INTERFERON: PROPERTIES AND CLINICAL USES
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INHIBITION OF INTERFERON INDUCTION AS A SCREEN FOR THE CARCINOGENIC POTENTIAL OF CHEMICALS*

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ABSTRACT

The induction of murine interferon by Newcastle disease virus has been shown to be inhibited by pre-treatment of fibroblasts with various carcinogens. The present study has extended the range of carcinogens to include chloroacetaldehyde. Chloroethanol and chloroacetic acid, rarely carcinogenic analogs of chloroacetaldehyde, had no significant effect on interferon induction by Newcastle disease virus. When polyriboinosinic-polyribocytidylic acid was used as an interferon inducer, induction of interferon was also inhibited by pretreatment of the cells with chloroacetaldehyde. Addition of reduced glutathione, which can trap active metabolites of carcinogens, abrogated the effect of a carcinogen on interferon induction, suggesting that fibroblasts can activate carcinogens in the inhibition of interferon induction system. Following further verification of the ability of the inhibition of interferon induction system to discriminate among chemicals on the basis of carcinogenic potential, this test could become part of a comprehensive battery of tests for chemical carcinogenicity.

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INTRODUCTION

Many different tests have been developed over the past several years to determine the carcinogenic potential of chemicals. Several of the early tests, such as the Ames Salmonella test (1), were able to determine the mutagenic potential of chemicals, but could not consistently discriminate between mutagens and carcinogens. Several recently developed assays, which involve determination of the activation of "SOS" repair in bacteria, have apparently been able to differentiate between carcinogens and mutagens (2,3). However, these tests suffer from the drawback that they are carried out in prokaryotic cell systems which may not be analagous to the systems operative following exposure of a mammalian cell to a chemical.

Mammalian tests to determine the carcinogenic potential of chemicals have been developed, but also suffer from several drawbacks. Transformation of cell cultures or induction of tumors in animal models require extended periods of time for the carcinogenic potential of a chemical to be expressed. In addition, if a chemical is carcinogenic only at a very low rate, massive quantities of animals would be required to observe the carcinogenic event (4).

DeMaeyer and DeMaeyer-Guignard have shown in early studies that pre-treatment of rat fibroblasts with several carcinogens including benzo-(α)-pyrene and 3-methylcholanthrene resulted in reduced interferon production when the cultures were challenged with vaccinia virus (5,6). Benzo-(ε)-pyrene, a rarely carcinogenic mutagen and an analog of benzo-(α)-pyrene, had no effect on interferon induction. We have recently extended these studies to a murine system and have shown that several other suspected carcinogens, including Aflatoxin-B_1, 2-aminofluorene, styrene oxide, and methyl methanesulfonate, all inhibited interferon induction by Newcastle disease virus (NDV) in mouse embryo fibroblasts (7). Ethyl methanesulfonate, a rarely carcinogenic analog of methyl methanesulfonate, also had no effect on interferon induction.

The present study represents an extension of the examination of the effects of carcinogens on the induction of interferon. Pre-treatment of mouse embryo fibroblasts with the postulated carcinogen chloroacetaldehyde resulted in

inhibition of interferon induction when the cultures were challanged with NDV. Treatment of the cultures with chloroacetic acid and chloroethanol, rarely carcinogenic analogs of chloracetaldehyde, did not significantly affect interferon induction. These data suggest that the inhibition of interferon induction system may be useful as an assay for the carcinogenic potential of chemicals, following more extensive studies and confirmation of results. In addition, chloroacetaldehyde treatment inhibited interferon induction by polyriboinosinic-polyribocytidylic acid (poly I:C), suggesting that carcinogen treatment does not disrupt the interaction of virus with the cell membrane resulting in inhibited interferon production. Finally, application of reduced glutathione to the cell cultures in conjunction with the carcinogen benzo-(α)-pyrene resulted in abrogation of the effects of the carcinogen on interferon induction. These data suggest that a system exists in fibroblasts whith can activate carcinogens in the interferon inhibition system.

MATERIALS AND METHODS

Mouse Embryo Fibroblast Cultures: C57Bl/6 derived mice were bred and maintained in our laboratory. Fifteen to eighteen day old embryos were surgically removed from pregnant mice, minced, trypsinized in 0.25% trypsin 1-300 (ICN Pharaceuticals, Cleveland, OH) and then suspended in minimum essential medium (Grand Island Biological, Grand Island, NY) supplemented with 10% fetal calf serum, penicillin and streptomycin and glutamine. Second or third passage culutres were used in all experiments and were plated in 25cm^2 tissue culture flasks (Falcon Plastics, Oxnard CA).

