DNA DAMAGE AND MAMMALIAN CELL KILLING

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DNA DAMAGE AND MAMMALIAN CELL KILLING

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ABSTRACT DNA lesions--particularly single- and double-strand breaks--are induced in considerable excess by doses required to register one lethal "hit." In the case of X-ray cell killing, the disposition of such damage has been determined in the progeny of surviving cells. Fewer than 0.2% of the single- and 6.0% of the double-strand breaks are propagated. Hence, it is likely that in surviving cells all breaks are repaired. A mechanism of X-ray cell killing based upon misrepair of DNA breaks is proposed.

INTRODUCTION

Diverse agents capable of producing DNA damage are also lethal to mammalian cells. Focusing on surviving cells, I compare the lethal and DNA damaging properties of X-rays, ultraviolet (UV) light, and the fluorescent light (FL) exposure of cells grown in a medium containing 5-bromodeoxyuridine (BUdR), BUdR/FL.

DNA Damage. To compare relative rates of break induction (1,2), dose must be normalized to an equivalent biological effect. This can be done if the unit of dose is the D_0 dose (i.e., the dose that reduces survival by $1/e$ along the exponential, final portion of a survival curve) as in Table I.

BUdR/FL Lethality. V79 Chinese hamster cells, labeled overnight with BUdR, are killed close to exponentially (10). In contrast to X-ray killing, dose fractionation gives no evidence of sublethal damage repair and treatment with anisotonic buffer does not alter single-dose survival. Nevertheless, ssb's are rapidly repaired (3). It is estimated that ~1% of the ssb's are dsb's. But even if most of the latter are not frank scissions, it is probable that $dsb/D_0 \gg 1$.

X-Ray Lethality. X-irradiated cells rapidly repair sublethal damage. They may also suffer potentially lethal damage which they can repair even more rapidly (6). As with breaks induced by BUdR/FL, X-ray-induced breaks are rapidly repaired. However, X-ray-induced breaks either differ qualitatively, or are registered in different DNA-containing structures since $1/10$ th as many are produced than by BUdR/FL.

TABLE I

DNA DAMAGE VERSUS SURVIVAL OF V79 CHINESE HAMSTER CELLS

Treat- ment	ssb ^a /D ₀	ssb repair	dsb ^a /D ₀	Survival curve	SLD ^e repair	PLD ^f repair (6)
BUdR/FL	50,000 (3) ^b	rapid	500 (5)	exponen- tial	none	none
X-rays	1,000 ^c	rapid	40 ^d	thresh- old	rapid	rapid
UV	100 (4)	slow/ none	<<ssb/D ₀	thresh- old	slow	none

^assb = single-strand break; dsb = double-strand break.

^bNumbers in parentheses indicate references.

^cBased on D₀ ≈ 100 rads, 60 eV/ssb, and 1 × 10⁻¹¹ g DNA/cell.

^dBased on ≈25 ssb/dsb (Elkind, unpublished data; published values for mammalian cells range from 12 (7) to 46 (8), and recently ≈23 was reported for E. coli (9).

^eSLD = sublethal damage; repair usually measured by dose fractionation.

^fPLD = potentially lethal damage; enhanced damage expressed by postirradiation treatment with anisotonic buffer (6).

UV Lethality. The cell killing properties of UV light are a mixture of those due to BUdR/FL and those due to X-rays. The photolesions that appear as ssb's are repaired slowly after doses corresponding to high survival levels (>10T). After low survival level doses, the number of ssb's due to UV light actually increases (11) whereas ssb's are rapidly repaired following X-irradiation (4). The ratio of ssb's per D₀ or "one-hit" dose is equal to the ratio of total DNA to unique sequences (12).

A Search for Breaks in X-Ray Survivors. Although X-ray lethality very likely involves DNA damage (13,14), it is clear in Table I that X-rays do not kill cells efficiently.

