PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, 146 401-405 (1974)

DNA Repair Inhibition: A New Mechanism of Action of Steroids with Possible Implications for Tumor Therapy[1] (38114)

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The observation that a number of different steroids can inhibit DNA repair replication in normal human lymphocytes represents a new mechanism of action of these compounds which is potentially exploitable in the field of tumor therapy. Earlier work from this laboratory indicates that this same mechanism can also explain the mechanism of cocarcinogenesis by some steroids as well as by numerous other cocarcinogens (1, 2).

In recent years, our studies of the biological importance of DNA repair and of inhibitors of this process have indicated that it is a subject which should receive increasing attention because of its potential importance in the field of tumor therapy involving X-rays and alkylating agents (3, 4). Other workers have shown that mammalian cells have the ability to carry out excesion repair of their DNA (5–7). This process involves the excision of a chemically altered segment from one strand of the DNA double helix followed by resynthesis of this segment using the complementary strand as a template. The repair process is then completed by a ligase which closes the ends of the chain, thereby converting a chemically altered DNA back to a biologically active form which we presume to be identical to that existing prior to the damage. This process is of importance to the field of tumor therapy because the kinds of damage caused by alkylating agents and X-rays are among those which can be repaired by the excision repair process (5, 8). Thus, it is reasonable to conclude that DNA repair plays an important role in the resistance shown by tumor cells toward the alkylating agents and X-rays. Additional support for this concept has been provided by earlier work from this laboratory in which it was demonstrated that inhibitors of the repair process can sensitize experimental animal tumors toward these agents (3, 4, 9). Tumors used in these experiments included both cyclophosphamide-resistant and cyclophosphamide-sensitive plasmacytomas and melanomas implanted in hamsters. The repair-inhibiting drugs used in our earlier experiments included such agents as chloroquine and quinacrine which probably exerted their effects as a result of their ability to bind to DNA. Our work with DNA repair-inhibiting drugs has now been extended to a number of naturally occurring and synthetic steroids. Many of these steroids, as shown below, have been demonstrated to be inhibitors of DNA repair. Also, as indicated in the discussion, these drugs may be of use in tumor therapy when used in conjunction with alkylating agents or X-rays.

Methods. These methods are modifications of those originally used to demonstrate the presence of a DNA repair capability in normal human lymphocytes (10), and involve measurement of the increased uptake of tritiated thymidine into the DNA of lymphocytes after uv irradiation. Hydroxyurea is included in the reaction mixtures to inhibit semiconservative DNA synthesis but not repair, thereby permitting measurement of thymidine incorporation due to repair without a high background of incorporation from semiconservative synthesis (11). After a 2-hr incubation in the presence of hydroxyurea and tritiated thymidine, the cells were lysed with the counting solution of Stewart and Ingram (12). The nuclei which remained were col-

[1] This work supported by NCI Grants CA 13148, CA 12763, and CA 12538.

lected on 0.8 μm "Nuclepore" filters, then washed with phosphate-buffered saline, 5% trichloroacetic acid containing 1% sodium pyrophosphate, and 95% ethanol. The air-dried filters were then transferred to scintillation vials for counting. Cell counts were also taken at the ends of the incubation periods to ensure that none of the inhibition observed arose from cell lysis. The steroids used were dissolved in dimethylsulfoxide and then diluted into the assay mixtures. Controls were performed to determine that the highest concentration of DMSO (1%) did not inhibit the repair process. Details of these experiments have already been published and will not be repeated here (9). A small amount of incorporation into unirradiated controls was subtracted from each of the uv-irradiated samples, and the incorporation into the samples containing added steroid hormones calculated as a percentage of that in the uv-irradiated samples without added steroid. These results are presented below as graphs of the percentage of inhibition versus the steroid concentration.

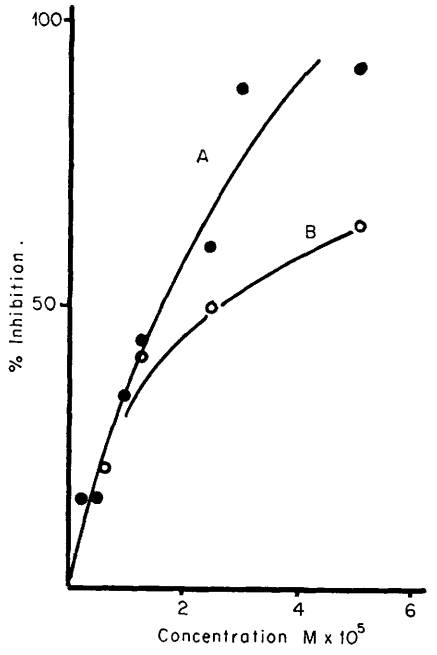

FIG. 1. The percent inhibition of DNA repair replication in normal human lymphocytes is plotted as a function of the concentrations of diethystilbestrol (curve A) and estradiol (curve B).

