exposure (52). Slow acetylator NAT2 genotype was also a significant risk factor for bladder cancer in moderate and heavy smokers, but had no effect in nonsmokers (53).

GSTs are another group of metabolic detoxification enzymes that have attracted a great deal of interest in recent years because of their association with risks for different types of cancers. Based on their sequences, these enzymes are divided into five classes. Three of these classes – GSTM1, GSTT1 and GSTP1 – are important in the context of tobacco-related cancers. Extensive studies on the relationship of these genes to cancer risks have shown that most populations studied have very high frequencies (20% to 50%) of homozygous GSTM1 and GSTT1 deletion carriers. GSTM1 and GSTT1 may be involved in the etiology of cancer at more than one site. Furthermore, the risk to individuals who carry homozygous deletions is generally small but increases significantly on interaction with cigarette smoking (54). Among all metabolic cancer susceptibility genes, the association of GSTM1 deficiency with cancer risk is the most consistent and unidirectional. Various experimental and epidemiological observations support the role of this gene in tobacco-related cancers. For example, it has been observed that the excretion of urinary mutagens and the number of lung tissue DNA adducts in GSTM1-deficient smokers is significantly greater than those carrying the wild-type allele (55-57). Various epidemiological studies also support the premise that deficiency of this enzyme predisposes for lung and bladder cancers (58). Furthermore, low activity alleles of GSTP1 have been often found in association with different types of human cancers (59,60).

In addition to anomalies of biotransformation enzyme genes, inactivation of tumour suppressor genes such as *p53*, and activation of the proto-oncogene *K-ras* are also involved in tobacco-related cancers. Various mutated forms of tumour suppressor gene *p53* have been commonly detected in lung tumours and it has been found that these mutations are predominantly located in exons 5 to 8. The nature of point mutations in this gene has been extensively investigated and studies show that the most common mutant allele of the *p53* gene possesses a G:C to A:C transversion (61), which is associated with tobacco use (62,63).

The above studies show that several genetically controlled polymorphic enzymes and enzyme systems are linked to tobacco carcinogen activation and deactivation. Some of these genes have been identified and characterized, but others remain undiscovered. Not only the independent effects of single gene polymorphisms, but an interplay of multiple gene interactions appear to be involved. The complexity of epidemiological studies, which have many uncontrollable variables, makes it difficult to study such interactions and their control in human studies. Additionally, many of the enzymes involved in tobacco carcinogen metabolism are also induced by other environmental factors such as alcohol use, dietary constituents, pesticide and xenobiotic exposure, hormonal status, etc, further complicating the interpretation of data. The interaction of many of these genes with each other and the effect of environmental factors are just beginning to be examined. Experimental studies in specifically constructed transgenic and knock-out animals will be important

for a systematic evaluation of the contribution of specific cancer genes and/or cancer susceptibility genes to the tobacco carcinogenic process, and to help identify the mechanisms through which environmental agents, such as cigarette smoke, influence these processes.

SECONDHAND SMOKE

The adverse effects of cigarette smoke on human health are widely recognized. It is the main etiological agent in chronic obstructive pulmonary disease and lung cancer, and is a known human carcinogen. While the risks to human health from active smoking are accepted, evidence supporting the risk of involuntary exposure to environmental tobacco smoke (ETS) has accumulated in recent years. It is the main source of toxicant exposure by inhalation in nonsmokers. Despite recent regulations, smoking in public enterprises is not uncommon. However, despite an occasional report on the effect of secondhand smoke in nonsmokers, little attention was given to this aspect of smoking until about 1970. ETS is now regarded as a risk factor for development of lung cancer, cardiovascular disease and altered lung functions in passive smokers (64). In general, children exposed to ETS show deterioration of lung function, more days of restricted activity, more pulmonary infections, more days in bed, more absences from school and more hospitalization than children living in nonsmoking homes (65).

Passive smoking is also implicated in increasing atherosclerosis in individuals 15 to 65 years of age. Children exposed to ETS are at higher risk of developing cardiovascular disorders. Quantitative risk estimates were obtained by measuring the intimal-medial thickness of the carotid artery in a large longitudinal atherosclerosis risk study of 10,914 individuals. Increases of 50%, 25% and 20% were shown over nonsmokers in current, ex- and passive smokers, respectively, thus suggesting a role of all types of tobacco smoke exposure in the progression of atherosclerosis (66). A recent meta-analysis (67) of 18 epidemiological studies (10 cohort and eight case-control) further showed an increased RR of CAD in ETS-exposed individuals. These investigators also identified a significant dose-response relationship between the intensity of smoke exposure and risk of CAD in passive smokers. Cardiovascular health risks of smoke-exposed women are of particular concern. Although the exposure to ETS is a current topic of debate in tobacco-related cancers and other lung diseases, the limited research at the basic experimental level provides a strong argument for launching experimental studies to support human data and explore disease mechanisms.

