CIGARETTE SMOKE TOXICOLOGY

Introduction

The inhalation toxicity of tobacco smoke has become one of the major public health problems of the 20th century. The chemical complexity of tobacco smoke confounds the task of identifying its toxic constituents. Tobacco smoke is comprised of thousands of chemical components arising primarily from volatilization and pyrolysis of the tobacco leaf (Stedman 1968; Green 1977). The chemical gamut runs from traces of elemental metals, such as cadmium, to nonvolatile whole tobacco leaf components that have escaped degradation during the burning process (USDHHS 1981). Approximately 90 percent of the individual constitutents are organic compounds associated with both the particulate phase and the gas phase (Guerin 1980). It is not surprising that chronic inhalational exposure to this diverse mixture of potentially bioactive compounds can evoke a wide variety of toxicologic responses. Over the years, scientific and public concern has centered primarily on the carcinogenic and atherogenic effects of tobacco smoke. In contrast, relatively little is known about the involvement of tobacco smoke constituents in the pathogenesis of chronic obstructive lung disease (COLD) (USDHHS 1981).

For the most part, smoke constituent toxicity studies, both epidemiologic (Dean et al. 1977; Higenbottam et al. 1980) and toxicologic (Walker et al. 1978; Lewis et al. 1979; Coggins et al. 1980), have been confined to a comparison of the varying amounts of particulate matter or tar delivered by smoke. In studies of this nature, attempts have been made to distinguish between the relative toxicities of the vapor phase and the particulate phase of tobacco smoke. The general conclusion reached is that gas phase components that penetrate to the small airways and alveoli may play a significant role in the production of peripheral airway and parenchymal diseases such as emphysema, whereas particulate phase components that deposit in larger airways may play a role in the development of disorders of the more proximal airways such as chronic bronchitis (USDHHS 1981). This generalization may not always hold, however. For example, in a review of the effects of smoking on mucociliary clearance, Newhouse (1977) noted considerable disagreement among investigators with regard to whether the vapor phase or the particulate phase was the major factor in smoke-induced dysfunction of the mucociliary transport system. Also, Coggins and associates (1980) observed an increase in both peripheral and central airway goblet cell number in rats after exposure to tobacco smoke from which most of the vapor phase had been removed. Cohen and James (1982) found that the level of oxidants in tobacco smoke (oxidants have been implicated in the pathogenesis of

emphysema) correlated with the amount of particulate matter in smoke from various brands of cigarettes. At present, therefore, attempts to associate a specific toxicologic response solely with either the vapor phase or the particulate phase of tobacco smoke are not recommended.

Because so little is known regarding the role of specific constituents or phases of tobacco smoke in the pathogenesis of COLD, this section of the Report is organized on the basis of specific insults to the respiratory system that may be brought on by exposure to whole tobacco smoke and that may lead to structural and functional changes within the lung. Included, as available information permits, are data on the known contribution of individual smoke constituents or phases to a specific insult. Human and animal studies are described separately to provide a perspective on the extent to which animal research has verified or extended clinical research and vice versa.

Preliminary Considerations

Tobacco smoking is generally accepted as the major cause of COLD (USDHEW 1979; USDHHS 1981). COLD is often subdivided into three categories: (1) uncomplicated bronchitis, characterized by mucus hypersecretion and cough, (2) chronic bronchitis with bronchiolar inflammation and obstruction of distal airways, and (3) pulmonary emphysema, characterized by distal air space enlargement with loss of alveolar interstitium. These three pathologic conditions are often considered collectively within the context of COLD because they can coexist in the lungs of smokers and because signs and symptoms associated with one condition may presage the development of another.

Effects on Airway Function and Ventilation

Human Studies

Cigarette smoke appears to have both chronic and acute effects on airway function. In adults, smoking over a period of years leads to narrowing of and histopathologic abnormalities in small airways (Ingram and O'Cain 1971; Cosio et al. 1980; Suzuki et al. 1983). Even in teenagers, regular smoking for 1 to 5 years is sufficient to cause demonstrable changes in tests of small airway function in some smokers (Seely et al. 1971); the lungs of young cigarette smokers who die suddenly show definite pathologic changes in the peripheral airways (Niewoehner et al. 1974).

