INHIBITION OF DNA REPAIR BY COCARCINOGENS

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Summary:

DNA repair replication in normal human lymphocytes has been inhibited by every cocarcinogen tested to date, including anthralin, 12-O-tetradecanolyphorbol-13-acetate, and the neutral fraction from cigarette smoke. Such inhibition of DNA repair is proposed as the general mechanism of action of cocarcinogens.

Introduction

Cocarcinogens have the ability to enhance the tumorigenic action of various carcinogens (1). These substances are thought to act in a two stage process with the proximate carcinogen acting as the initiator of the tumorigenic response and the cocarcinogen acting as a promoter of that response. It is already known that most, if not all, carcinogens have the ability to interact covalently with DNA (2) and also that such chemical alteration of DNA is a substrate for the excision repair enzymes (3,4). Thus, the repair process serves to protect the cellular DNA genome against potentially mutagenic or carcinogenic damage.

In light of the facts cited above, it seemed reasonable to suggest that cocarcinogens may act by inhibiting the DNA repair process. In order to test this hypothesis, a number of cocarcinogens having a wide diversity of chemical structures were tested by us and found to be potent inhibitors of DNA repair replication in normal human lymphocytes (5). The cocarcinogens tested in our earlier experiments included a methanol extract of croton oil, Tween 80, Span 80, Arlacel A, vitamin A alcohol, diethylstilbestrol, various steroids, and acetylaminofluorene derivatives. Our earlier work was per-

formed with crude extracts of croton oil and did not indicate which compounds in this mixture were responsible for the DNA repair inhibition. We have extended our earlier observations to include an active ingredient of croton oil - 12-O-tetradecanoylphorbol-13-acetate. Another substance, anthralin (1,8-dihydroxyanthrone) has been reported to be a very strong cocarcinogen second only to the phorbol esters (6). Also, the neutral fraction of cigarette smoke has been shown to have not only carcinogenic but also co-carcinogenic activity (7). The experiments reported below were carried out to test the consistancy of our original hypothesis, and to show that the cocarcinogenic activities present in croton oil, the neutral fraction of cigarette smoke, as well as that of anthralin are all due to inhibition of DNA repair replication.

Methods

The assay procedures are based on the experiments carried out by Evans and Norman (8) to demonstrate the presence of a DNA repair capability in normal human lymphocytes. These procedures involve measurement of the UV stimulated uptake of tritiated thymidine by lymphocytes in the presence of hydroxyurea. Addition of hydroxyurea to the incubation mixture permits measurement of tritiated thymidine uptake due to DNA repair replication without the higher background which would otherwise result from semiconservative synthesis of DNA. After UV irradiation, the lymphocyte preparation was incubated for two hours at 37°C in Spinner modified Eagle's minimal essential medium containing hydroxyurea and tritiated thymidine. The cells were then washed and the thymidine uptake determined by scintillation counting. The uptake into the UV irradiated lymphocytes was corrected for a small amount of uptake into unirradiated controls. The activities in samples containing DNA repair inhibitors are expressed as a percentage of the activity in the UV irradiated lymphocytes without added inhibitors. Details of the experimental methods have already been published (5) and will not be repeated in detail here.

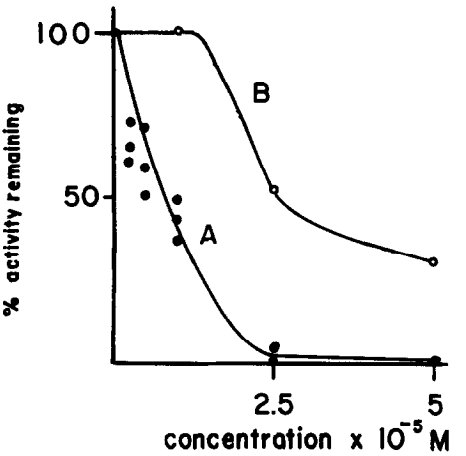

Figure 1. The effect of anthralin (curve A) and of 12-O-tetradecanoylphorbol-13-acetate (curve B) on DNA repair replication in normal human lymphocytes. The activity in the UV-irradiated samples containing repair inhibiting drugs is expressed as a percentage of the activity in UV-irradiated samples without the drugs. A small amount of incorporation into unirradiated controls was subtracted from all of the UV-irradiated samples before calculating the percentages.

Since the materials used as inhibitors of repair are not readily soluble in water, stock solutions of the compounds were prepared in dimethylsulfoxide (DMSO) just before use and then diluted into the assay mixture. The highest DMSO concentration reached in the assay mixture was 1%. Control experiments run with DMSO alone showed that this concentration of DMSO does not cause any detectable inhibition of DNA repair replication.

Cigarette smoke from 120 cigarettes was collected in a dry ice trap. The neutral fraction from this whole smoke condensate was then prepared according to the procedure of Day (9). A weighed amount of the neutral fraction was extracted with DMSO. The DMSO extract was then added to the lymphocyte assay mixture to test for its DNA repair inhibiting capability.

Results and Discussion

From figure 1, it can be seen that both the phorbol ester and the anthralin

are potent inhibitors of the UV stimulated uptake of tritiated thymidine into normal human lymphocytes. Thus, these cocarcinogens appear to act by overcoming the normal protective mechanism against agents which can cause a chemical alteration to cellular DNA. Similarly, DMSO extracts of the neutral fraction from cigarette smoke can also inhibit DNA repair replication. The DNA repair was reduced to 50% of the control value by dilution of the neutral fraction to less than 0.01%. Since the neutral fraction from cigarette smoke is a complex mixture, the concentration and chemical structure of the repair inhibitor are not known. Also, there may be more than one repair inhibiting compound present in the mixture. Therefore, identification of the inhibitory compound or compounds and the concentrations at which they are effective must remain the subject of future experiments. It is hoped that these results will help to lead to a better understanding of the harmful effects of cigarette smoke.

Since the phorbol esters stimulate DNA synthesis (10,11,12) while the anthralin is cytostatic (13), DNA repair inhibition is probably a more important factor in the mechanism of cocarcinogenesis than whether or not DNA synthesis is stimulated in the affected cells. However, stimulation of DNA synthesis by phorbol esters suggest that these compounds play a dual role in cocarcinogenesis, first by direct inhibition of the repair process and secondly by decreasing the length of time available to the cells for carrying out repair before they attempt to replicate the DNA which had been chemically altered. The importance of the time factor is also demonstrated by the fact that cells which have been stimulated to undergo cell division as a result of wound healing (12) show an increased susceptibility to carcinogens. Actinomycin D, which has the ability to prevent this DNA synthesis by inhibiting the RNA and subsequently the protein synthesis necessary for cell division, decreases the cocarcinogenic effects of croton oil (14,15). Similarly, the survival of irradiated cells in the plateau phase of growth where there is a greater time available for repair is increased over those cells in log phase growth (16).

Anthralin has been shown to improve the response of psoriasis patients treated with UV light (17). Although the picture is somewhat confused by the fact that anthralin alone is beneficial in the treatment of psoriasis (18), it seems likely that its repair inhibitory properties also play an important role in improving the UV treatment.

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