# DNA REPAIR INHIBITION: A POSSIBLE MECHANISM OF ACTION OF CO-CARCINOGENS

by

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Summary:

Co-carcinogens with a wide diversity of chemical structure have the common property of inhibiting DNA repair replication in normal human lymphocytes. This evidence suggests that inhibition of DNA repair may be an important factor in the mechanism of action of co-carcinogens.

Co-carcinogens are substances which have the property of increasing the carcinogenic effect of other compounds (1). These co-carcinogens are analogous to DNA repair inhibitors in that each of these groups of compounds increases the effect of other agents which can interact covalently with DNA. In an extensive review article, Miller (2) has pointed out that all of the carcinogens which have been studied adequately to date have been found to interact covalently with cellular macromolecules. The significance of the similarities between co-carcinogens and DNA repair inhibitors is made more apparent by the observation that primary carcinogens are lost from cellular DNA at a rate which suggests that the chemically altered DNA resulting from its covalent interaction with carcinogens is a substrate for DNA repair (3, 4).

In typical experiments which characterize the two stage process of carcinogenesis, treatment of an animal with a primary carcinogen is followed by a prolonged treatment with a co-carcinogen to increase the frequency and accelerate the onset of tumor appearance (1). If the

chemical alteration of DNA resulting from its covalent interaction with a carcinogen is a substrate for DNA repair, then it seems likely that the role played by a co-carcinogen is that of a DNA repair inhibitor. The experiments reported below were carried out to test this hypothesis. As will be shown in the following data, repair replication in normal human lymphocytes was inhibited by every co-carcinogen we have tested to date as well as by some carcinogens.

Methods:

These experiments are based on the assay procedures of Evans and and Norman (5) who used the uptake of tritiated thymidine into the DNA of UV-irradiated lymphocytes to demonstrate the presence of a DNA repair capability in these cells. Inclusion of hydroxyurea in the incubation mixture permits the measurement of this repair replication without the higher background incorporation which would otherwise result from ordinary DNA synthesis. For these experiments, the methods of Evans and Norman were followed except that the cells were lysed with the counting solution of Stewart and Ingram (6) prior to filtering and washing. Incubation was carried out for two hours in Eagle's minimal essential medium (Spinner modified) (Baltimore Biological Laboratories). Uptake of tritiated thymidine in the UV-irradiated cells was corrected for a small amount of uptake in the unirradiated controls. Water insoluble inhibitors were dissolved in dimethylsulfoxide (DMSO) and added in small volume to the incubation mixtures. An equal amount of DMSO was added to control and UV-irradiated samples.

Active extracts of the croton oil were obtained by extraction with an equal volume of methanol followed by evaporation of the methanol under a stream of nitrogen. This extract was then diluted with DMSO and added to the assay mixture.

Results:

A variety of compounds which are chemically diverse, but have the

common property of being co-carcinogens in different experimental situations were examined for their ability to inhibit post-irradiation uptake of tritiated thymidine by normal human lymphocytes in the assay system described above. Table I lists those compounds which were found to be inhibitors of the DNA repair process and the concentrations required to produce 50% inhibition.

Croton oil has long been used as the classic example of a co-carcinogen. In this series of experiments, methanol extracts of the crude oil were inhibitory to the DNA repair mechanism. Since the oil is a mixture of substances, a precise identification of the phorbol esters as the active inhibitors and a quantitative estimate of their degree of inhibition must await further experiments with the purified esters. The other inhibitors used in these experiments do not present such problems.

One of the most interesting of these inhibitors, because of its common pharmaceutical use, is Tween 80. Table I shows that Tween 80 is a potent inhibitor of repair, producing 50% inhibition at a concentration of 0.002%. Since the Tween 80 is high molecular weight polymer, the stock solution was dialyzed overnight against two changes of saline and tested again for its repair inhibitory properties. The dialyzed material inhibited repair to the same extent as the undialyzed. The white cells did not appear to be damaged by the detergent properties of the Tween 80, and after the incubation period appeared morphologically intact and were not decreased appreciably in number as a result of the two hour incubation. The monomeric detergents, the Spans and Arlacel A also inhibited repair.