The acute response to cigarette smoke has been reported to involve large airways, small airways, or both. Costello and associates (1975), in a study of asymptomatic smokers and nonsmokers, found that

tests of small airway function (maximum expiratory flow volume curves, closing volume, and frequency dependence of compliance) were unaltered after smoking one cigarette; however, specific airways conductance, a measure of large airway function, fell significantly in both groups. Essentially the same results were obtained by Gelb and associates (1979), who reported a decrease in airways conductance but little or no change in volume of isoflow (another test of small airway function) in healthy nonsmokers after smoking one cigarette. Likewise, McCarthy and colleagues (1976), using several different tests, found no evidence for an acute effect of intensive cigarette smoking on small airways, but did demonstrate increased large airways resistance. The decrease in conductance caused by cigarette smoke has been shown to occur within 7 or 8 seconds of a single inhalation (Rees et al. 1982), and filtration seems to reduce the degree of bronchoconstriction (Da Silva and Hamosh 1980). Irritant effects of tobacco smoke are not limited to the particulate phase, because exposure to oxides of nitrogen at levels present in cigarette smoke also can precipitate acute bronchospasm (Tate 1977). Nicotine does not appear to be responsible for the acute bronchoconstriction that accompanies cigarette smoking (Nadel and Comroe 1961).

Zuskin and coworkers (1974) showed that in healthy human subjects, smoking one or two cigarettes decreased flow rates on maximum and partial flow-volume curves, and concluded that smoking causes acute narrowing of small airways. Da Silva and Hamosh (1973) and Sobol and colleagues (1977) measured airways conductance, as well as maximum mid-expiratory flow rates, and concluded that both large and small airways are probably affected by the acute inhalation of cigarette smoke.

Though the bronchoconstrictive response to cigarette smoke has most often been attributed to a cholinergic reflex originating with stimulation of irritant receptors in the airways, there are data suggesting that histamine may also be involved. Walter and Walter (1982b) found a significant increase in the number of degranulated basophils in the blood of smokers 10 minutes after smoking compared with just before smoking. There is also some evidence to indicate that smokers may differ from nonsmokers in their responsiveness to inhaled histamine (Brown et al. 1977; Gerrard et al. 1980) as well as methacholine (Malo et al. 1982; Kabiraj et al. 1982; Buczko et al. 1984), but smoking immediately prior to an inhalation test does not appear to affect bronchial responsiveness to either histamine or methacholine (McIntyre et al. 1982).

Asthmatic subjects have a greater than normal susceptibility to the bronchoconstrictive effects of cigarette smoke when the smoke is actively inhaled. The question whether tobacco smoke plays a role in

allergic asthma has yet to be resolved completely (USDHEW 1979; Shephard 1982; Burrows et al. 1984).

Animal Studies

Binns and Wilton (1978) studied the acute ventilatory response to cigarette smoke in rats. They found that within the first 3 minutes after initiation of smoke exposure, tidal volume fell to 80 percent of the preexposure level, then rose to 160 percent after 9 minutes of exposure; respiratory rate dropped to 40 percent of the preexposure level within 1 minute and remained there for the duration of the exposure period. There was no adaptation of the acute ventilatory response after 4 weeks of daily smoke exposures. In a similar study, Coggins and associates (1982) found that rats exposed to a relatively low dose of cigarette smoke demonstrated a persistent depression in tidal volume and breathing frequency, whereas animals exposed to a relatively high dose exhibited an increase in tidal volume with no change in frequency.