Chemicals: Chloroacetaldehyde, chloroethanol and chloroacetic acid were generous gifts of Dr. John Wong, Department of Chemistry, University of Louisville. Polyriboinosinic and polyribocytidylic acids were obtained from P-L Biochemicals, Milwaukee, WI. Benzo-(α)-pyrene was obtained from Aldrich Chemical Company, Milwaukee, WI. Reduced Glutathione was obtained from Calbiochem, La Jolla, CA.

Interferon Induction: Mouse Type I interferon was produced in fibroblasts with the Herts strain of Newcastle disease virus, as described elsewhere (8). After inactivation of residual inducing virus by pH 2 treatment at 4°C for four days, the tissue culture supernatants were assayed for antiviral (interferon) activity. Polyriboinosinic and polyribo-

cytidylic acid were complexed to poly I:C by heating at 45°C for one hour. Type I interferon was induced with poly I:C by adding 50 µg of poly I:C to tissue cultures for 90 min., and adding additional fresh medium to the mouse embryo fibroblast cultures. DEAE-Dextran was included with the poly I:C to insure maximum of induction of interferon (9). After twenty-four hours of incubation, the culture supernatants were harvested and assayed for antiviral activity.

Interferon Assay: Interferon titers were determined by plaque reduction on mouse L-929 cells using the Indiana strain of bovine vesicular stomatitis virus (10). The interferon titer corresponded to the reciprocal of the highest dilution of test sample that reduced virus plaques by 50%. One interferon unit in this assay equals 0.88 NIH-G-002-904-511 reference units.

RESULTS

Chloroacetaldehyde, chloroethanol, and chloroacetic acid were solublized in dimethyl sulfoxide (DMSO) and diluted to appropriate concentrations in tissue culture medium. The chemicals were then added to different confluent monolayers of mouse embryo fibroblasts. Following twenty-four hours of incubation at 37°C, the culture supernatants were removed and interferon was induced with NDV. The results shown in Table 1 indicate that only treatment with chloroacetaldehyde resulted in decreased interferon production of 50% or greater.

TABLE 1

EFFECT OF PRETREATMENT OF CELL CULTURES WITH CHLORO-ACETALDEHYDE AND ITS ANALOGS ON INTERFERON INDUCTION BY NDV

Treatment*	Interferon Titer	% Decrease
NDV Only	300	—
DMSO + NDV	500	—
Chloroacetaldehyde + NDV	69	77%
Chloroacetic acid + NDV	225	25%
Chloroethanol + NDV	209	30%

*All chemicals were applied at a concentration of 0.005 µM

Since the interferon assay has an innate two-fold variability due to the biological nature of the assay (11), only differences of 50% or greater were considered to demonstrate an effect of a carcinogen on interferon induction.

When chloroacetaldehyde, chloracetic acid and chloroethanol were applied to confluent monolayers and interferon was induced with poly I:C, inhibition of interferon induction of 50% or greater was again only observed in chloroacetaldehyde treated cultures (Table 2). Chloroacetic acid and chloroethanol had much less of an effect on the induction of interferon.

TABLE 2

EFFECT OF PRETREATMENT OF CELL CULTURES WITH CHLOROACETALDEHYDE AND ITS ANALOGS ON INDUCTION OF INTERFERON BY POLY I:C

Treatment*	Interferon Titer	% Decrease
Poly I:C only	430	—
DMSO + poly I:C	385	10%
Chloroacetaldehyde + poly I:C	187	57%
Chloroacetic acid + poly I:C	268	37%
Chloroethanol + poly I:C	304	29%

*All chemicals were applied at a concentration of 0.005 µm.

Benzo-(α)-pyrene was solublized in DMSO and applied to confluent monolayers of mouse embryo fibroblasts at a concentration of 0.5 µm. As has been previously reported for induction of interferon by NDV (7), this treatment resulted in a significant drop in the titer of interferon induced by poly I:C (Table 3). Addition of reduced glutathione with the benzo-(α)-pyrene resulted in abrogation, at least in part, of the inhibitory effects of benzo-(α)-pyrene on interferon induction by poly I:C (Table 3). Glutathione itself had minimal if any effect on the induction of interferon (Table 3).