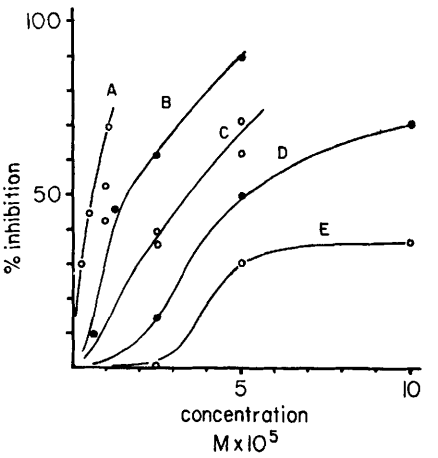

FIG. 2. The effect of progestrone and several of its derivatives on DNA repair replication. Curve A, progesterone; B, 2-α-methylprogesterone; C, medroxyprogesterone acetate (17α-hydroxy-6α-methylprogesterone acetate; D, 11-dehydroprogesterone; E, 11-ketoprogesterone.

Results. Diethylstilbestrol (DES), proved to be one of the best DNA repair inhibitors of the hormone analogs which we have examined, with 50% inhibition being produced between 1 and 2 $\times$ 10^{-5} M. Because of the estrogenic activity of DES, estradiol was also tested and found to be inhibitory (Fig. 1). These results as well as the ones reported below were all obtained using blood from normal healthy volunteers. Other steroids proved to differ widely in their ability to inhibit the repair process. This is true even in cases in which there are only small subtle differences in the structure of the steroid molecules. Examples of such effects can be seen in the case of progesterone and its derivatives. Of these, progesterone proved to be the most effective inhibitor, producing 50% inhibition of the repair process at about 5 $\times$ 10^{-6} M, (Fig. 2). Small changes in the progesterone structure result in marked decreases of inhibitory capability. For example, the 11-keto and the 11-dehydro derivatives are much less effective as inhibitors. Medroxyprogesterone was also less effective as an inhibitor of repair than progesterone, although it is not known whether it must be activated by hydrolysis of the acetyl groups for its inhibitory activity to be expressed. An additional point of interest lies in the fact that S-shaped curves were obtained

with some of the progesterone derivatives. This phenomenon is not restricted to the steroids, but has also been observed in the case of DNA repair inhibition by the neutral fraction from whole cigarette smoke condensate and by 12-*O*-tetradecanoylphorbol-13-acetate (2).

In contrast to the estrogen and progesterone derivatives, testosterone and its derivatives were generally much poorer inhibitors of repair. The best of these inhibitors was dihydrotestosterone which resulted in 50% inhibition of repair replication at about 4×10^{-5} *M*. Testosterone was slightly less inhibitory in our assay system. Other androgen derivatives were essentially noninhibitory. These compounds included Δ1,4-androstadien-3, 17-dione, androstenedione, androsten-6-bromo-3, 17-dione, and "Testolactone" (17α-Oxa-D-homo-1,4-androstadien-3,17-dione).

In addition to naturally occurring estrogens, a number of 17-α-ethynyl derivatives were also tested. These compounds were effective inhibitors, as were the diethylstilbestrol and estradiol mentioned previously. The compounds tested included 17α-ethynylestradiol (Fig. 3), norethindrone (19-nor-17α-ethynylandrosten-17β-ol-3-one), and norethynodrel (17α-ethynyl-17β-hydroxy-5(10)-estren-3-one) (Fig. 4).

Because these 17α-ethynyl compounds are commonly found in birth control pills, blood samples were drawn from women during the third week of the period during which they were taking the pills. No detectable decrease of repair capability was found in the white blood cells from these individuals. These experiments differed in that the white cells were resuspended in their own serum for incubation rather than in the Spinner modified minimal essential medium used in the other experiments. Thus, the cells were incubated in the presence of the same concentration of steroid as was present *in situ*.

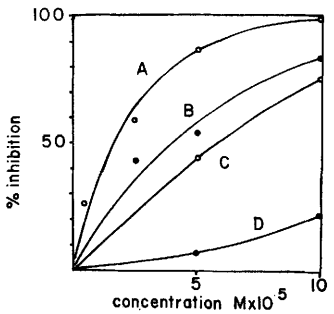

FIG. 3. A comparison of the repair inhibitory capability of 17α-ethynylestradiol (curve A) with that of dihydrotestosterone (curve B), testosterone (curve C), and "Teslac" (17α-Oxa-D-homo-1,4-androstadien-3,17-dione), (curve D).

