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ABSTRACT

A comparison was made of the extent of UVR of gamma ray-induced lethal damage (mitigation of gamma ray-induced lethal effects by appropriate administration of low UV doses) in fish, amphibian, reptile and mammalian tissue culture cell lines. A significant level of UVR was detected in the non-mammalian lines, but the mammalian cells appeared to have lost this ability. Associated mitotic index data is interpreted as supporting the notion that low UV doses, appropriately administered, may aid repair processes in some cells (indirectly) by hindering the antagonistic metabolic processes which convert gamma ray-induced lesions to a non-reversible state.

INTRODUCTION

Ultraviolet light reactivation (UVR) has been observed in many microorganisms. When UV-inactivated phage are allowed to infect appropriate host cell bacteria and these infected systems receive appropriate UV exposures, significant increases in phage survival are observed. This UVR of phage has been observed repeatedly (Weigle, 1953; Rupert and Harm, 1966). Elkind and Sutton (1959) and Calkins and Todd (1968) have described UVR of X-ray induced lethal damage in yeast. Calkins and Griggs (1969) observed a rather marked degree of UVR of x-ray induced lethal damage in protozoa. This report deals primarily with an attempt to determine whether UVR of ionizing radiation-induced lethal damage extends to mammalian and other vertebrate cell cultures.

MATERIALS AND METHODS

The two mammalian tissue culture lines used were the H3 HeLa human cancer line and V79 hamster *(Cricetulus griseus)* line, obtained from Dr. Joel Bedford of Colorado State University. Both lines were routinely maintained in closed tissue culture flasks at 37°C in F10 medium supplemented with 10 percent fetal calf serum and buffered with HEPES. Three non-mammalian vertebrate cell lines, derived from lines furnished by Dr. James Regan of Oak Ridge National Lab were used: the GFI fish line derived from a *Haemulon scuirus* line, the A8W243 amphibian line derived from a *Xenopus laevis* line, and the THI reptile line derived from a *Terrapene carolina* line. These non-mammalian lines were routinely maintained in essentially the same manner as has been described previously for A8W243 cells (Griggs and Bender, 1972).

Monolayers of cells in vigorous log phase growth in plastic petri plates were used in all experiments. Gamma ray was administered at a dose rate of 40 rads/minute by a custom designed Mark IV Cesium 137 irradiator. This machine is designed so that the same monolayer samples prepared for UV exposure can also be conveniently exposed to gamma ray. The techniques employed in UV exposures, incubation, single cell isolations, colony assays, and other aspects of dose-survival determinations have been previously described (Griggs and Bender, 1972; Orr and Griggs, 1976).

The UVR experiments for each cell line involved the following. The gamma ray dose-survival relationship was determined to enhance selection of an appropriate gamma ray dose level for the UVR detection test (i.e., a gamma ray dose level at which any significant change in cell survival due to subsequent administration of low UV doses could be detected). Medium was removed from a series of plates containing the desired monolayers and replaced with a thin layer (0.5 mm in depth) of balanced salt solution. Immediately following administration of the same appropriate gamma ray dose to all members of the series, each member was exposed to a different dose of UV in the range 0 to 20 ergs/mm². These "doubly exposed" monolayers were then converted to single cell suspensions and the remainder of the experiment carried out essentially as described by Griggs and Bender (1972).

RESULTS AND DISCUSSION

The shapes of the gamma ray dose-survival responses in Figures 1 and 2 indicate that dose levels which result in surviving fractions between about 0.1 and 0.6 would be appropriate levels at which to test for UVR ability in all five cell lines. Thus, the 0.5 survival point was used. Consideration of the progression of radiation events (up the evolutionary scale) from fish to human cells in Figures 1 and 2 indicates a definite decrease in gamma ray resistance. The LD50 (dose that is lethal to 50 percent of the cells) ranges from about 1000 rads in the fish line to near 500 rads in the human cells. It is interesting that UVR ability seems to roughly parallel the gamma ray resistance in that a marked degree is present in fish and amphibian cells, less in reptile cells and little, if any, in mammalian cells.

The mechanism of UVR has not been adequately described. Ono and Shimazu (1966) suggest that UVR results from a form of enzyme induction. Kneser et al (1965) and Calkins and Todd (1968) have published data which they interpret as supporting the notion that UVR results from induction of an enzyme repair system by appropriate UV administration. These suggestions have led to little elucidation of UVR since techniques for exploring the nature and extent of such intracellular mechanisms have not been developed.