Acute airway responses to cigarette smoke have not been studied as extensively in animals as they have been in man. Experiments in anesthetized dogs (Aviado and Palecek 1967), rabbits (Sellick and Widdicombe 1971), and cats (Boushey et al. 1972) indicate that acute smoke exposure elicits reflex bronchoconstriction. There also is evidence from experiments with isolated monkey lungs that smoke exposures stimulate histamine release (Walter and Walter 1982a). Histamine appears to be responsible, at least in part, for mediating the increase in collateral resistance in dogs following administration of cigarette smoke (Gertner et al. 1982).

The chronic effects of cigarette smoking on pulmonary function in dogs were studied by Park and coworkers (1977). Active inhalation of 100 and 200 puffs of diluted (1:4) smoke 5 days per week for periods of 6 months and 1 year, respectively, did not produce any noteworthy changes in pulmonary function. The effects of chronic cigarette smoke exposure on ventilation in rats were studied by Loscutoff and coworkers (1982). Animals exposed for up to 24 months to cigarette smoke containing various amounts of tar and nicotine were found to have higher tidal volumes and lower respiratory rates than sham-exposed animals. The most pronounced changes were seen in animals exposed to smoke from low tar, high nicotine cigarettes. Histopathologic examination of lungs taken from these animals revealed primarily granulomatous lesions with no evidence of emphysematous changes (Wehner et al. 1981). Roehrs and colleagues (1981) used operant conditioning techniques to get baboons to smoke 30 cigarettes per day for 3 years. Measurements of lung volumes, compliance, and expiratory flow showed no differences between smoking animals and sham animals, but airway reactivity to inhaled methacholine was decreased in animals that had smoked. Subse-

quently, Wallis and associates (1982) reported that both acute and chronic inhalation of nicotine mimicked this effect of cigarette smoke on bronchial reactivity in baboons.

Effects on Permeability of the Pulmonary Epithelium

The pulmonary epithelium functions to protect underlying structures from injurious agents deposited in the airway lumen. Cigarette smoke has been shown to diminish this protective function by increasing epithelial permeability in all regions of the tracheobronchial tree (Simani et al. 1974). In the airways, irritant receptors located just beneath epithelial tight junctions are more accessible following exposure to tobacco smoke. Stimulation of these receptors is thought to initiate rapid changes in ventilation and to induce bronchoconstriction (Widdicombe 1977). Likewise, mast cells, a source of potent bronchoconstrictive mediators, become more accessible to inhaled toxicants after smoke exposure (Guerzon et al. 1979). In the alveolar region, an increase in epithelial permeability may promote the transfer of noxious smoke constituents and endogenous proteases to the interstitium, thereby facilitating disruption of alveolar septa.

Human Studies

Subepithelial structures of the lung are important targets for smoke-induced injury, and research has shown that tobacco smoke alters epithelial permeability to allow offending agents to gain access to these structures. Minty and colleagues (1981) showed that, compared with nonsmokers, smokers had significantly shorter half-time lung clearance as measured with inhaled radiolabeled aerosols. After cessation of smoking, half-time clearance increased, but at 21 days it was still significantly less than that reported in nonsmokers. Using similar techniques, Kennedy and colleagues (1984) compared pulmonary epithelial permeability and bronchial reactivity to inhaled histamine in smokers and nonsmokers. These researchers found increased permeability in smokers, but could find no evidence of increased reactivity. Although the mechanism by which cigarette smoke induces an increase in alveolar epithelial permeability is not fully understood, it has been suggested that carbon monoxide may play an important role, with possible additional contributions from nicotine and oxides of nitrogen (Jones et al. 1980).

