Depressant Effect of Marihuana Smoke on Antibacterial Activity of Pulmonary Alveolar Macrophages

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Other than the potentially therapeutic bronchodilatory influences of marihuana, very little is known of its biologic effects on the lung. To evaluate this problem, alveolar macrophages were harvested from rats by broncho-pulmonary lavage and incubated in vitro with Staphylococcus albus and marihuana smoke of standardized 2.2-percent tetrahydrocannabinol content in graded amounts. After three hours, control alveolar macrophages inactivated 78.0 ± 5.0 percent of the staphylococcal challenge. There was a dose-dependent depression of alveolar macrophage bactericidal activity, with 66.7 ± 7.1 percent, 23.7 ± 7.0 percent, 20.5 ± 7.0 percent, and 11.4 ± 7.6 percent of the bacteria killed following exposures to 2 ml, 4 ml, 6 ml, or 8 ml of marihuana smoke, respectively. Differential filtration of marihuana smoke revealed that the alveolar macrophage cytotoxin was present in the gas phase of the smoke and was water-soluble. Studies on purified tetrahydrocannabinol and on tetrahydrocannabinol-extracted marihuana revealed that the impairment in alveolar macrophage function was not related to the psychomimetic or bronchodilatory components of marihuana.

In this country 24 million Americans have smoked marihuana, 2.5 million use it regularly, and over 13 million consume it occasionally.¹ Under the federal code, possession of marihuana is a misdemeanor. Unfortunately, little scientific data are available on health effects of marihuana consumption in general, and its specific biologic effects on the lung are virtually unknown. Marihuana is among the oldest and most widely used drugs of man. It was used in the eighth century BC in northern Syria, was entered in the Chinese compendium of the Emperor Shen Nung in 2737 BC, and was included in the United States Pharmacopoeia until the first "marihuana crisis" and the introduction of the Marihuana Tax Act of 1937.² Marihuana originates from the Indian hemp plant, Cannabis sativa, an annual dioecious plant that has widespread distribution. Until the introduction of synthetic fibers, this plant supplied the world with rope and twine. Marihuana is consumed primarily by smoke inhalation. It is important, therefore, to evaluate the direct effect of marihuana smoke on the key cellular component of the pulmonary host defense system, the alveolar macrophage. The purpose of this communication is to report that a water-soluble component of the gas phase of fresh marihuana smoke is toxic to the alveolar macrophage and impairs the bactericidal activity of this cell in a dose-dependent manner.

Materials and Methods

Phagocytosis and intracellular inactivation of bacteria were quantitatively assayed in a system used by Green and Carolyn³ to evaluate the effects of tobacco smoke on alveolar macrophage bactericidal activity in the rabbit. Male rats** weighing 200 to 250 gm were used in all experiments as a source of alveolar macrophages. The techniques for cell harvest, in vitro culture, and macrophage bactericidal function have previously been described.³,⁴ The smoke from marihuana cigarettes† (tetrahydrocannabinol content of 2.2 mg/100 ml), marihuana-placebo cigarettes‡ (all tetrahydrocannabinol extracted), and reference-tobacco cigarettes was introduced into our in vitro bioassay system in variable amounts. In addition, 0.10 mg of purified tetrahydrocannabinol† in a volume of 0.02 ml, the amount of tetrahydrocannabinol present in an 8-ml puff of fresh marihuana smoke, was added by itself to the bioassay system to test the isolated effect of the active psychomimetic ingredient of marihuana on macrophage function in the absence of other fresh smoke components.

To evaluate the effects of these products on macrophage bactericidal activity, volumes of fresh marihuana or tobacco smoke in graded amounts of 2.0, 4.0, 6.0, or 8.0 ml were

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†Marihuana, marihuana-placebo units, and purified tetrahydrocannabinol were obtained from the National Institute of Drug Abuse, Bethesda, Md.
‡KR-1 reference-tobacco cigarettes, University of Kentucky, Lexington.

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added to the tissue culture flasks after the zero-hour samples were taken; and the flasks were immediately stoppered and allowed to incubate for one, two or three hours at 37°C before resampling for quantitative bacterial cultures. Flasks with bacteria, fresh serum, and strain-specific antibody, but without macrophages or smoke, were prepared as control samples. Additional "controls" were prepared by incubating flasks with the bacteria, fresh serum, strain-specific antibody, and various smoke products, but no macrophages.

