B19

THE INHIBITION OF DNA REPAIR PROCESSES

Kendric C. Smith

Department of Radiology, Stanford University School of Medicine,
Stanford, California 94305, U.S.A.

There are four main characteristics of an optimum radiation sensitizer that works by inhibiting repair. First, its inhibitory action or the cellular consequences of temporary inhibition should be irreversible. Second, it should be non-toxic to unirradiated cells. Third, it should be toxic to irradiated cells when added after irradiation. (As a control for the specificity of action of the drug, it should be non-toxic to irradiated cells genetically deficient in the repair system inhibited by the drug.) Fourth, to be most useful therapeutically, the inhibitor should be preferentially taken up by tumor tissue.

Several compounds are known to sensitize bacterial cells to killing by radiation when added after irradiation, and many of these have been shown to inhibit specific repair processes irreversibly. There are fewer data of this type available for mammalian cells, however.

Before discussing the inhibition of repair, I will briefly describe the different repair systems known to operate in bacterial and mammalian cells. Our laboratory has demonstrated the existance of two major pathways for the repair of X-ray-induced DNA single-strand breaks and postulated the existance of a third, ultra-fast pathway (1). I will restrict my description here to the pathways for which there are solid genetic and molecular data. In bacterial cells the repair of X-ray-induced DNA single-strand breaks can occur by two major pathways (Fig. 1A); one can proceed when the cells are suspended in buffer but the second can only occur when the cells are in complete growth medium. The growth-medium-independent repair system is very fast with a half time of about 1 minute at 37°C. It repairs about 95% of the X-ray-induced DNA single strand breaks whether produced in the presence or absence of oxygen. This repair system is mostly dependent upon DNA polymerase I, coded for by the polA gene, but in the absence of this enzyme DNA polymerase III, coded for by the polC gene, can substitute but at a much slower rate and repair is not as complete. We have had little success in finding inhibitors of this repair system that aren't extremely toxic to cells (1).

The growth-medium-dependent repair system is slow and requires 40-60 minutes at 37°C for completion. This repair system depends upon the recA, recB, and recC genes (3, 4, 6), genes originally described as controlling the process of genetic recombination (7). You will recall that in genetic recombination, the DNA injected by a male bacterium into a female bacterium is cut into pieces and combined with the DNA of the recipient female bacterium such that a new chromosome is formed carrying the genetic characteristics of both parents. This growth-medium-dependent repair process also depends upon the exrA gene (2, 5), whose function at the molecular level is not known and on the polC gene (6). While this repair process appears to be important to the survival of irradiated cells, as witnessed by the radiation sensitivity of cells that are either genetically deficient in this repair system, or whose repair system has been inhibited by drugs, it actually repairs a very small number of chain breaks (1, 6). It is this slow, growth-medium-dependent system for the repair of X-ray-induced DNA single-strand breaks that is so easily inhibited in bacteria by drugs of diverse pharmacological action (Table 1).

At present it is not easy to assay for the repair of base damage produced by X-irradiation (14). It is easy to assay for the repair of base damage after UV irradiation, however, and we can draw analogies from the mechanisms known for the repair of UV-induced base damage. In one type of repair of UV-induced base damage, called excision repair, the damaged bases are cut out of the DNA and the resultant gaps are filled with undamaged bases (15). Since the first step in the uvr gene

Reprinted from Excerpta Medica International Congress Series No. 353
Vol. 5, Surgery, radiotherapy and chemotherapy of cancer
Proceedings of the XI International Cancer Congress, Florence 1974
Excerpta Medica Amsterdam
ISBN 90 219 0287 7

TABLE 1 Inhibitors of the growth-medium-dependent repair of X-ray-induced DNA single-strand breaks in bacteria.