Animal Studies

In studies of rabbit tracheal rings exposed in vitro, just a few puffs of diluted cigarette smoke have caused ultrastructural changes in tracheal epithelial cells and an increase in the size of intracellular spaces, but junctional complexes between cells remain intact (Davies

and Kistler 1975). Boucher and associates (1980) found that, compared with controls, guinea pigs exposed to 100 or more puffs of cigarette smoke exhibited a significantly faster transfer rate for horseradish peroxidase across tracheal epithelium. These animals also demonstrated a progressive disruption of epithelial tight junctions as a function of the dose of tobacco smoke. Hulbert and associates (1981) reported that smoke-induced increases in guinea pig airway permeability were transient, reaching maximum levels at 30 minutes after acute exposure to 100 puffs and returning to control levels 12 hours later. Gordon and associates (1983) reported that acute exposure (48 hours) of hamsters to NO_2 caused a marked increase in bronchiolar and alveolar epithelial permeability to horseradish peroxidase. Restoration of the epithelial barrier was noted 48 hours after exposure in these animals. Ranga and associates (1980) demonstrated a similar increase in guinea pig tracheal epithelial permeability upon exposure to NO_2 for 14 days.

Effects on Mucociliary Structure and Function

The mucociliary system provides the lung with one of its most effective lines of defense against inhaled pollutants. Disruption of this system enables pollutants to remain in contact with the respiratory membranes for prolonged periods and increases the risk of toxic damage. Tobacco smoke can adversely affect mucociliary function by increasing the amount or viscosity of respiratory tract secretions or by depressing ciliary activity directly (Newhouse 1977; Wanner 1977).

Effects on Cells: Pulmonary Alveolar Macrophages and Polymorphonuclear Leukocytes

Pulmonary emphysema is believed to result from the slow degradation of the elastin framework of lung parenchymal tissue. Degradation of elastin is most likely initiated by elastolytic enzymes released locally in the lung and not adequately inhibited by endogenous antiproteases. Recent studies of the effects of cigarette smoke on these cellular sources of elastolytic enzymes have provided additional insights into the relationships between smoking and pulmonary emphysema. (See chapter 5.)

Human Studies

Normally, pulmonary alveolar macrophages (PAMs) function as a defense mechanism against particulate material deposited on the respiratory surfaces of the lung. However, cigarette smoke can induce a number of changes in PAMs that may promote excessive degradation of native lung tissue. For example, PAMs from smokers

have elevated elastase levels, and these cells may secrete elastase in vitro (Harris et al. 1975; Rodriguez et al. 1977; Hinman et al. 1980). Further, PAMs from smokers have been shown in vitro and in vivo to bind and internalize elastase released from polymorphonuclear leukocytes (PMNs) (Campbell et al. 1979; White et al. 1982). It has been suggested that during the phagocytosis of smoke particulates by PAMs, elastolytic enzymes may be released into extracellular spaces (Hocking and Golde 1979; Brain 1980; Kuhn and Senior 1978). Numerous investigators have reported significantly greater yields of PAMs from the lungs of smokers compared with nonsmokers (Green et al. 1977; Roth et al. 1981; Hoidal and Niewoehner 1982). The effects of smoking on PAM mobility are unclear. Some researchers have reported a significant increase in chemotactic migration of PAMs from smokers versus PAMs from nonsmokers (Warr and Martin 1974), but others have been unable to observe such an effect (Demarest et al. 1979).

Macrophages from smokers exhibit various morphologic, metabolic, and functional abnormalities. Structural changes noted in the PAMs of smokers include a slight increase in cellular diameter, the presence of "smokers inclusions" consisting predominantly of kaolinite particles (which have been shown to be cytotoxic to human PAMs in vitro (Green et al. 1977)) and increased numbers of lysosomes and phagolysosomes (Brody and Craighead 1975). Perturbations in several metabolic pathways have been observed in PAMs from smokers, and acrolein in smoke has been implicated in this toxicity (Green et al. 1977; Laviolette et al. 1981). The production of superoxide radicals and hydrogen peroxide (H_2O_2), both of which inhibit lung antiproteases, has been reported to be enhanced in the PAMs of smokers (Hoidal et al. 1981). Smokers' PAMs have also been shown to release chemotactic substances for PMNs (Gadek et al. 1978; Hunninghake et al. 1980). Additionally, there is evidence suggesting that PAMs from smokers secrete factors that promote the release of elastases from PMNs (Cohen et al. 1982).