Another co-carcinogen of interest is vitamin A alcohol. This substance is also an inhibitor of the repair process and gives 50% inhibition at a concentration of about $5 \times 10^{-5}$ M. Because of its insolubility in aqueous solutions, stock solutions of the vitamin A alcohol were prepared in DMSO and diluted into the assay medium. Control samples containing the DMSO but no vitamin A demonstrated that

TABLE I

| Inhibitor | Concentration required to Produce 50% inhibition of Repair replication |
|---|---|
| Croton Oil | 0.005% |
| Tween 80 | 0.002% |
| Span 80 | .01% |
| Arlacel A | .01% |
| Vitamin A Alcohol | $1.7 \times 10^{-5}$ M |
| Diethylstilbestrol | $1.3 \times 10^{-5}$ M |
| Estradiol | $5 \times 10^{-5}$ M |
| Progesterone | $8 \times 10^{-6}$ |
| Testosterone | $2 \times 10^{-5}$ |
| 7-hydroxyacetylamino fluorene | $7 \times 10^{-6}$ |
| Azobenzene | $4 \times 10^{-5}$ |

the DMSO had little or no effect on repair replication.

Numerous steroids have also been found to inhibit the repair process at much higher than physiological concentrations. Studies with these steroids will form the basis for a separate paper, but some examples are listed in Table I.

Discussion:

From our earlier experiments (7,8), it would appear that inhibitors of the DNA repair process may find some use in the field of tumor therapy when used in conjunction with either alkylating agents or X-rays. However, inhibition of DNA repair is not without possible harmful side effects as shown by these and other experiments with co-carcinogens.

Vitamin A alcohol, for example, has the property of enhancing the anti-leukemic effect of bis-chloroethylnitrosourea (9) and of acting as a co-carcinogen (10). When these two pieces of evidence are combined with the results reported here, it seems likely that the antitumor effect and the co-carcinogenic effect may be explained by the same phenomenon, namely the inhibition of DNA repair. Here we have two analogous situations where the effect of an agent which results in chemical alteration of cellular DNA is enhanced by the presence of a DNA repair inhibitor. Similarly, diethylstilbestrol, which increases the tumorigenic effect of X-rays (11) has been shown by us to be an inhibitor of DNA repair replication. Other co-carcinogenic steroids have also been found to inhibit repair.

Tween 80 is another example of a co-carcinogen which is a DNA repair inhibitor. It would appear that the high molecular weight fraction is a repair inhibitor as well as the monomeric Spans and Arlacel A. The latter compounds are emulsifiers of the type used in Freund's incomplete adjuvant which has been found to potentiate the carcinogenic effect of benzpyrene (12). These compounds are of particular interest because of their widespread human use as emulsifiers of water insoluble pharmaceuticals.

Some carcinogens which have become bound to cellular DNA are lost from that DNA at a rate indicating that this chemical alteration of DNA is repairable (3, 4). When coupled with our observation that all of the co-carcinogens which we have studied to date are repair inhibitors, it would appear that the process of co-carcinogenesis may, at least in large part, be explained on the basis of DNA repair inhibition.

There are other interesting analogies between the action of co-carcinogens and the effect of DNA repair inhibitors on the survival of cells which have been treated with agents such as X-rays, ultraviolet light, and alkylating agents, all of which exert their cytotoxic effect as a result of the chemical alteration which they cause to cellular DNA. Varying the time available for repair by increasing or decreasing the generation time of the cells has a

pronounced effect on cell survival. In the case of experiments which demonstrate the two stage character of carcinogenesis with croton oil, we see a similar situation. This co-carcinogen not only inhibits the repair process as reported here, but by stimulating the early onset of DNA synthesis (13) also decreases the effective time available for repair and can result in the fixing of premutational damage in the DNA genome as a result of the replication. The time available for repair may be regarded as the period between the occurence of the damaging event and the time when the cell tries to replicate the chemically altered DNA. Actinomycin D, which can prevent the shortening of the time available for repair by preventing the RNA and protein synthesis necessary for cell replication, has been shown to have an antitumor effect in experiments demonstrating two stage carcinogenesis using croton oil (14).

Inhibition of repair replication by acetylaminofluorene derivatives reported here is also interesting since persistance of acetylaminofluorene for prolonged periods has been observed in neoplastic nodules of liver but not in adjacent normal liver cells (15).

The evidence presented here implicates inhibition of the DNA repair process in co-carcinogenesis, and provides a rational chemical basis for further studies of the enzymic mechanisms involved.

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