Drug* or treatment	Other metabolic effects
Quinacrine (8)**	Inhibits DNA synthesis Uncouples oxidative phosphorylation
Dinitrophenol (9)	Uncouples oxidative phosphorylation
Chloramphenicol (10)	Inhibits protein synthesis
Impurity in hydroxyurea (11)	(?)
Acriflavine (12)	Binds to DNA
UV radiation*** (UV-X-ray synergism) (13)	Inhibits DNA synthesis, etc.

*When added after X-irradiation and incubated for 60-90 minutes, they sensitize the killing of wild-type cells and irreversibly inhibit growth-medium-dependent repair, but they do not sensitize the killing of mutants deficient in this type of repair.
**References. ***UV-irradiated just before X-irradiation.

dependent excision repair process is the enzymatic induction of a DNA single-strand break adjacent to the damaged base, we wondered if the same repair systems found to operate in the repair of X-ray-induced DNA single-strand breaks might also repair the strand breaks induced as a consequence of the excision of damaged bases from the DNA of UV-irradiated cells. This proved to be the case (16).

There is a rapid system for the repair of excision breaks in DNA that can occur in buffer (Fig. 1B) that depends mainly upon DNA polymerase I, and in the absence of this enzyme DNA polymerase III can partially substitute. There is also a pathway of repair that requires complete growth medium and functioning rec, exrA, and polC genes. It is the growth-medium-dependent repair of UV-induced damage that is irreversibly inhibited by drugs of diverse pharmacological action such as impurities in agar (18), chloramphenicol (16), and dinitrophenol (19). Therefore, the systems under the same genetic and physiological control that repair X-ray-induced single-strand breaks also repair enzymatically-induced DNA strand breaks after UV irradiation.

An early step in excision repair, i.e., the release of dimers as acid soluble products, has been shown by Setlow and co-workers to be inhibited to various degrees by starvation for a carbon source, or by treatment with KCN, caffeine, and acriflavine (20), but was not inhibited by treatment with chloramphenicol (21) or by starvation for thymine (20). The effects of these treatments on survival were not tested, however.

A second method for the repair of DNA base damage is called post-replication repair (22, 23). In this process the initial base damage in the parental strands of DNA is not repaired, but rather, gaps are left in the newly synthesized daughter strands opposite this damage. Subsequently, the gaps in the daughter strands are repaired by molecular processes not yet well understood. Recently, however, we have shown by genetic studies that there appears to be at least four separate pathways of post-replication repair (24). A recA mutation appears to inhibit all post-replication repair, whereas, mutations in recB, uvrD, and exrA appear to control independent pathways of post-replication repair. There is evidence for a fourth pathway that requires the cooperative effort of the recB, uvrD, and exrA gene products. Chloramphenicol inhibits this cooperative pathway (6). Since the recF gene has been shown by Clark and co-workers (25) to act independently of recB in post-replication repair, the recF gene may control a fifth pathway.

It should be emphasized that whenever any one of these many pathways of DNA repair are inhibited either by mutation or by drug action, the cells are rendered much more sensitive to killing by radiation. In most cases, however, drugs show less of a sensitizing effect on cell killing than do mutations, suggesting that the drugs either are not 100% effective as inhibitors of repair or else they affect only one pathway of repair while the mutation may affect several pathways of repair (e.g., rec genes). In only one case thus far has a drug proven to be as effective a sensitizer as a mutation (9).

What is operationally defined as post-replicational repair is observed in mammalian

cells and it is inhibited by caffeine (26). UV irradiated rodent cells are sensitized by caffeine (27), but human HeLa cells are not (28). Skin cells from patients with the so-called variant type of the disease xeroderma pigmentosum appear to be partially deficient in post-replication repair (29).

Certain types of mammalian cells appear to be capable of excision repair after UV irradiation while other types appear to be deficient (Table 2). While there has been the tendency to consider that mammalian cells are all alike, Table 2 demonstrates clearly that they are not all alike in their repair capacity. The different mammalian cell lines, therefore, should be considered analogous to different mutant bacterial strains, and their properties should be studied, as bacteriologists study the properties of different bacterial mutants. The great advances in our understanding of repair mechanisms in bacteria could not have occurred if each laboratory had worked on only one cell line at a time.

