Effect of Carcinogenic Components of Cigarette Smoke on \textit{in Vivo} Production of Murine Interferon

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\textbf{ABSTRACT}

Mice were treated with several different potentially carcinogenic components of tobacco smoke. The chemicals used were: 4-aminobiphenyl and aniline-HCl, which are found in high concentrations in sidestream tobacco smoke; hydrazine sulfate, which is found in high concentrations in mainstream tobacco smoke; and 2-methylquinoline, which is found in intermediate concentrations in both sidestream and mainstream smoke. The chemicals were injected i.p. into mice, and then $\alpha/\beta$ interferon was induced in the mice by i.v. injection of polyriboinosinic-polyribocytidylic acid. The interferon was induced either 2, 24, or 48 hr after treatment with the tobacco smoke components. Mice treated with 4-aminobiphenyl showed some depression of interferon production 2 hr after treatment, maximum inhibition of interferon induction 24 hr after treatment, and a return to control levels of interferon 48 hr after treatment. Mice treated with hydrazine sulfate showed maximum inhibition of interferon induction 24 hr after treatment but no effects at any other treatment time. These components were the most carcinogenic chemicals of those utilized in this study. Treatment of mice with aniline-HCl, a chemical whose carcinogenic potential is still debated, resulted in marginal depression of interferon induction 24 hr after treatment. 2-Methylquinoline, the chemical with the lowest carcinogenic potential in this study, had no effect on interferon induction after administration to mice. \textit{In vivo} interferon induction was, therefore, inhibited by treatment of mice with chemical carcinogens found in tobacco smoke. The efficacy of the chemical in inhibiting interferon induction was not influenced by the mainstream or sidestream smoke predominance of the chemical.

\textbf{INTRODUCTION}

Several studies have indicated that the \textit{in vitro} production of interferon could be inhibited by the pretreatment of cell cultures with carcinogens (1, 4–6, 8, 10, 16, 17). Non- or poorly carcinogenic analogues of the chemicals had little or no effect on interferon induction. These studies have recently been extended to carcinogens that are found in tobacco smoke (15). These studies indicated that the inhibition of interferon induction was related to the carcinogenic potential of the component and not to the predominance of the chemical in mainstream (direct) or sidestream (passive) smoke (15).

These findings are of interest, as considerable debate is currently in progress as to the potential danger of exposure to sidestream smoke. Experimental and epidemiological evidence has been accrued suggesting that there is potential high risk of development of cancer after exposure to sidestream smoke (2, 12, 18). The validity of some of those studies has recently been challenged (7). Therefore, it was of interest to study and to compare the effects of carcinogenic chemicals that are found in large amounts in either mainstream or sidestream smoke for their effects on a biological system that may be involved in defense against cancer, \textit{i.e.}, the interferon system (9, 13).

The results of the present study suggest that carcinogens found in tobacco smoke can inhibit \textit{in vivo} $\alpha/\beta$ interferon induction. Treatment of mice with 4-aminobiphenyl, a carcinogen found in high concentrations in sidestream smoke, or with hydrazine sulfate, a carcinogen found in high concentrations in mainstream smoke (14, 18), resulted in severe inhibition of interferon induction by poly(l-C).\textsuperscript{3} Treatment of mice with aniline-HCl, a possible carcinogen found in high proportions in sidestream tobacco smoke (14, 18), had marginal effects on \textit{in vivo} $\alpha/\beta$ interferon production. Treatment of mice with 2-methylquinoline, the chemical with the least carcinogenic potential of all those used in this study, had no effects on interferon induction. 2-Methylquinoline is found in intermediate amounts in both mainstream and sidestream smoke (14, 18). These data suggest that the effects of the tested chemicals on interferon induction are related to the carcinogenic activity of the chemical and are not related to the mainstream or sidestream smoke predominance of the chemical.

\textbf{MATERIALS AND METHODS}

\textbf{Mice.} Female 18- to 25-g Swiss/Webster mice were obtained from Laboratory Supply, Inc., Indianapolis, Ind. The mice were housed according to American Association for Accreditation of Laboratory Animal Care guidelines in an accredited animal care facility.

