MASTER

ANNUAL TECHNICAL PROGRESS REPORT
on
STUDIES ON THE RADIATION AND CHEMICAL-INDUCED
MOLECULAR DAMAGES, THEIR REPAIR AND
BIOLOGICAL EXPRESSION IN MAMMALIAN CELLS*

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PROPOSED TECHNICAL SCOPE

Work in progress is designed to delineate the molecular basis for UV-induced mutagenesis, cell killing and cancer in human cells. The kinetics and characteristics of the dark repair mechanism will be studied in human cells. The biological and molecular consequences of environmental agents on the DNA repair process will be examined. Also, a search for a recombination-type of repair process in mammalian cells will be made.

In this 4th annual progress report, I will outline the types of the research activities engaged in this year, results obtained and new programs to be pursued.

This year's activities, albeit curtailed from last year's proposal due to a lack of funds, was centered around how different exogenous environment agents affect the repair processes in higher organisms and how these effects are manifested biochemically and biologically.

During this last year, I was able to continue my collaborative research efforts with several investigators (Dr. E.H.Y. Chu, Oak Ridge National Laboratory; Dr. R.B. Setlow, Oak Ridge National Laboratory; Dr. R.B. Painter, Laboratory of Radiobiology, University of California; Dr. Barnett Rosenberg, Biophysics Department, Michigan State University; and Dr. Park Gerald, Harvard University).

Because of this last years contract, I was able to spend five weeks at Oak Ridge National Laboratory (in a training and research session), working with Dr. R.B. Setlow and Dr. E.H.Y. Chu. This enabled me⁽¹⁾ to learn new techniques in tissue culture and physical biochemistry⁽²⁾, to get caught up with the literature in radiobiology, and ⁽³⁾ most importantly, to gain new insights to new and old problems in radiobiology in human cells. Because of that five weeks training session, I now have the capacity⁽¹⁾ to do quantitative mutagenesis work in mammalian tissue culture, as well as in replica-plating of mammalian cells in culture; (2) to do repair enzyme assays in vitro (endonuclease), and (3) to do alkaline sucrose gradient analysis of DNA from human cells.

I have also hired a new technician, Miss Jacquie Christian, who replaced Mrs. Miriam Isoun. Mrs. Isoun left with her husband to go to Nigeria. I had two pre-doctoral students, who although they were not technically under my mentorship, completed their Ph.D. thesis research in my laboratory. Mr. Harold Harder received his Ph.D. from the Michigan State University Biophysics Department, studying the molecular basis for platinum-inhibition of cancer. Miss Noemi Diaz received her Ph.D. from the Food Science Department studying the biochemical effects of irradiated sugars on human cells in culture.

In general, I was able to finish several projects started a couple of years ago (i.e., sunlight-induced pyrimidine dimers in human cells; lack of photoreactivation in human cells; molecular basis for psoralen-photosensitization of human cells), as well as continuing work on the characterization of repair of DNA in human cells.

I. Synergistic effects of ultraviolet light and caffeine in mammalian cells.

It has been demonstrated that caffeine can sensitize UV-irradiated mammalian cells¹⁻². Caffeine has been shown to inhibit excision of UV-induced pyrimidine dimers in bacteria³⁻⁵. However, caffeine does not inhibit excision in human cells in which it can cause sensitization in Chinese hamster cells in which do not excise pyrimidine dimers. Several mechanisms of caffeine sensitization have been proposed; one suggests that caffeine inhibits the ligase-step of dark repair⁷ and another infers that it inhibits a recombination-type of repair^{8,9}. Dr. Chu and I, using his 8-Azaquanine sensitive-resistant mutant assay system in Chinese hamster cells, tested the effects of UV-posttreatment of caffeine on UV-induced mutations in Chinese hamster cells

(see enclosed pre-publication manuscript). The results of our experiment indicate that caffeine [at a certain concentration] is synergistic, in the sense that it sensitizes cells to UV-induced killing and in the sense that it reduces the frequency of UV-induced mutations in the survivors (see Table I). The molecular^{ar} basis for these results will be undertaken, as well as further extensions of these results (see Renewal Proposal).

II. In vitro repair of UV-induced molecular damage in Chinese hamster cells by bacterial repair enzymes.

Dr. R.B. Setlow and I wanted to test the purified endonuclease enzyme preparation that he and W. Carrier had, on isolated nuclei from Chinese hamster cells which do not excise or monomerize UV-induced pyrimidine dimers. Using labelled, UV-irradiated isolated nuclei, incubated for various times in various concentrations of the purified UV-endonuclease, the amount of repair in these nuclei was measured by alkaline-sucrose gradient centrifugation of the nuclei. The greater the amount of DNA molecular weight reduction, the greater the amount of repair^{10, 11}. Our results indicated that both purified bacteria UV-endonuclease and yeast photoreactivating enzyme could get into the nuclei and initiate repair. This work still has to be completed. (see Renewal Proposal).