As with PAMs, PMNs have been found in elevated numbers in the lungs of smokers (Reynolds and Newball 1975; Hunninghake et al. 1980a; Hunninghake and Crystal 1983). Exposure of PMNs to cigarette smoke condensate in vitro has been shown to promote the release of elastolytic enzymes (Blue and Janoff 1978). Hutchison and coworkers (1980) found that the particulate phase of cigarette smoke stimulated the release of lysosomal enzymes from human PMNs, but they did not quantitate this release specifically for elastases. A recent study by Totti and colleagues (1984) showed that nicotine was chemotactic for human PMNs and that it enhanced PMN responsiveness to other chemotactic factors. These results are in contrast with a previous study by Bridges and coworkers (1977) showing that nicotine, when used in higher concentrations than those employed

by Totti and colleagues, inhibited the chemotactic response of PMNs to casein.

In a preliminary laboratory study, Janoff and colleagues (1983) found that smokers have elevated levels of PMN elastase in their lung fluids compared with nonsmokers. This finding is of particular interest in that human PMN elastase has been shown to induce emphysema in animals (Janoff et al. 1977; Senior et al. 1977; Snider et al. 1984). There is also evidence that cigarette smoke alters PMN metabolism in such a way as to favor the release of toxic oxygen metabolites. PMNs from smokers with an elevated white blood cell count show a marked increase in the release of superoxide anions compared with PMNs from nonsmokers or with PMNs from smokers with a normal white cell count (Ludwig and Hoidal 1982). These unstable oxygen metabolites have harmful effects on various cells and tissues in vivo and in vitro (Sachs et al. 1978; Fridovich 1978), and are capable of injuring phagocytes and promoting the release of proteolytic enzymes (Hoidal and Niewoehner 1982). Oxidants derived from PMNs are also capable of inactivating lung antiproteases in vitro; this may be yet another mechanism by which smoke-affected PMNs contribute to the development of emphysema (Zaslow et al. 1983).

Animal Studies

Laboratory animal studies of the effects of cigarette smoke on lung free-cell population and integrity have yielded results similar to those obtained from human studies. Recruitment of PAMs to the lungs following cigarette smoke exposure has been demonstrated in a number of animal species, including mice (Matulionis and Traurig 1977; Guarneri 1977); hamsters (Hoidal and Niewoehner 1982), and monkeys (DeLucia and Bryant 1980). In the rat, PAM recruitment in response to smoke exposure appears variable. In two relatively similar studies, one group of investigators (Drath et al. 1978) found a depression in the number of PAMs in the lungs of rats exposed to smoke for 30 days, whereas another group (Walker et al. 1978) reported a significant increase in the number of PAMs after 6 weeks of smoke exposure.

Several authors have described the effects of cigarette smoke exposure on the morphology of rat PAMs. Observed changes include increases in PAM size, lipid vacuoles, and lysosomes, as well as the presence of "smokers inclusions" (Walker et al. 1978; Davies et al. 1978; Lewis et al. 1979). Cigarette smoke exposure of mice induces a similar pattern of morphological changes in PAMs (Matulionis 1977).

Alterations of PAM phagocytic capacity have been demonstrated in animals exposed to cigarette smoke. Fogelmark and colleagues (1980) reported a dose-related increase in the rate of phagocytosis of fungal spores in vitro by PAMs from hamsters and rats that had

been exposed to cigarette smoke. The ability of rats to mobilize PAMs in response to a bacterial challenge does not appear to be altered by cigarette smoke exposure (Guarneri 1977), nor is there a significant effect upon PAM phagocytosis of Staphylococcus aureus in rats after 30 days of smoke exposure (Drath et al. 1981). Macrophages from mice exposed to cigarette smoke for 4 weeks secrete significantly higher amounts of elastase than PAMs from controls. However, it is not known whether this effect is due to the stimulation of resident macrophages or to the recruitment of a highly exudative population of macrophages to the lungs (White et al. 1979).