TABLE 2 Photoproduct excision in various eukaryotic cell types (30).

Excision detectable	No excision detectable
Human fibroblasts	Human XP cells
Human HeLa cells	Mouse L cells
Bovine fibroblasts	Chinese hamster V79 and V7979 cells
Chick fibroblasts	Chinese hamster CH461 cells
Mouse ehrlich ascites cells	Pig PS cells
Chinese hamster CHEF125 cells	Rabbit fibroblasts

Several drugs have been tested for their inhibitory action on the excision of thymine dimers from UV-irradiated mammalian cells (30). Acriflavine, crystal violet, actinomycin D, chloroquin and iodoacetate were inhibitory, but hydroxyurea, arabinosyl cytosine, 5-aminouracil, 5-fluorodeoxyuridine, caffeine, and cycloxheximide were not inhibitory. Unfortunately neither the toxicity of these drugs at the concentrations used nor the reversibility of their inhibition was reported.

We have observed that most X-ray-induced DNA single-strand breaks are rapidly repaired by Chinese hamster cells in buffer. There is also a much slower repair process that appears to require complete growth medium (31). Thus, both bacterial and mammalian cells seem to possess growth-medium-independent and dependent pathways for the repair of X-ray-induced DNA single-strand breaks.

Table 3 lists drugs that have been shown to sensitize mammalian cells to X-rays when added post-irradiation. Here again we see differences in the response of different cell lines, e.g., hydroxyurea, hydroxylamine and puromycin did not sensitize Chinese hamster cells but did sensitize HeLa cells. This may be a true genetic difference or it may only represent a difference in technique. It would be very beneficial if a single laboratory would compare different cell lines under one set of conditions. The molecular basis for the sensitization by most of these drugs is not known. For those few that have been tested, the results are either negative, or equivocal or else the molecular biology has been done after supralethal doses of radiation.

One technique that is arousing current interest is the use of heat to sensitize cells to X-irradiation. It should be recalled that bacterial mutants that contain temperature sensitive repair enzymes can be made more sensitive to irradiation by holding them for a short time at a temperature at which the enzyme cannot function (44). Because mammalian cells can be sensitized to irradiation by heat, they may normally contain such temperature sensitive enzymes.

The length of the list of sensitizers in Table 3 is not very impressive, yet in theory repair inhibitors should be very beneficial adjuncts to the therapeutic use of radiation in the treatment of cancer. Why then has progress been so slow in turning up effective radiation sensitizers that function by inhibiting repair?

Unfortunately, most experiments on radiation sensitization have been performed with the drug present at the time of irradiation. While experiments of this design may be optimum for the screening of potential sensitizers they give no clue as to the mechanism of sensitization, i.e., whether they are acting as a potentiator of

TABLE 3 X-ray sensitizers when added post-irradiation.

Chinese hamster cells	HeLa cells
Actinomycin D (34, 35, 39)	Actinomycin D (42)
Acriflavine (35)	Lucanthone (Miracil D) (33)
Ethidium bromide (35)	5-Fluorodeoxyuridine (36, 40)
Quinacrine (32)	Cytosine arabinoside (40)
Streptozotocin (34)	Puromycin (37)
N-ethylmaleimide (43)	Puromycin aminonucleoside (41)
Temperature (41.5°C) (38)	Hydroxyurea (36, 40)
	Hydroxylamine (40)
NO SENSITIZATION: (35) hydroxyurea, hydroxylamine, puromycin, chloramphenicol, cyclohexamide, caffeine	PROTECTION: cyclohexamide (36)

radiation chemistry or as a repair inhibitor or both. Clearly the only way that a drug can be unambiguously tested as a repair inhibitor is for it to be added after irradiation.