III. Synergistic effects of drugs on the repair of UV-induced pyrimidine dimers in human cells.

With the demonstration that non-repair of pyrimidine dimers in human cells is somehow related to the appearance of skin cancers in patients lacking the UV-endonuclease enzyme^{12, 13}, one could infer that certain conditions, agents or mutations that would interfere with any of the four enzymatic steps of dark repair in human cells would lead to cancer. Along this¹⁵ general hypothesis, I tested LSD's possible effect on DNA repair. Since LSD has been shown to complex with DNA, I reasoned it might

interfere with normal DNA synthesis and/or repair. Results indicated that LSD at large concentrations in human cells does not interfere with normal synthesis of DNA, with normal excision of pyrimidine dimers, or with the stability of pre-existing DNA [see figure 2,3; Table 2]. Along these lines, we concluded our studies of 8-methoxypsoralen on human cells (see enclosed manuscript).

IV. Molecular basis of platinum-tumor inhibitions.

Dr. Harold Harder, working in my laboratory, was able to derive a theoretical model for the inhibition of cancers by several platinum compounds. Certain platinum compounds are potent inhibitors of Sarcoma 180 tumors and solid S - 180 tumors in mice¹⁴. Dr. Harold Harder was able to show that the molecular effects of tumor-inhibiting platinum compounds were shown to mimic closely those of bifunctional alkylating agents, especially HN₂, in terms of preferential inhibition of DNA synthesis, giant cell formation, inhibition of de novo purine synthesis, and enhanced DNase and DNA polymerase activities in extracts of treated cells¹⁵.

On the basis of the size of the platinum molecule, he proposed a hypothesis that the mechanism of action of tumor-inhibiting platinum compounds involves the creation of platinum coordinated intrastrand cross-links between two adjacent purines. In essence, the effects of the platinum coordinated purine dimers would mimic the effects of UV-induced pyrimidine dimers.

We are now trying to test whether platinum-coordinated purines are repaired and whether they might be mutagenic.

V. Biochemical effects of irradiated sugars on human cells in culture.

Dr. Noemi Diaz, Food Science Department, finished her research in my laboratory

The summary of her results are as follows [see thesis]. The synthesis of DNA, RNA and protein in AV₃ cells was inhibited when the cells were incubated with 3Mrad irradiated sucrose, glucose and fructose solutions (Fig. 4).

The synthesis of DNA in AV₃ cells was impaired most by sucrose and fructose. The toxicity induced by the radiolysis products of sucrose and fructose was most accentuated at the dose level of 3Mrad. The synthesis of RNA and protein in AV₃ cells was less affected than that of DNA in the same cells. Irradiated glucose was less harmful to AV₃ cells in culture than sucrose or fructose. Sucrose solutions irradiated at three and four Mrad and stored for seven months at room temperature did not reduce the inhibitory effects of the synthesis of DNA in AV₃ cells. A concentration of 40 µg/ml of catalase only partly eliminated the deleterious effects of 3 Mrad irradiated sucrose to human amnion cells. The activity of DNA polymerase from an AV₃ cell extract was not effected by the presence of irradiated sucrose in the substrate.

Also, under the conditions of the experiments, it was apparent that the damage to human amnion cells by the radiolysis products of sucrose and fructose cannot be repaired¹⁶.

VI. Molecular basis of Franconi's disease (Leukemia)

Collaboring with Dr. Park Gerald of Harvard University, I examined whether cells of patients suffering from Franconi's disease could excise UV-induced pyrimidine dimers. The results [Table 3] indicate that these cells can perform the first endonuclease step of dark repair. We have decided to terminate this line of investigation.

VII. UV-irradiated medium studies.

It is known that after skin has been severely sunburned, cells in basal region

of the epidermis proliferate to replace those that were killed by the ultraviolet radiation. One could hypothesize that the irradiation of cells leads to the release of some products which could trigger cell division in normally non-dividing cells. Since it has been shown that UV-irradiated medium can induce chromosome breaks¹⁷ and that products of ultraviolet irradiated tissue induce biological damage¹⁸, we hoped to demonstrate these effects on a molecular biochemical level. Our results were negative however (Fig. 5).