A number of metabolic abnormalities have been noted in PAMs from smoke-exposed animals (Low et al. 1977). Among these is an increase in oxidative metabolism resulting in an increased production of superoxide anions. Hoidal and Niewoehner (1982) showed that enhanced oxidative metabolism in hamster PAMs was diminished if the particulates were filtered from the smoke. However, other workers (Drath et al. 1981) studying smoke enhancement of rat PAM oxidative metabolism have attributed this effect to the vapor phase of smoke. In another study of PAM oxidative metabolism in rats exposed to cigarette smoke for 180 days (Huber et al. 1980), it was reported that metabolism was activated after 30 days, and at the same time PAM superoxide dismutase (an enzyme that detoxifies superoxide radical) activity was depressed by 30 percent.

Animal PAMs, like human PAMs, can secrete PMN-directed chemotactic factor in response to various stimuli. For example, Gadek and colleagues (1980) demonstrated that noninfectious particulate material stimulated the release of PMN-specific chemotactic factor from guinea pig PAMs. Perhaps tobacco smoke particulates might evoke a similar response.

Owing to the ease with which PMNs can be harvested from peripheral blood, most research concerning the effects of tobacco smoke on PMNs has been conducted using human cells. In one laboratory study, hamsters exposed to cigarette smoke for 2, 8, and 20 hours showed a progressive recruitment of PMNs to the lungs. Control saline aerosol and filtered smoke did not stimulate recruitment of PMNs, suggesting that this effect of cigarette smoke resides in the particulate phase (Kilburn and McKenzie 1975).

Effects on Protease Inhibitors

In addition to efforts to characterize the effects of tobacco smoke on cellular sources of elastolytic enzymes, considerable research has gone into delineating the effects of tobacco smoke on the protease inhibitor defense mechanism in the lungs. While several protease inhibitors have been identified, α_1-protease inhibitor (α_1Pi) is consid-

ered to be the most important in neutralizing the effects of elastase (Gadek et al. 1981). Chemical oxidants are known to inactivate α_1Pi and diminish its capacity to inhibit elastase both in vitro and in vivo (Abrams et al. 1980). Cigarette smoke is an abundant source of chemical oxidants that can exert the same effect on α_1Pi and thereby reduce lung defenses against endogenous elastases.

Human Studies

The toxic effect of tobacco smoke on protease inhibitors has been demonstrated in a variety of experimental situations. Janoff and Carp (1977) reported that tobacco smoke condensate suppressed the inhibitory action of human serum, purified α_1Pi, and bronchopulmonary lavage fluids on both porcine and human elastase. The suppression of human serum elastase inhibitory capacity by smoke condensate solutions in vitro has also been demonstrated by others (Ohlsson et al. 1980).

Comparison of the protease inhibitory capacity of serum samples from smokers and nonsmokers has revealed a significant depression in smokers that is correlated with smoking history (Chowdhury 1981; Chowdhury et al. 1982). The latter studies also reported that the depression of serum protease inhibitors was related to an effect of smoke on the inhibitors per se, and not to a decrease in serum antiprotease concentration. Still, the effect of tobacco smoke on serum and lung lavage fluid antiprotease concentration and activity remains controversial. Several investigators have reported that smokers have elevated serum protease inhibitor levels (Rees et al. 1975; Ashley et al. 1980); others (Olsen et al. 1975; Warr et al. 1977), like Chowdhury and colleagues, have shown no difference in serum or lavage fluid protease inhibitor concentrations between smokers and nonsmokers.

Gadek and associates (1979) compared α_1Pi activity of lung lavage fluids taken from smokers and nonsmokers and found that smokers had a twofold depression of functional α_1Pi activity. The activity of α_1Pi in this study was tested against porcine pancreatic elastase. In a similar study (Carp et al. 1982), in which human neutrophil elastase was used, bronchoalveolar lavage fluids obtained from smokers had 40 percent less α_1Pi activity than fluids from nonsmokers. However, Stone and colleagues (1983) found that smokers' bronchoalveolar lavage fluids did not exhibit decreased functional α_1Pi activity when tested against either porcine pancreatic elastase or human neutrophil elastase, and suggested that increased elastase derived from neutrophils may be the main factor in the genesis of emphysema in smokers.