Follow-up of news stories, and local and state ordinances, leads to the conclusion that more communities and states are restricting exposure to secondhand smoke.

NATIONAL INSTITUTES OF HEALTH RESEARCH FUNDING FOR STUDIES OF HEALTH EFFECTS OF CIGARETTE SMOKE

To determine the extent of federal support for experimental studies in the area of health effects of cigarette smoke, the National Institutes of Health (NIH) database of all R01 research grant awards was searched for titles and abstracts containing the words 'cigarette smoke' from 1985 to 1998. The results are summarized below. A total of 127 hits were obtained and a careful review of the abstracts provided the following distribution:

- Grants involving experimental animal studies = 12 (9.4%)
- Grants involving experimental animal studies in which whole tobacco smoke was used = 3 (2.3%)
- Grants involving experimental animal studies using smoke components (nicotine, PAH, cadmium and quinones) = 8 (6.2%)
- One grant involved aging

A similar search of the NIH database from 1999 to 2006 revealed 907 grants in all award categories. The grant distribution by category was as follows:

- Total number of R01s = 383
- Grants involving experimental animal studies = 77 (20.1%)
- Grants involving experimental animal studies in which whole tobacco smoke was used = 29 (7.6%)
- Grants involving experimental animal studies using smoke components (nicotine, PAH, cadmium and quinones) = 29 (7.6%)

All the remaining grants generally supported behavioural and epidemiological studies in humans or other systems. Although the number of grants supporting animal studies increased between 1999 and 2006 compared with 1985 to 1998, a significant portion of NIH funding still went to research projects in the area of tobacco use and smoking behaviour, tobacco use among youth and interventions, nicotine addiction and neurobiology of nicotine (areas not covered in this review), presumably in agreement with the NIH's recent goal of finding effective smoking cessation programs to reduce tobacco usage in the general population. Thus, it is clear that the need for basic experimental research in the field of smoking-associated diseases and the mechanisms through which tobacco smoke causes various diseases remain as important as they ever were. The escalation of health care costs makes it even more necessary to find ways to protect the health of smokers and smoke-exposed individuals with any dietary or therapeutic interventions that hold promise.

DIRECTIONS FOR FUTURE RESEARCH

The most benefit is likely to result from detailed epidemiological studies complemented by specific molecular genotyping of various populations. Ideally, studies of this type will re-evaluate the prevalence of smoking and tobacco use and determine the exact nature of tobacco-related disease incidence, the role of contributory factors such as dietary habits, exposure to other substances and the genetic composition of subpopulations most at risk. Various biochemical and molecular assays will need to be applied to screen nonsmoker and smoker populations for a variety of health risks. Analysis of the results from such studies will help identify the main interacting factors for various health risks and define relationships among various epidemiological parameters. It would appear necessary to assemble teams of multidisciplinary investigators to perform these coordinated human studies in the field and in the laboratory. By nature, such studies are expensive and will involve commitment of resources, time and substantial amounts of funds to obtain meaningful results. Given the limited resources and competing priorities

for research funding, it is not easy to undertake such human studies. Hence, the experimental studies in animal models using inhalation exposure to whole smoke, and not individual constituents of smoke, is probably the next best approach for smoking and health programs.

The human epidemiological studies described in the present review have identified a number of genes that appear to have a distinct role in various tobacco-related diseases, and cancers in particular. Inability to control all the different variables in human studies has made it difficult to clearly define the contribution of various suspect genes in tobacco carcinogenesis. With the recent commercial availability of a variety of transgenic and knock-out animals for research, it would be most desirable, as a first step, to use these animals to establish experimental models of various tobacco-related diseases which can then be used for determining the contribution of different genes to disease processes and for elucidation of the mechanism(s) of disease development. Furthermore, these animal

models can be used to identify various agents possessing protective and therapeutic potential.

Research efforts in the area of smoking and health would benefit by focusing on studies of the in vivo effects of inhaled whole cigarette smoke in animal models of known specific genetic composition. Selection of the genetic composition would also require a thorough consideration of the information available from human molecular epidemiological studies. As indicated earlier, there are a number of genes that clearly influence the development of smoke-related diseases. In this context, many relevant transgenic and knock-out animals that can be effectively used for the study of tobacco-related diseases are now becoming available.

CONCLUSION

Tobacco abuse is a major public health problem and includes secondhand smoke exposure. Continued efforts to control and eliminate this abuse are a medical necessity.

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