In some experiments, phagocytic activity was evaluated by washing the cell monolayer in the culture flasks with balanced salt solution three hours after incubation. The phagocytes and bacteria were stained, and monolayers were prepared for light microscopic evaluation. Phagocytic indices were determined in all groups. A 2.5-percent solution of crystalline trypsin§ was added at the end of incubation to separate the cells from the surface of the flask, and the resulting suspension was cultured quantitatively. The viability of alveolar macrophages was ascertained by exclusion of trypan blue.4

To determine if the potentially toxic components of tobacco or marihuana smoke were labile, 35 ml of fresh smoke were held in the syringe for 15 minutes before transfer to the tissue culture flask. In additional experiments, whole smoke was partitioned to form a gas phase by passage through an absolute all-glass filter disk and to form a water-filtered phase by further passage through a fritted dispersion filter in 8 ml of distilled and deionized water to remove all water-soluble components. The effect of these partitioned smoke components on macrophage bactericidal activity was quantitatively determined by individually adding each component to the bioassay system.

All values for bacterial inactivation are presented as the arithmetic mean (± 1 SE) of bacterial colony-forming units remaining at an indicated time or following a specific dose of smoking product, expressed relative to the zero-hour or inherent control value for each individual experiment.5 In each case the effect of smoke or a smoke component on macrophage function was corrected for the effect of that smoke or smoke component on in vitro bacterial growth rates in duplicate paired experiments. Comparisons were made according to Student's t-test for unpaired data.5

RESULTS

Greater than 95 percent of the cells recovered were mononuclear, and 90 to 95 percent were viable by the exclusion of the supravalvital stain. Addition of tobacco or marihuana smoke or of tobacco or marihuana smoke partitioned components did not alter macrophage viability. There were no differences in the number of recoverable viable bacteria obtained by resuspending the adherent phagocytes following the addition of trypsin to the tissue culture flasks. Previous studies in our laboratory using lysostaphin to inactivate extracellular staphylococci have demonstrated that phagocytosis of Staphylococcus albus by pulmonary alveolar macrophages in this system is equivalent to intracellular killing.4 Therefore, all data presented were derived from the culturable colony-forming units on pour-plate dilutions of sampled specimens and reflect both events.

§Nutritional Biochemical Corporation, Cleveland.

![Figure 1. Effect of fresh marihuana smoke on bacterial growth rates in vitro in absence of alveolar macrophages. Number of culturable bacteria remaining after three hours of incubation is expressed as percent of initial colony-forming units (CFU) at time zero. This independent effect of each smoking product was corrected for when bioassay system was used to evaluate response of alveolar macrophages to each agent.](image-url)

The effect of marihuana smoke on bacterial growth rates in vitro in the absence of phagocytes is shown in Figure 1. Over the three-hour period of evaluation, bacterial replication in the absence of any smoke product occurred such that there were 131.0 ± 6.5 percent of the control or zero-hour organisms. Addition of 8 ml of fresh marihuana smoke depressed bacterial growth to 88.6 ± 7.6 percent. Therefore, to correct for this factor when expressing the effects of either tobacco or marihuana smoke on macrophage killing of staphylococci in vitro, each inactivation value was expressed in reference to incubation of bacteria and smoke alone.

Comparative dose-response effects of marihuana and tobacco smoke on macrophage bactericidal function are shown in Figure 2. Following the addition of 2, 4, 6, or 8 ml of fresh marihuana smoke, there was a progressive and significant depression of macrophage bactericidal activity, with inactivation rates of 66.7 ± 7.1 percent, 23.7 ± 7.0 percent, 20.5 ± 7.0 percent, and 11.4 ± 7.6 percent, respectively. Comparative values for the same amounts of fresh tobacco smoke were 50.4 ± 9.4 percent, 37.4 ± 13.0 percent, 22.6 ± 9.1 percent, and 17.7 ± 0.7 percent, respectively.