Smokers may have a functional deficiency in bronchial mucus protease inhibitor (BMPi) activity. A comparison of BMPi obtained from tracheal aspirates of smokers with BMPi from nonsmokers

revealed that smokers' BMPi was 20 percent less active against PMN elastase than nonsmokers' BMPi (Carp and Janoff 1980a).

Specific cigarette smoke constituents that may be responsible for the inactivation of lung protease inhibitors have not been identified. Nicotine and acrolein were studied for their ability to suppress α_1Pi activity and were found to be ineffective (Janoff and Carp 1977). Several studies have shown that oxidizing compounds such as chloramine-T and N-chlorosuccinimide can oxidize methionine groups on α_1Pi and reduce its activity against porcine pancreatic and human PMN elastases (Cohen 1979; Johnson and Travis 1979; Satoh et al. 1979; Abrams et al. 1980; Beatty et al. 1980). This has led to the current belief that oxidants in cigarette smoke may be involved in the inactivation of protease inhibitors (Janoff et al. 1983). In addition to free radicals (Pryor 1980), cigarette smoke contains oxides of nitrogen possessing their own free radical properties and able to react with olefins in the gas phase or with peroxides to generate potent oxy-radicals (Dooley and Pryor 1982; Pryor et al. 1983).

As mentioned previously, smoke condensate solution suppresses the elastase inhibitory capacity of serum α_1Pi in vitro. This suppression can be prevented by the incorporation of phenolic antioxidants into the test media (Carp and Janoff 1978). Similarly, BMPi suppression by smoke condensate can be prevented by antioxidants (Carp and Janoff 1980a). Cohen and James (1982) used o-dianisidine oxidation to quantify the levels of oxidants in tobacco smoke condensates from various brands of cigarettes and found that oxidant levels correlated with capacity to suppress α_1Pi deactivation of elastase. Further, this study provided evidence that peroxides and superoxide anions were responsible for the loss of α_1Pi activity, because inclusion of catalase and superoxide dismutase in the test system reduced smoke condensate effects on α_1Pi activity. Bronchoalveolar fluid from smokers contains some amount of oxidant-inactivated α_1Pi, as evidenced by the presence of methionine sulfoxide residues (Carp et al. 1982).

In addition to the numerous oxidizing agents present in cigarette smoke, byproducts of smoke-stimulated phagocyte metabolism represent another potential source of oxidants capable of inactivating lung protease inhibitors. Carp and Janoff (1979) demonstrated that phagocytosing human PMNs produce activated oxygen species that diminish the elastase inhibitory capacity of human serum and pure α_1Pi in vitro. These workers presented evidence to show that hydroxyl radicals resulting from the reaction between superoxide anions and H_2O_2 were responsible for this effect. The inactivation of α_1Pi by a myeloperoxidase-mediated reaction was also described in the study, which concurs with other studies demonstrating that purified myeloperoxidase, in conjunction with H_2O_2 and a halide ion, can inactivate α_1Pi in vitro (Matheson et al. 1979, 1981). Further

work has shown that activation of human blood monocytes, PMNs, and PAMs by use of a membrane-perturbing agent (as opposed to phagocytosis) results in the release of superoxide anions and H_2O_2 and the suppression of serum elastase inhibitory capacity (Carp and Janoff 1980b). Clark and colleagues (1981) have demonstrated that the myeloperoxidase-H_2O_2-halide system from chemically stimulated PMNs oxidizes α_1Pi in vitro. Similar evidence ascribing inactivation of BMPi to the myeloperoxidase-H_2O_2-halide system has been reported (Carp and Janoff 1980a). In the studies noted above, phagocyte-derived oxidants were shown to be capable of inactivating α_1Pi when porcine pancreatic elastase was used as the substrate. These findings were recently extended to include the more pathophysiologically relevant protease, human neutrophil elastase (Zaslow et al. 1983). Little is known about in vivo inactivation of protease inhibitors by phagocyte-derived oxidants, other than that inactive α_1Pi (in the oxidized state) has been found in the synovial fluid of patients with inflamed joints (Wong and Travis 1980). The extent to which oxidants from stimulated phagocytes play a role in the suppression of lung α_1Pi activity in smokers is at present unknown.