The experimental results depicted in Figure 3 suggest an alternative, and perhaps simpler, mechanism for UVR. Comparison of the data clearly indicate that a gamma ray dose of 600 rads induces a drop in mitotic index in the A8W243 cells that persists for about 20 hours in log phase A8W243 cells. When a low dose of UV is administered in conjunction with the gamma ray, cell progression is practically stopped (arrows pointing downward) for about 12 hours and the mitotic index is drastically decreased for more than 32 hours. However, the same combination of exposures does not induce such a pronounced decrease in mitotic index in the V79 cell line. Intracellular mechanisms capable of repairing radiation-induced, potentially lethal damage have been detected in vertebrate cells (Elkind and Whitmore, 1967; Griggs and Bender, 1972). It has also been shown that low temperatures and a number of chemical agents can, under proper circumstances, alter the rate of metabolic activities in such a manner as to enhance repair of radiation damage (Elkind and Whitmore, 1967). These facts, coupled with the data from Figure 3, suggest that appropriate administration of low UV doses to some vertebrate cells, following ionizing radiation exposures, alters metabolic activities in such a manner as to significantly delay cells in their pro-

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gression through the cell cycle. This delay allows repair mechanisms more time to operate, resulting in the increased level of survival which has been called UVR.

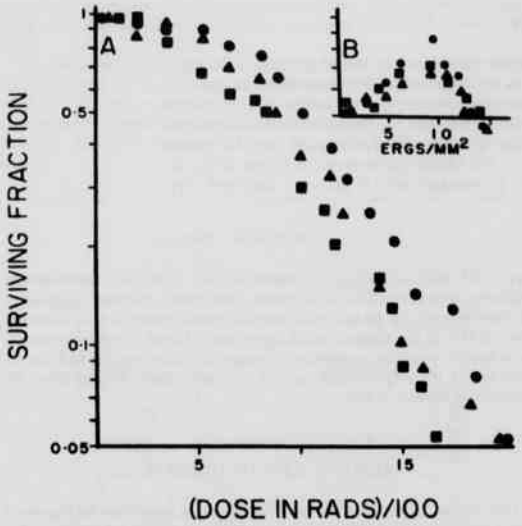

Figure 1. (A) Gamma ray survival curves; (B) Gamma ray plus UV survival curves (UVR); filled circles = GFI cells, filled triangles = A8W243 cells, filled squares = THI cells.

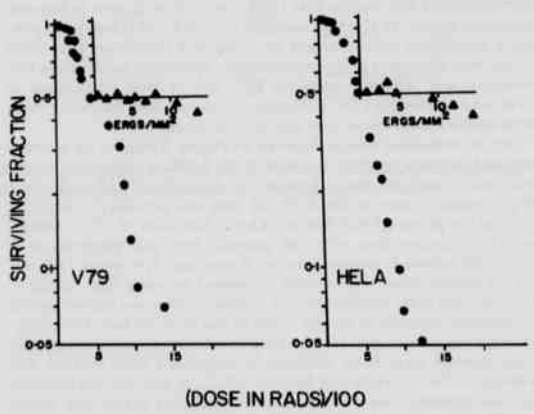

Figure 2. Gamma ray survival curves (filled circles) and gamma ray plus UV survival curves (UVR) (filled triangles) for hamster (V79) and human (HeLa) cell lines.

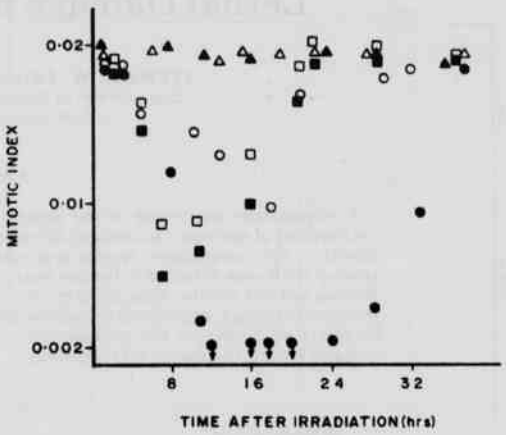

Figure 3. Radiation induced mitotic delay in log phase A8W243 (filled symbols) and V79 (open symbols) cells. Triangles = controls, squares = 600 rad gamma ray, circles = 600 rad gamma ray plus 15 ergs/mm² UV.

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