The effect of differential filtration of the marihuana smoke on macrophage bactericidal function is
shown in Figure 3. The filtered gas phase from 8 ml of marihuana smoke impaired function, with an inactivation rate of 24.8 ± 4.1 percent, not statistically different from an equivalent amount of unfiltered smoke. Passage of the gas phase through distilled water removed the cytotoxin (72.6 ± 1.4 percent inactivation, with 78.8 ± 1.4 percent in controls). Similar results were obtained by the partition of tobacco smoke (17.7 ± 0.7 percent inactivation for whole smoke, 21.9 ± 3.4 percent for filtered gas phase, 76.9 ± 15.1 percent following water filtration, and 78.6 ± 4.1 percent for controls). Stale smoke also lost most of the active cytotoxin (64.5 ± 13.6 percent inactivation for marihuana and 59.5 ± 2.5 per-

The effects of marihuana, marihuana placebo, and pure tetrahydrocannabinol on macrophage function are shown in Figure 4. There were no differences between macrophage impairment by the placebo (inactivation rate, 14.0 ± 0.3 percent), filtered gas phase of the placebo (inactivation rate, 20.7 ± 4.8 percent), and whole marihuana smoke (inactivation rate, 11.4 ± 7.6 percent). Addition of an amount of tetrahydrocannabinol equivalent to that contained in 8 ml of marihuana smoke had no effect whatsoever on macrophage bactericidal activity (an inactivation rate of 69.3 ± 5.4 percent compared to 73.5 ± 4.6 percent in controls).

**Discussion**

The implication that marihuana is harmless has enjoyed considerable acceptability without concrete support. Our data demonstrate that fresh whole marihuana smoke is cytotoxic to the alveolar macrophage, the key defense cell of the lung. This cytotoxicity is dose-dependent and depresses macrophage bactericidal capacity by impairing phagocytosis of the bacterial challenge. Analyses of in vitro bacterial growth rates further demonstrate that marihuana is also cytotoxic to staphylococci. Our results indicate that marihuana did not differ in its cytotoxicity from tobacco cigarette smoke, confirming the results of Green and Carolyn. The dose-response effect appears to reach a threshold maximum at 4 to 6 ml of fresh marihuana or tobacco smoke, a dose beyond which cell function declines rapidly to a minimum bactericidal activity. Additional work from our laboratory has demonstrated that this cytotoxin in fresh smoke needs to be in contact with the alveolar macrophage bioassay system for only a mat-
tter of seconds to exert its effect and that it induces maximum cytotoxicity within minutes of initial exposure. Our results further indicate that the effect of smoke from tetrahydrocannabinol-extracted placebo did not differ significantly from whole marijuana or tobacco smoke.

There are 2,000 or more components in fresh tobacco smoke. Although similar analytic data do not exist for marijuana, it would not be illogical to assume that the number of individual components generated by its pyrolysis is also extensive. The macrophage cytotoxin in marijuana is present in the gas phase of the smoke, as removal of all particulate matter with an absolute filter does not reduce the phagocytosis-imparing factor. The Leuchtenbergers and their associates have also emphasized the cytotoxicity of the gas phase of marijuana smoke, reporting abnormalities in DNA synthesis, mitosis, and growth of human fibroblastic cells derived from lung explants following in vitro exposure.

Further partitioning of the gas phase revealed that the cytotoxin is water-soluble and can be removed by one passage through a water filter. The cytotoxin is also “unstable” in that it is present only in fresh, but not in stale, marijuana or tobacco smoke. Although Green and Carolyn have implied that the dosage of smoke needed to induce macrophage impairment in this bioassay system is relatively small, it may be that absorption of a water-soluble gas-phase component by the incubation mixture under the conditions of this system may selectively and inappropriately increase the potential cytotoxin to unrealistically high concentration within the tissue culture flask. Experimental models and limited studies in man have suggested that such water-soluble components of fresh smoke are potentially totally absorbed high in the upper respiratory tract and, thus, may not reach the alveoli, where the vast majority of macrophages function, in any biologically significant concentration. Isolation of the gas-phase cytotoxin and determinations of absolute concentrations in this bioassay system and relative concentrations in the airways of man are far beyond the scope of this contribution and will be the subject of further studies.

It has been suggested that tetrahydrocannabinol, the primary psychomimetic component of marijuana, may have a potentially therapeutic role exerted through its bronchodilatory effects, and limited studies in asthmatic patients have been promising. Our studies indicate that the macrophage cytotoxin is present as one of the products of marijuana pyrolysis and is not related to the psychomimetic activity of the material. Fresh smoke from marijuana-placebo cigarettes, from which all tetrahydrocannabinol had been extracted, had the same relative cytotoxicity in this bioassay system as whole marijuana smoke or whole tobacco smoke. Furthermore, the addition of purified tetrahydrocannabinol to the tissue culture flasks did not by itself alter macrophage function.