Animal Studies

Although most of what is known about cigarette-smoke-induced oxidant injury to lung protease inhibitors has been derived from human studies, some work has gone into the effects of cigarette smoke on lung protease inhibitors in laboratory animals. It has been demonstrated that very brief exposure of rats to cigarette smoke can cause a significant reduction in the elastase inhibitory capacity of α_1Pi obtained from lung lavage fluid (Janoff et al. 1979). Very likely this toxic effect of cigarette smoke is caused by oxidant damage to protease inhibitors, because treatment of the lavage fluid with a reducing agent partially restored normal elastase inhibitory capacity and because animals rendered oxidant tolerant by preexposure to ozone did not exhibit a significant reduction in α_1Pi activity following exposure to cigarette smoke.

Effects on Lung Tissue Repair Mechanisms

A preponderance of the research to elucidate mechanisms by which cigarette smoking induces emphysema has focused on the factors that initiate lung tissue degradation. Recent studies suggest, however, that the increased risk of emphysema associated with cigarette smoking may be due partially to the effects of smoke on lung repair mechanisms.

Human Studies

For the most part, work concerning the effects of cigarette smoke on lung repair mechanisms has been conducted in experimental animals. It has been shown, however, that cigarette smoke contains an inhibitor that can prevent the cross-linking of human fibrin polymers and thereby impede normal tissue repair (Galanakis et al. 1982). Smoke and smoke constituents have also been shown to induce membrane damage in human lung fibroblasts (Thelestam et al. 1980). Of 464 smoke constituents tested, approximately 25 percent caused membrane damage. The most active constituents were amines, strong acids, and alkylated phenols; nitriles and polycyclic aromatic hydrocarbons were inactive.

Animal Studies

Cigarette smoke has been shown to affect elastin synthesis in vitro and elastin repair in vivo. Laurent and coworkers (1983) determined the effect of solutions of smoke condensate on elastogenesis in vitro by measuring the formation of desmosine (one of the major cross-linking amino acids of elastin) during conversion of tropoelastin to elastin. Using a cell-free system of purified tropoelastin from chick embryo aorta or porcine aorta and lysyl oxidase purified from chick embryo or bovine lung, these investigators found that desmosine synthesis was inhibited from 80 to 90 percent in the presence of an aqueous solution of the gas phase of cigarette smoke. Elastin repair in vivo has been reported to be retarded by tobacco smoke. Osman and colleagues (1982) showed that hamsters with elastase-induced lung injury resynthesized elastin at a reduced rate if they were exposed to six or seven puffs of whole cigarette smoke hourly for 8 hours per day during the repair period.

Summary and Conclusions

1. The mass median aerodynamic diameter of the particles in cigarette smoke has been measured to average approximately 0.46 μm, and particulate concentrations have been shown to range from 0.3×10^9 to 3.3×10^9 per milliliter.
2. The particulate concentration of the smoke increases as the cigarette is more completely smoked.
3. Particles in the size range of cigarette smoke will deposit both in the airways and in alveoli; models predict that 30 to 40 percent of the particles within the size range present in cigarette smoke will deposit in alveolar regions and 5 to 10 percent will deposit in the tracheobronchial region.
4. Acute exposure to cigarette smoke results in an increase in airway resistance in both animals and humans.

5. Exposure to cigarette smoke results in an increase in pulmonary epithelial permeability in both humans and animals.
6. Cigarette smoke has been shown to impair elastin synthesis in vitro and elastin repair in vivo in experimental animals (elastin is a vital structural element of pulmonary tissue).

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