Still other studies have been reported in which the effects of the drugs on specific repair processes were followed at the molecular level. However, most of these biochemical studies did not include companion studies on the toxicity of the drugs on unirradiated cells or their effects on the survival of irradiated cells. Furthermore, the repair processes were usually not followed after removing the drug to see if the inhibitory effects were reversible. Experiments without such controls convey little useful information since, e.g., putting irradiated cells in a refrigerator slows repair but this inhibition is freely reversible at 37°C and the treatment has no effect on survival and hence no therapeutic value. As a more dramatic example, sulfuric acid is an excellent repair inhibitor but will probably find little therapeutic use.

If a drug sensitizes cells to killing by radiation, when added after irradiation, then it probably acted on repair in an irreversible manner. However, because there are now so many different pathways of repair, each requiring a different series of enzymatic steps, it is quite possible that a drug could inhibit reversibly one pathway while inhibiting another irreversibly, with only the irreversible inhibition having an effect on the viability of the cells. Therefore, depending upon which of these two pathways of repair was being studied, a wrong conclusion could easily be reached concerning the true molecular mechanism by which the drug enhanced the lethality of the radiation if reversibility was not checked.

Therefore, molecular biological repair inhibition studies should not be performed without also testing for the toxicity of the drugs to unirradiated cells, their effects on the survival of irradiated cells, and the reversibility of the inhibition on the particular repair system being studied.

There are unique technical problems that become apparent when studying DNA repair processes in mammalian cells compared with bacteria. For one thing, mammalian cell biologists do not yet have the luxury of a large catalog of strains mutant in the different pathways of repair. The amount of DNA present in mammalian cells is about 10^3 times greater than that in bacteria, and there are about 40 chromosomes in mammalian cells compared with one for bacteria. These differences have caused considerable difficulty in the assay of DNA single-strand breaks in mammalian cells.

There are also technical problems related to testing the effects of drugs on the survival of mammalian cells. For example, if the cells are trypsinized following the drug treatment for the purposes of cloning, sensitization may not be observed, but is observed if the cells are treated with the drug after attachment to Petri dishes (31). In addition, the concentration of the drug per cell is more important than the absolute molar concentration of the drug. This problem is made all the more acute when the drug also binds to serum and other constituents in the growth medium. Their position in the cell cycle also affects the response of cells to drugs (41, 43).

Apart from these technical problems there may be an even more fundamental difference

between mammalian and bacterial cells. There is growing evidence to suggest that the repair systems so easily inhibited in bacteria by drugs of diverse pharmacological action (i.e., the rec and exr gene controlled pathways) may be induced by irradiation (45). Therefore, drugs which interfere with the formation of these induced enzymes would be expected to sensitize cells to radiation. If in mammalian cells these repair systems preexist, i.e., do not need to be induced, then general metabolic inhibitor drugs would not be expected to sensitize mammalian cells, as seems to be the case (46). I emphasize that the inducibility of repair in bacteria and the lack of same in mammalian cells is presently only a working hypothesis, but it could help explain some of the apparent differences in the response of bacterial and mammalian cells to different drugs.

The first successful molecular biological experiments on the repair of X-ray-induced damage were only performed about 8 years ago (47). Therefore, we may have been overly naive to hope that clinically useful repair inhibitor drugs could be found before we gained a better understanding of the molecular biology of repair. Exciting progress has been made over the past few years in elucidating mechanisms for the repair of DNA, but perhaps the major revelation has been that the biochemistry of repair is much more complicated than anyone had previously imagined. Even now, flow diagrams are necessary to chart the multiple pathways of repair; each pathway requiring a different subset of enzymes.

The ultimate success in finding clinically useful repair inhibitor drugs will probably have to await more precise information on the enzymology of the repair of DNA strand breaks and base damage in mammalian cells. Then agents will have to be found that inhibit those enzymes that are mainly, if not exclusively, involved in repair processes. I still remain optimistic, however, that the use of selective drugs to inhibit the repair of damaged DNA offers great promise as a beneficial adjunct to the radiation treatment of cancer.