To follow the fate of ssb's in mammalian cells by sedimentation, invariably doses of radiation are used that are biologically large. The superstructure of the DNA must be sufficiently damaged (e.g., 15) to facilitate the release of DNA whose sedimentation properties are readily interpretable. Consequently, studies of the repair of breaks suffer from several shortcomings: 1) the fate of breaks in dying, as opposed to surviving, cells is observed; 2) since the superstructure may reform during the process of repair, the completeness of repair may not be assessable; and 3) depending on the circumstances (e.g., dose, quality of radiation, etc.), as part of the pathology of a dying cell, DNA breakdown may set in so that repair becomes offset by DNA degradation. Hence, measurements of the rate of completeness of DNA repair may be confounded by artifacts and in any case such data do not

indicate the fate of damage in surviving cells.

To circumvent these problems, the following experiment was performed. V79 cells were exposed to repeated ~ 1000 rad X-ray doses. Between each fraction, cells were incubated to permit the survivors to multiply and the killed cells to lyse. After a total of 20 krads and again after 50.8 krads, surviving cells and their offspring were examined and compared to controls to determine: 1) if ssb's were inherited; 2) if the break-induction curve had changed; 3) if the rate of repair after a large dose was different; and 4) if the survival curve was altered. Only the results after 50.8 krads are described since they do not differ from those after 20 krads.

For control cells, 2.4 krads are required to produce one ssb/ 2×10^8 daltons (number average). Using the technique of the cosedimentation of DNA from control and test cells, it was found that (16): 1) the 21 ssb's in 2×10^8 dalton DNA due to 50.8 krads were not inherited; 2) the induction curve of new breaks was not changed; 3) 50.8 krad survivors repair ssb's equally as rapidly as control cells; and 4) although the 50.8 krad survivors were a little more resistant than controls, the D_0 increase was modest, 36%. Of the 21 ssb's, 1.7 would have come from 0.85 dsb's; thus, repair of duplex scissions is also indicated. An analysis of the resolution available in the method used shows that a difference of at least 0.05 ssb/ 2×10^8 daltons can be detected. Hence, fewer than 1:420 ssb's and fewer than 1:17 dsb's remained unrepaired following an accumulated dose of 50.8 krads.

Cell Killing Hypotheses. The foregoing results do not indicate when breaks are repaired in surviving cells or their progeny. Nevertheless, it shows that radiation survivors repair the vast majority (if not all) of the breaks registered. For example, in the 1 out of 100 cells surviving a 1000 rad dose increment, $\sim 10,000$ ssb's and ~ 400 dsb's are registered. Remaining unrepaired would be fewer than 24 out of 10,000 ssb's and fewer than 24 out of 400 dsb's. Since the repair of DNA in lethally irradiated cells is rapid, it is probably rapid in surviving cells as well.

Whatever may be the molecular basis for a threshold on an X-ray survival curve, beyond the threshold a D_0 dose registers one lethal "hit". In the ensuing competition between damage repair and damage fixation, this hit could be the one ssb or the one dsb that loses the race. Thus, even if we discount the effectiveness of ssb's produced by one D_0 dose, because we assume that all such breaks will be repaired faithfully by a pre-replicative process, we must conclude, at least, that one dsb fails to be repaired at all.

A second hypothesis derives from the apparently large amount of repair that surviving cells effect. Whereas the

lack of repair of one or a few ssb's and/or dsb's is not excluded, the limit of the unrepaired damage is sufficiently low to support the likelihood that all DNA breaks are repaired in surviving cells. As already noted, lethally affected cells rapidly repair ssb's and presumably, therefore, dsb's. Consequently, following X-radiation the rejoining of strand breaks takes place in the entire population. This suggests the possibility that lethality results from a misrepair of one or more ssb's or one or more dsb's per D_0 . Since misrepair would apply only to 0.1% of the ssb's or only to 2.5% of the dsb's (i.e., 1.25% of the ssb's resulting from the dsb's would suffice), it is clear that the repair of strand breaks is on the whole a faithful process.

CONCLUSION

The data discussed for the properties of X-ray survivors leads to two main conclusions. First, the induction of X-ray resistant cells is not a likely event or if it is, such cells are selected against by the growth conditions used. And second most, if not all, of the double-strand breaks (as well as the single-strand breaks) are repaired in surviving cells. The latter conclusion is contrary to what has been generally believed. The ability of cells to effect double-strand rejoinings makes understandable processes which involve major transpositions of genetic material (e.g., sister-chromatid exchanges) or less drastic rearrangements which may result in mutation or neoplastic transformation and represent, therefore, misrepair compatible with survival.

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