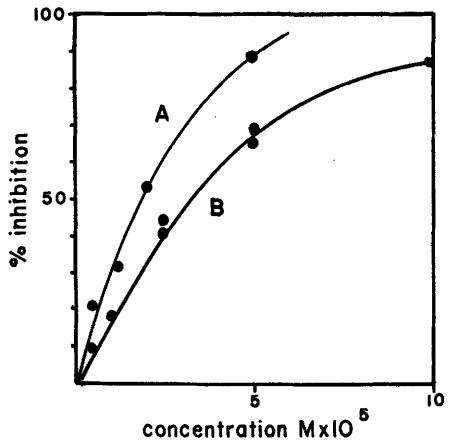

FIG. 4. The percentage of inhibition of DNA repair by norethindrone (19-nor-17α-ethynylandrosten-17β-ol-3-one) (curve A) and norethinodrel (17α-ethynyl-17β-hydroxy-5(10)-estren-3-one (curve B).

A number of other steroids of potential therapeutic interest were also tested. These included pairs of compounds such as cortisone and hydrocortisone, and prednisone and prednisolone. In each case the 11-keto compound proved to be a more effective inhibitor than the corresponding 11-hydroxy derivative.

In order to test the possibility that the observed inhibition may have resulted from nonspecific detergent effects of the steroids at the high concentrations used, numerous bile salts were also examined and found to be noninhibitory. These compounds included deoxycholic acid, taurodeoxycholic acid, cholesteryl sulfate, cholic acid, sodium taurocholate, and sodium glycocholate.

Discussion. Among the wide diversity of effects attributed to steroids, the ability to inhibit DNA repair replication is one which has not been previously recognized. Diethylstilbestrol, the first steroid-like compound to be studied by us, was examined because of its cocarcinogenic properties. Earlier work from this laboratory has demonstrated that the ability to inhibit DNA repair is a property

common to every cocarcinogen tested (1, 2). This repair inhibition is probably the general mechanism of action of such compounds.

As a result of the experiments with DES, other steroids were subsequently examined and also found to be DNA repair inhibitors. Quite obviously the concentrations used in these experiments are much higher than the normal hormone concentrations. However, it must also be recognized that the lymphocytes used in these experiments are not the natural target tissue for the steroids used. Thus, it remains to be determined whether there is any target tissue selectivity of these steroids with respect to DNA repair inhibition. Some steroid-induced selective sensitization of tissues toward carcinogens may already have been observed (13, 14). Reports in the literature of therapeutic enhancement of radiation therapy by high concentrations of steroids open up some very exciting new areas of investigation. For example, progesterone has been found to sensitize endometrial carcinoma to radium implants (15). Similarly, ethynylestradiol has been observed to enhance the effects of X-rays on breast tumors (16) and DES has been used to enhance the effect of cyclophosphamide on breast tumors (17). The observation that steroids will inhibit the process of DNA repair replication may give us a rational chemical basis for the explanation of these effects. Although other types of hormone action can not be excluded on the basis of our experiments, the existing evidence indicates that serious consideration should be given to the role of DNA repair inhibition when repair-inhibiting drugs are administered in conjunction with either X-rays or alkylating agents. This ability to increase the cell-killing effect of these agents combined with the possibility that this might be accomplished with a relative degree of selectivity suggests that this is an area worthy of further investigation.

It should also be recognized that potential harmful side effects might result when a DNA repair-inhibiting drug is administered along with either alkylating agents or X-rays. However, if further work demonstrates that sensitization can be accomplished with a degree of selectivity, then the increased cell killing of one type of tissue might be accompanied by a relative sparing action on other sensitive tissues such as the hematopoietic system. Experiments are being conducted to determine if this can be accomplished with neoplasms of breast, endometrium, prostate, and with lymphocytic leukemia.

The observation that the bile salts did not inhibit repair combined with the finding that small subtle changes in the steroids have marked effects on their repair-inhibitory capability suggests that the observed inhibition did not result simply from a nonspecific detergent action.

The S-shaped inhibition curves seen with some of the steroids are generally interpreted in terms of cooperative or allosteric effects. However, the repair system involves more than one enzyme and diffusion of the steroid into the cells must also be taken into account. Furthermore, we do not as yet know the particular enzyme in the sequence which is inhibited nor whether the action is a direct one of the steroid or the result of a complex between the steroid and a cytoplasmic protein. Thus, many questions about these effects remain to be answered.

Summary. A number of steroids have been demonstrated to be inhibitors of DNA repair replication in normal human lymphocytes. This inhibitory capability is a previously unrecognized mechanism of action of these compounds. Because the alkylating agents and X-rays used in tumor therapy cause chemical alterations to cellular DNA of a type which can be repaired by the excision repair process, it is suggested that repair inhibitory steroids may be useful in conjunction with treatment involving alkylating agents or X-rays.

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Received Jan. 12, 1974. P.S.E.B.M., 1974, Vol. 146.