Cells can be sensitized to radiation by three major mechanisms: 1.) The efficiency of the radiation itself in producing chemical changes in DNA can be increased by electron affinic compounds and sulfhydryl binding agents. 2.) The intrinsic radiation sensitivity of the DNA can be increased by analog substitution, e.g., the replacement of thymine with 5-bromouracil. 3.) The repair of radiation-induced damage to DNA can be inhibited by chemicals. In all probability, the optimum approach to the successful destruction of tumor cells with radiation plus sensitizers will depend upon the use of a combination of radiation potentiating agents plus repair inhibitors.

REFERENCES

(1) Town, C.D., Smith, K.C. and Kaplan, H.S. (1973): Current Topics in Radiat. Res. Quart., 8, 351.
(2) Youngs, D.A. and Smith, K.C. (1973): J. Bacteriol., 114, 121.
(3) Morimyo, M., Horii, Z. and Suzuki, K. (1968): J. Radiat. Res. (Japan), 9, 19.
(4) Kapp, D.S. and Smith, K.C. (1970): J. Bacteriol., 103, 49.
(5) Sedgwick, S.G. and Bridges, B.A. (1972): Mol. Gen. Genet., 119, 93.
(6) Youngs, D.A. and Smith, K.C. (1974): Unpublished observations.
(7) Howard-Flanders, P. and Boyce, R.P. (1966): Radiat. Res. Suppl. 6, 156.
(8) Fuks, Z. and Smith, K.C. (1971): Radiat. Res., 48, 63.
(9) Van der Schueren, E., Smith, K.C. and Kaplan, H.S. (1973): Radiat. Res., 55, 346.
(10) Ganesan, A.K. and Smith, K.C. (1972): J. Bacteriol., 111, 575.
(11) Kapp, D.S. and Smith, K.C. (1971): Int. J. Radiat. Biol., 19, 255.
(12) Kapp, D.S. (1970): Ph.D. Thesis, Stanford University.
(13) Martignoni, K.D. and Smith, K.C. (1973): Photochem. Photobiol., 18, 1.
(14) Hariharan, P.V. and Cerutti, P.A. (1972): J. Mol. Biol., 66, 65
(15) Howard-Flanders, P. (1968): Ann. Rev. Biochem., 37, 175.
(16) Youngs, D.A., Van der Schueren, E. and Smith, K.C. (1974): J. Bacteriol., 117, 717.
(17) Braun, A. and Grossman, L. (1974): Proc. Nat. Acad. Sci. U.S.A., 71, 1838.
(18) Van der Schueren, E., Youngs, D.A. and Smith, K.C. (1974): Photochem. Photobiol., 20, 9.
(19) Van der Schueren, E. and Smith, K.C. (1974): Photochem. Photobiol., 19, 95.
(20) Setlow, R.B. and Carrier, W.L. (1968): In: Replication and Recombination of Genetic Material, Australian Academy of Sciences, Canberra. pp. 134-141.