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COMPILATION OF EQUIPMENT IN LABORATORY OF

Dr. James E. Trosko

Microvoid transfer hood

Hotpack CO₂ incubator

Hotpack CO₂ standup tissue culture incubator

New Brunswick shaker incubator

Zeiss inverted phase microscope

Zeiss research microscope

Waterbaths (2)

Freezer

Refrigerator

pH meter

Branson sonifier

Chromatography equipment

Clinical centrifuge

Yellow Springs radiometer

High Temperature oven

Packard scintillation counter

Coulter Cell Counter

FIGURE LEGENDS

- Fig. 1 Alkaline-sucrose gradient profile of UV-irradiated and control nuclei from Chinese hamster cells that were treated with bacterial UV-endonuclease enzyme.
- Fig. 2 Incorporation of ^3H -thymidine into LSD-treated human amnion cells.
- Fig. 3 Alkaline sucrose gradient of ^3H -thymidine containing material from human amnion cells which were treated with LSD (Fig. 3). Alkaline sucrose gradient of ^3H -thymidine material synthesized in LSD and non LSD treated human amnion cells. Estimated single stranded molecular weight at peaks, using conditions reported by Setlow, et.al., is greater than 10^8 daltons.
- Fig. 4 ^3H -thymidine uptake in DNA synthesis by AV₃ cells incubated with 3 Mrad irradiated sucrose, glucose and fructose solutions (5.8×10^{-2} M in MEM at pH 7.2).
- Fig. 5 Effect of UV-irradiated medium on the incorporation of ^3H -thymidine into non-irradiated human amnion cells.

Fig. 1

Fig. 2

3a²

3b

Fig. 3

Figure 4. -- ^3H -thymidine uptake in DNA synthesis by AV_3 cells incubated with 3 Mrad irradiated sucrose, glucose and fructose solutions ($5.8 \times 10^{-2}\text{M}$ in MEM at pH 7.2).

Fig. 5

TABLE LEGENDS

- Table 1. Effect of caffeine on UV-induced mutation frequency in Chinese hamster cells.
- Table 2. Excision of ultraviolet-light induced thymine-containing dimers in LSD treated human amnion cells.
- Table 3. Excision of ultraviolet-light induced thymine-containing dimers in cells from patients suffering from Franconi's disease.

TABL... Effect of caffeine posttreatment on the frequency of UV-induced mutations from azaguanine sensitivity to resistance in Chinese hamster cells in culture.

EXPERIMENT	UV DOSE (erg/mm ²)	TREATMENT	PERCENT SURVIVAL	NO. OF MUTANT COLONIES	TOTAL NO. OF VIABLE CELLS	MUTANT FREQUENCY PER SURVIVORS
1	0	No caffeine	100	25	15 X 10 ⁵	1.67 X 10 ⁻⁵
	0	Caffeine	90	9	13.5 X 10 ⁵	0.67 X 10 ⁻⁵
	65	No caffeine	50	69	15 X 10 ⁵	4.60 X 10 ⁻⁵
	65	Caffeine	26	30	7.8 X 10 ⁵	3.85 X 10 ⁻⁵
2	0	No caffeine	100	32	15 X 10 ⁵	2.13 X 10 ⁻⁵
	0	Caffeine	97	9	14.6 X 10 ⁵	0.60 X 10 ⁻⁵
	65	No caffeine	80	733	22.8 X 10 ⁵	67.88 X 10 ⁻⁵
	65	Caffeine	19	57	5.7 X 10 ⁵	10.00 X 10 ⁻⁵
3	0	No caffeine	100	1	30 X 10 ⁵	0.03 X 10 ⁻⁵
	0	Caffeine	100	0	30 X 10 ⁵	0
	65	No caffeine	57	27	17.1 X 10 ⁵	24.3 X 10 ⁻⁵
	65	Caffeine	4	1	1.1 X 10 ⁵	0.05 X 10 ⁻⁵
4	0	No caffeine	100	5	30 X 10 ⁵	0.17 X 10 ⁻⁵
	0	Caffeine	100	1	30 X 10 ⁵	0.03 X 10 ⁻⁵
	65	No caffeine	46	382	13.9 X 10 ⁵	27.55 X 10 ⁻⁵
	65	Caffeine	3	2	0.8 X 10 ⁵	0.27 X 10 ⁻⁵

TABLE II

Treatment	Time After UV Dimers Analyzed	$\frac{\hat{X}\hat{T}^*}{T}$	%Thymine As Dimer
UV ¹	0 hour	688/1,276,700	0.05
UV	24 hours	300/860,900	0.03
UV + 20 erg/ml LSD	24 hours	307/881,700	0.03
UV + 0.002 μ g/ml LSD	24 hours	310/1,023,700	0.03

1. Cells were irradiated with 200 ergs/mm² of 2537 Å ultraviolet light.

* $\hat{X}\hat{T}$ refers to both $\hat{T}\hat{T}$ and $\hat{U}\hat{T}$ dimers, since chromatography procedures used here do not separate them from each other.

TABLE III

Experiment	Time after UV*	π/T	$\pi\pi\%$
1.	0 hour	250/227,600	0.11 %
	24 hour	234/303,800	0.07 %
2.	0 hour	428/393,200	0.10 %
	24 hour	154/254,800	0.06 %

*Cells were irradiated with 250 ergs/mm² of 2537 Å UV light.