\textbf{Chemicals.} 4-Aminobiphenyl, aniline-HCl, hydrazine sulfate, and 2-methylquinoline were all obtained from Sigma Chemical Co., St. Louis, Mo. DMSO was also obtained from Sigma.

\textbf{Interferon Production.} $\alpha/\beta$ interferon was induced in mice by i.v. injection of $10 \mu$g of annealed poly(l-C) into mice. The poly(l-C) was formed by annealing polyriboinosinic acid and polyribocytidylic acid (P-L Biocemicals, Milwaukee, Wis.) by heating at 45° for 1 hr (3). The animals were bled 6 hr after injection of poly(l-C), the peak time of interferon induction. Interferon produced in this fashion has been characterized as the $\alpha/\beta$ type because of pH 2 stability, 56° lability, and sensitivity to an antibody directed against $\alpha/\beta$ interferon (20).

\textbf{Interferon Assay.} The antiviral titer of the test serum believed to contain interferon activity was determined by means of a plaque reduction assay (11). The Indian strain of vesicular stomatitis virus was used as the test virus. The interferon titer corresponded to the reciprocal of the greatest dilution of test serum that reduced virus plaques by 50%. One interferon antiviral unit in this assay was equivalent to 0.88 NIH G-002-904-511 reference units.

\textsuperscript{3} The abbreviations used are: poly(l-C), polyriboinosinic-polyribocytidylic acid; DMSO, dimethylsulfoxide.
RESULTS

4-Aminobiphenyl, aniline-HCl, and 2-methylquinoline were solubilized in DMSO. Hydrazine sulfate was water soluble and dissolved in tissue culture medium. All of the chemicals were diluted in tissue culture medium (Grand Island Biological Co. minimal essential medium). Several different dilutions of chemical were i.p. injected into different mice. The lowest dilution of each chemical used was equivalent to 100 μM dosages used for tissue culture studies (15). Control mice for studies with 4-aminobiphenyl, aniline-HCl, and 2-methylquinoline were given i.p. injections of equivalent volumes of DMSO only. Control mice for the study with hydrazine sulfate were given injections of equivalent volumes of tissue culture medium. The mice were i.v. challenged with poly(l-C) to induce α/β interferon at one of 3 time intervals after application of the carcinogens, i.e., 2, 24, or 48 hr. Six hr after poly(l-C) challenge, the mice were bled, and then interferon titrations were performed on the sera.

Treatment of mice with 4.2 mg of 4-aminobiphenyl resulted in a significant depression of interferon production when interferon was induced 2 hr after treatment (Chart 1). Interferon titers (units/ml) were reduced from control values of 651 ± 227 (S.E.) to treated values of 73 ± 43 (p < 0.05) (Chart 1). Since the interferon assay was a titration, a statistically significant decrease of 50% or greater of interferon titers from controls was required for treatment with a chemical to be considered effective (1). Treatment of mice with 4.2 mg or 850 μg of 4-aminobiphenyl resulted (Chart 1) in significant inhibition of interferon induction 24 hr after treatment. Titers were reduced from 2671 ± 567 to 487 ± 136 and 701 ± 188 (p < 0.05), respectively. No significant effect of 4-aminobiphenyl treatment on interferon induction was observed 48 hr after treatment (Chart 1).

No effect of hydrazine sulfate treatment was observed when interferon was induced in mice 2 hr after treatment (Chart 2). When interferon was induced 24 hr after hydrazine treatment, significant inhibition of interferon induction was observed with 3.25 mg and 650 μg of hydrazine sulfate (Chart 2). Interferon titers were reduced from control value of 2054 ± 209 to 528 ± 82 and 699 ± 78 (p < 0.05), respectively. No effect of hydrazine sulfate treatment was observed on interferon induction 48 hr after treatment (Chart 2).

