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UNITED STATES ATOMIC ENERGY COMMISSION

on

"RADIOTOXICITY OF IODINE-125
IN MAMMALIAN CELLS: DIVISION
DELAY IN SUSPENSION CULTURES"

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Radiotoxicity of Iodine-125 in Mammalian Cells:

Division Delay in Suspension Cultures

S. Herbert, E.W. Prince and S.J. Adelstein

1. Introduction

The radiotoxicity of iodine-125 on the clonal survival of cultured mammalian cells, when the radionuclide is incorporated into DNA as iododeoxyuridine (IUdR), has been documented in this laboratory (Prince and Adelstein, 1972). We have also studied division delay caused by intranuclear iodine-125 when $^{125}\text{IUdR}$ is added to the medium of suspension cultures. In this document, preliminary results are presented which demonstrate that small amounts of $^{125}\text{IUdR}$ (2.5 nCi/ml) in the medium produce significant division delays.

2. Materials and methods

The cell line employed was the Chinese hamster ovary (CHO) obtained from Dr. David Baltimore's laboratory at M.I.T. where it had been adapted to spinner suspension culture.

Joklik's medium (GIBCO) supplemented with 1% NEAA and 10% fetal calf serum was employed after it had been shown to give the most reliable growth curves. The medium was made from powdered stock and finally passed through a 40 μ (Millipore) filter.

Cells were grown in 250 ml flasks with magnetic chain-stirrers (Kontes) placed in an eight-station portable incubator maintained at 37⁰. The volume of cell suspensions used was 100 ml. To obtain reproducible growth curves it was found necessary to have the initial inoculum 1.5×10^5 cells/ml or greater. The cell doubling time was found to range between eight and ten hours with logarithmic growth ending at cell concentrations greater than 5.0×10^5 cells/ml.

Cell counts were determined in two ways. Aliquots (0.2 ml) of the cell suspension were added to WBC Unopettes (Fisher) and counted in a Fisher autocyto meter which was calibrated daily. The autocyto meter values were extremely reproducible. Hemacyto meter counts were used to check the autocyto meter counts and also to visualize the cells for morphological changes which might indicate adverse conditions in the media. Whichever method was used, care was taken to minimize the amount of time the incubator was opened in order to prevent division delay due to lowering the temperature.

The cells were irradiated by placing the incubator upright beneath the tube of a 250 KvP x-ray machine with a filter of 1 mm Cu and 1 mm Al. The dose rate was found to be 30R/min as determined by Fricke dosimetry.

The iodine-125 labeled iododeoxyuridine (New England Nuclear) was obtained with a specific activity of 25 Ci/mM and added directly to the suspension medium.

3. Results

Figure 1 shows the results of a typical experiment in which logarithmically growing CHO cells were irradiated with x-rays at 30 R/minute. The x-ray experiments are summarized in Table I and shown graphically in Figure 2. The division delay produced by x-rays is about 1.6 hours per 100 R exposure.

The results of exposure to varying concentrations of $^{125}\text{IUdR}$ with constant specific activity are given in Table II and typical growth curves are shown in Figure 3. A concentration of 10^{-10}M IUdR ($2.5\text{ nCi }^{125}\text{I/ml}$) has slightly less effect on division delay than 100 R of x-rays. At concentrations above $5 \times 10^{-10}\text{M}$ the division delays observed were in excess of five hours.

4. Discussion

These experiments demonstrated that short-term exposure of suspension cultures to low concentrations of $^{125}\text{IUdR}$ in the medium produce significant division delay. It is presumed that this is due to intracellular iodine-125 but appropriate control experiments will have to be performed with unlabeled IUdR and $\text{Na }^{125}\text{I}$ as an extracellular radiation source.

It is rather surprising that continuous exposure to 10^{-10} M ^{125}I UdR results in the rather rapid onset of mitotic delay that lasts only 1 to 1.5 hours, an interval considerably shorter than the S period of these cells (about 4 hours). The transient delay in the face of continued irradiation suggests that some sort of tolerance (perhaps induced repair) is developed at this low concentration.

Drew and Painter (cited by Feinendegen, 1967) have found that incubation of cultured cells with tritiated thymidine also results in mitotic delay. Incubation of HeLa-S3 cells with ^3H -TdR ($2\mu\text{Ci/ml}$) for 3 hours produced 4.1 dpm per nucleus and resulted in a 1 hour delay, 6 hours of incubation resulted in a 4 hour delay and 9 hours of incubation resulted in a 7 hour delay. It is difficult to compare these results with those we have obtained with ^{125}I UdR but on a microcurie/ml basis the iodine-125 labeled nucleoside is relatively more effective than the hydrogen-3 one. This is particularly striking when one compares the x-ray sensitive sensitivity of the HeLa-S3 line, 2.4 hours per 100 rad, with the CHO line, 1.2 hours per 100 rad (Walters and Petersen, 1968).

5. Conclusions

Irradiation of CHO cells, grown in suspension, with x-irradiation produced a dose related mitotic delay of 1.6 hours per 100 R exposure. This compares favorably with the value of 1.2 hours per 100 rads obtained elsewhere (Walters and Petersen, 1968).

Incubation of CHO cells with $^{125}\text{IUdR}$ at a concentration of 2.5 nCi/ml in the medium produced a 1.3 hour delay, while concentrations of 12.5 and 125 nCi/ml produced a division delay in excess of five hours.

6. References

- E.W. Prince and S.J. Adelstein. Radiotoxicity of intracellular iodine-125 in mammalian cells: effects on the survival curve. U.S.A.E.C. Document C00-3229-1, 1972.
- L.E. Feinendegen. Tritium-labeled molecules in biology and medicine. Academic Press, New York, 1967, p. 325.
- R.A. Walters and D.F. Petersen. Radiosensitivity of mammalian cells I. Timing and dose-dependence of radiation-induced division delay. Biophysical J. 8, 1475-1486, 1968.

Table I Division Delay (hours) Following External Irradiation

exp. no.	Control	Dose 125R	Dose 300R
1	0	3 hrs.	5 hrs.
2	0	2 hrs.	4 hrs.
3	0	1.75 hrs.	---
4	0	2 hrs.	3.5 hrs.
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Average	0	2.2 hrs.	4.2 hrs.

Table II Division Delay (hours) After Continuous Exposure
to $^{125}\text{IUdR}$ (sp. act 25 Ci/m mole)
Molarity $^{125}\text{IUdR}$

exp. no.	Control	1×10^{-9}	5×10^{-10}	1×10^{-10}	1×10^{-12}
1	0	>5 hrs.	>5 hrs.	---	---
2	0	---	---	1.5 hrs.	---
3	0	---	---	1.5 hrs.	0
4	0	---	---	1 hr.	0

Figure 1. Mitotic delay in logarithmically growing CHO cells exposed to 250 KvP x-rays at a dose rate of 30R/minute.

Figure 2. Mitotic delay in CHO cells following external irradiation.

Mean ± 1 S.D.

Figure 3. Mitotic delay in logarithmically growing CHO cells exposed to iodine-125 labeled IUdR.