(21) Swenson, P.A. and Setlow, R.B. (1966): J. Mol. Biol., 15, 201.
(22) Rupp, W.D. and Howard-Flanders, P. (1968): J. Mol. Biol., 31, 291.
(23) Smith, K.C. (1971): In: Photophysiology, 6, 209.
(24) Youngs, D.A., Van der Schueren, E. and Smith, K.C. (1975): In: Molecular Mechanisms for the Repair of DNA (P.C. Hanawalt and R.B. Setlow, eds.), Plenum, New York (in press).
(25) Rothman, R.H., Kato, T. and Clark, A.J. (1975): In: Molecular Mechanisms for the Repair of DNA (P.C. Hanawalt and R.B. Setlow, eds.), Plenum, New York (in press).
(26) Cleaver, J.E. and Thomas, G.H. (1969): Biochem. Biophys. Res. Commun., 36, 203.
(27) Rauth, A.M. (1967): Radiat. Res., 31, 121.
(28) Wilkinson, R., Kiefer, J. and Nias, A.H.W. (1970): Mutat. Res., 10, 67.
(29) Lehman, A.R. (1975): In: Molecular Mechanisms for the Repair of DNA (P.C. Hanawalt and R.B. Setlow, eds.), Plenum, New York (in press).
(30) Cleaver, J.E. (1974): Adv. Radiat. Biol., 4, 1.
(31) Roots, R. and Smith, K.C. (1975): Int. J. Radiat. Biol.
(32) Voiculetz, N., Smith, K.C. and Kaplan, H.S. (1974): Cancer Res., 34, 1038.
(33) Bases, R. (1970): Cancer Res., 30, 2007.
(34) Roots, R. and Smith, K.C. (1974): Unpublished observations.
(35) Arlett, C.F. (1970): Int. J. Radiat. Biol., 17, 515.
(36) Phillips, R.A. and Tolmach, L.J. (1966): Radiat. Res., 29, 413.
(37) Djordjevic, B. and Kim, J.H. (1969): Radiat. Res., 37, 435.
(38) Ben-Hur, E., Elkind, M.M. and Bronk, B.V. (1974): Radiat. Res., 58, 38.
(39) Elkind, M.M., Sutton-Gilbert, H., Moses, W.B. and Kamper, C. (1967): Nature, 214, 1088.
(40) Weiss, B.G. and Tolmach, L.J. (1967): Biophys. J., 7, 779.
(41) Djordjevic, B., Kim, J.H. and Kim, S.H. (1968): Int. J. Radiat. Biol., 14, 1.
(42) Bases, R. (1959): Cancer Res., 19, 1223.
(43) Sinclair, W.K. (1973): Radiat. Res., 55, 41.
(44) Youngs, D.A. and Smith, K.C. (1973): Nature New Biol., 244, 240.
(45) Witkin, E. and George, D.L. (1973): Genetics, 73(Suppl.), 91.
(46) Terasima, T. and Yasukawa, M. (1969): In: Comparative Cellular and Species Radiosensitivity (V.P. Bond and T. Sugahara, eds.), Igaku Shoin Ltd., Tokyo pp. 79-86.
(47) McGrath, R.A. and Williams, R.W. (1966): Nature, 212, 534.

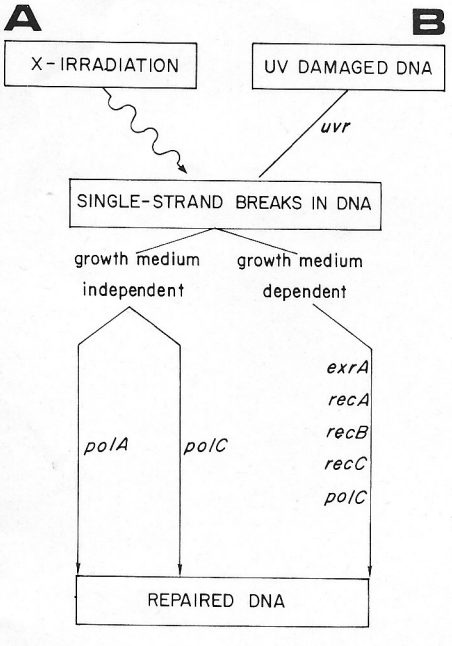

FIG. 1A. Separate pathways for the repair of X-ray induced single-strand breaks in the DNA of bacteria. The polA and polC genes code for DNA polymerase I and III, respectively. The recB and recC genes code for an exonuclease (Exo V). The recA and exrA gene products are not known at present (based upon references 1-6).

FIG. 1B. Separate branches of the uvr gene-dependent excision repair process in ultraviolet-irradiated Escherichia coli K-12 (16). The uvrA and B genes code for an endonuclease that makes the first incision in the excision repair process (17).

Presented at Conference 9 - Session 1: Molecular biology relevant to radiotherapy