Treatment of mice with aniline-HCl resulted only in marginal inhibition of interferon induction when 3.25 mg of aniline was injected into the mice and interferon was induced 24 hr later (Chart 3). The interferon titer was reduced from a control value of 399 ± 110 to 199 ± 12 (p < 0.05), a decrease of approximately 50%. No effect of 2-methylquinoline treatment was observed on interferon induction in mice (Chart 4).

DISCUSSION

Several studies have suggested a possible increased risk for the development of cancer in animals and individuals exposed to sidestream (passive) smoke (2, 12, 18). Recent debate has raised some question of the reliability of those studies (7). Therefore, it was of interest to determine the effects of carcinogenic components of sidestream and mainstream tobacco smoke on the interferon system, a possible defense against cancer. Previous in vitro studies had suggested that carcinogenic
components of mainstream and sidestream tobacco smoke inhibited the induction of α/β interferon in tissue cultures to an equivalent amount (15).

The results of the present study indicate that one i.p. dosage of various carcinogenic components of tobacco smoke can also inhibit interferon induction in mice. One dosage of the carcinogen 4-aminebiphenyl, a constituent of tobacco smoke found in relatively high amounts in sidestream tobacco smoke (sidestream to mainstream smoke ratio of 31) (14, 18) resulted in statistically significant large decreases in α/β interferon production by mice treated 2 and 24 hr prior to interferon induction. One i.p. dosage of hydrazine sulfate, a constituent of tobacco smoke found in relatively high amounts in mainstream smoke (sidestream to mainstream smoke ratio of 3) (14, 18) resulted in a significant large decrease in interferon induction in mice 24 hr after treatment.

Treatment of mice with aniline-HCl, a component of tobacco smoke found in large quantities in sidestream smoke (sidestream to mainstream smoke ratio of 30) (18) and a questionable carcinogen (14) resulted in marginal decreases in interferon induction 24 hr after treatment. Injection of mice with 2-methylquinoline, the least carcinogenic chemical of all those tested in this study and an intermediate level component of both sidestream and mainstream smoke (sidestream:mainstream smoke ratio of 11) (14, 18), had no effect on interferon induction in mice.

These results suggest that treatment of mice with carcinogenic components of tobacco smoke can result in inhibition of interferon induction in those mice. The degree of inhibition appears to relate to the carcinogenic potential of the chemical and not to the sidestream or mainstream smoke predominance of the chemical. The inhibition of interferon induction appears to begin as early as 2 hr after carcinogen treatment and to reach its peak 24 hr posttreatment. By 48 hr after carcinogen treatment, the mice regain their interferon-producing capacity, perhaps due to metabolism of the carcinogen.

Studies on the in vivo effects of carcinogens on interferon induction have been limited, although extensive in vitro studies have been carried out to indicate that carcinogens can inhibit interferon induction without being cytotoxic (1, 5, 8, 10, 16, 17). Mice given injections of methylcholanthrene (19) or urethane (6) also showed marked inhibition of interferon induction. The urethane-treated mice showed similar kinetics of the effects, with recovery of the mice 48 hr after carcinogen exposure (6). A recent study by Osborne and Archer4 has shown that mice given benzo(a)pyrene or ethyl methanesulfonate in their drinking water also exhibited suppressed interferon production. These results support the findings of the present study that carcinogens can inhibit interferon induction in vivo. In fact, the in vivo studies may be more selective for potent carcinogens than some previous in vitro studies which indicated that both aniline-HCl and 2-methylquinoline could inhibit in vitro interferon induction (15).

The present studies do indicate that components of tobacco smoke can inhibit in vivo interferon induction with just one exposure of mice to potent carcinogens; however, the present studies do not completely answer the question as to whether exposure to cigarette smoke itself can affect interferon induction. Additional studies utilizing chronic exposure of mice to very low dosages of carcinogens, which would more closely parallel exposure to the chemicals in tobacco smoke, or studies utilizing exposure of mice to actual sidestream or mainstream tobacco smoke are required to further explore the question. Such studies are currently in progress in our laboratory.

REFERENCES


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