TABLE 3

EFFECT OF CONCOMITANT ADDITION OF GLUTATHIONE AND BENZO-(α)-PYRENE ON INTERFERON INDUCTION BY POLY I:C

Treatment	Interferon Titer	% Decrease
Poly I:C only	266	—
Benzo-(α)-pyrene + poly I:C	76	71%
Benzo-(α)-pyrene + 0.1 μm glutathione + poly I:C	181	31%
Benzo-(α)-pyrene + 0.01 μm glutathione + poly I:C	150	43%
0.1 μm glutathione + poly I:C	347	—
0.01 μm glutathione + poly I:C	196	26%

Addition of glutathione to cultures of mouse embryo fibroblasts did not result in the induction of detectable levels of interferon.

DISCUSSION

The induction of Type I interferon has been shown to be inhibited by several carcinogenic chemicals (5-7). Of particular interest is the observation that when several pairs of highly carcinogenic chemicals and their rarely or non-carcinogenic analogs, e.g. benxo-(α)-pyrene and benzo-(ε)-pyrene, ethyl methanesulfonate and methyl methanesulfonate were tested, only application of the proven carcinogen resulted in the inhibition of interferon induction (5-7). We have now extended this observation to include the presumed carcinogen chloroacetaldehyde, and its rarely carcinogenic analogs chloro acetic acid and chloroethanol. After many additional pairs of carcinogens and analogs are tested in the future, the inhibition of interferon induction by chemicals may prove useful in the screening of chemicals for carcinogenic potential.

Dimethyl sulfoxide has previously been shown to have a non-statistically significant minimal effect on the induction of interferon by NDV (7). In the present study, no effect of DMSO on interferon induction was observed.

The mechanism of the inhibition of interferon induction was also examined in the present study. Previous work has shown that pre-treatment of rat fibroblasts with carcinogens did not inhibit the plaquing efficiency of vaccinia virus (5). Since poly I:C induction of interferon is also inhibited by pre-treatment of fibroblasts with carcinogens, it is not likely that the carcinogen treatment is affecting the binding of viruses to the cell membrane or the budding of viruses.

Many carcinogens must be activated by microsomal oxidases in order to form final active products before an effect in bacterial or mammalian systems can be observed (12). Benzo-(α)-pyrene is included among these chemicals. Reduced glutathione can trap these active products and prevent the occurence of a carcinogenic event (12). Application of reduced glutathione to the inhibition of interferon induction system resulted in at least partial abrogation of the effects of benzo-(α)-pyrene on interferon induction. These data suggest that fibroblasts must contain the type of activation system for carcinogens that renders them potent for the inhibition of interferon induction.

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DISCUSSION

UNKNOWN: Do you think that this effect is restricted only to these groups of carcinogens and works also with aflatoxins?

SONNENFELD: It does work with aflatoxin. We have shown it with aflatoxin previously as well and there has just been a report published using aflatoxins and the B1 aflatoxin.

UNKNOWN: And what about tumor promotors?

SONNENFELD: There have been reports using coal dust and also asbestos fibers as well, that there was reduced induction of interferon. The alfatoxin story is very interesting because these investigators at Western Virginia University used four different types of aflatoxin. One type being more potent carcinogen than the other and they found that the degree of inhibition of induction was related to the potency of the type of aflatoxin that they used.

DEGRE: Do you have any idea whether other cell types could be used for the same tests?

SONNENFELD: We have used fibroblast. DeMeyer has used fibroblasts. There has been one study in vitro, I cannot remember the chemical right off hand, but if mice were treated with this chemical they could not produce in vivo serum interferon. I do not know about other things. Of course, we would like to get into studies with Type II interferon as well in lymphoid cells but to this point this type of work has only been done using fibroblast.

GROB: You might have mentioned it but could you please repeat what the pretreatment schedule was before interferon induction.

SONNENFELD: What we would do was treat those cells with the carcinogens for twenty-four hours and then wash and make sure that there was no toxicity of the carcinogen.

UNKNOWN: Jay, just to support you, the emulsifiers that we used severely reduced the ability of cells to produce interferon. If you use them in very small concentrations in leukocyte culture, they cause chromosome breaks to a substantial degree higher than the normal controls.

SONNENFELD: Which might suggest some carcinogenic potential of the material.

UNKNOWN: Yes, I couldn't agree more on the need of shifting from a prokaryotic system to an eukaryotic system and I think that your approach is particularly exciting and promising, especially considering that you can use a human cell.

SONNENFELD: Yes, we have begun studies along those lines.