Exposure to the maximum cytotoxic concentration of fresh tobacco or marijuana smoke over the period of incubation did not affect macrophage viability. Thus, it appears that the marijuana cytotoxin impairs cellular function without killing the cell, and that this impairment appears to involve primarily a depression of phagocytosis rather than true intracellular killing. The alveolar macrophage derives part of its cellular energy for phagocytosis from glycolysis. There is a concurrent burst of hexosemonophosphate shunt activity occurring with phagocytosis, presumably generating the energy needed for intracellular killing. Cytochrome-linked respiration and oxidative phosphorylation in alveolar macrophages do not appear to be altered by gas-phase components or aqueous extracts of gas-phase tobacco smoke. Glycolysis and glyceraldehyde-3-phosphate dehydrogenase activity appear, on the other hand, to be directly impaired by gas-phase components of tobacco smoke, and Powell and Green have demonstrated a close parallel between the dose-dependent depression of phagocytosis by alveolar macrophages and the depression of glyceraldehyde-3-phosphate dehydrogenase activity with tobacco smoke. Iodoacetate, a sulfhydryl reagent, is a metabolic inhibitor of alveolar macrophage metabolism that directly impairs phagocytosis. In addition, cysteine and glutathione, sulfhydryl protectors, inhibit the cytotoxicity of tobacco smoke for alveolar macrophages. Although similar studies with marijuana smoke have not yet been undertaken, we would propose, as a pathophysiologic mechanism of cell injury, that the close similarities in the effects of marijuana smoke cytotoxicity to tobacco smoke cytotoxicity implicate a highly water-soluble component of the gas phase of both products modifying the sulfhydryl groups of glyceraldehyde-3-phosphate dehydrogenase activity, with a resultant impairment in phagocytosis.

One cannot extrapolate totally these in vitro studies on murine alveolar macrophages to the lungs of man or to the pathogenesis of any specific lung disease in man. One should emphasize, however, the role of the pulmonary alveolar macrophage in the normal physiology of the lung. This cell is the first line of the pulmonary defense network for all but the smallest fraction of the internal surface of the lung and has as its major function the uptake, transport, and elimination of all potentially pathogenic
agents that are inhaled. As the key cell for the phagocytosis and detoxification of inhaled poisons, the alveolar macrophage plays a central role in the pathogenesis of a wide variety of pulmonary disorders. It is the cornerstone of defense against both environmental and endogenous pathogenic influences. Our studies indicate that alveolar macrophage bactericidal function is directly impaired by marihuana smoke. The relevance of this observation to the development of disease in man may be very important and deserves further clarification.

In a report to the US Surgeon General, cigarette smoke was implicated as "...the most important of the causes of chronic bronchitis."

A similar data base on which these statistical associations for tobacco and chronic bronchitis were made does not exist for marihuana. As long as possession of marihuana remains illegal, reasonable epidemiologic data on its health effects in man may not be obtainable. In a limited survey of US Army soldiers in Europe, however, 25 percent of marihuana users complained of sore throats, and 6 percent had bronchitis. In addition, 24 of 30 bronchial biopsies of heavy marihuana smokers were abnormal, with pathologic evidence for bronchitis. Beyond that, one is limited by the literature primarily to smaller population samples or to case reports of acute bronchitis in individuals consuming very large amounts of marihuana smoke. An association of both tobacco cigarette smoke and an enhanced susceptibility to bacterial infection with chronic bronchitis implies that cigarette tobacco smoke may directly impair the antibacterial defenses in the lungs of man. The work of Green and coauthors5,15,16 may, indeed, support this hypothesis, but until quantitative measurements of potential alveolar macrophage cytotoxins in vitro bioassay systems, as well as in the airways of man, are determined, the results of tobacco smoke on cells harvested from experimental animals cannot be directly extrapolated to a causative role to the pathogenesis of human disease. The same reservation must hold true for our observations of the effects of marihuana smoke reported here. Their specific relevance deserves further study.

Marihuana may cause chromosomal damage in the chronic user, disrupt cellular metabolism that includes the synthesis of DNA, interfere with delayed-hypersensitivity immune reactions, mimic hormones or act on hormone regulators to alter hormone action, cause a wide variety of personality changes, and induce potentially irreversible brain damage.20 Our studies add to this list and indicate that a water-soluble component of the gas phase of fresh marihuana smoke is directly cytotoxic to the key host defense cell of the lung, the alveolar macrophage, and depresses in a dose-dependent manner its vital function of bacterial phagocytosis.

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REFERENCES