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FOREWORD

This report was prepared at the Pasadena Foundation for Medical Research, Pasadena, Calif., by—

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**DONALD E. ROUNDS
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The authors are grateful for the technical assistance of Jon Booher.

ABSTRACT

Yeast ribonucleic acid was hydrolyzed with NaOH to produce polynucleotide mixtures which were tested for radioprotective activity on cultured cells. The depolymerized RNA stimulated the growth rate of amnion cells during 5-day treatment periods, but had no effect on growth when the cells were treated for a 24-hour period. A 24-hour treatment of amnion cells with the nucleotide mixture prior to 700 r gamma radiation resulted in an increase in the surviving cell populations. This effect appeared to reach a maximum with a polynucleotide concentration of 0.3 mg./ml. The RNA hydrolysate had no significant effect on radiated KB cells, but surviving HeLa and amnion cell populations were increased by 19 percent and 63 percent, respectively, over controls. This radioprotective response was detected after a minimum of 4 hours of treatment of amnion cells with the test material.

This technical documentary report has been reviewed and is approved.

Robert B. Payne
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Colonel, USAF, MSC
Chief, Operations Division

RADIOPROTECTIVE AGENTS

The Effects of Depolymerized Yeast Ribonucleic Acid on Radiated Human Cells in Tissue Culture

Following reports of antiradiation effects of yeast ribonucleic acid (RNA) on mice and rats (1, 2), a similar response was described for radiated human cells in vitro (3). A survival pattern of these cells indicated that the polymerized nucleic acid produced a protective response which was quite different from that resulting from treatment with sulfhydryl-containing compounds, such as cysteine or aminoethylisothiuronium bromide hydrobromide (AET) (4). Recently, Maisin et al. (5) suggested that an alkaline hydrolysate of yeast RNA showed the same degree of protection of radiated rats as the polymerized form of RNA (6). Preliminary experiments, using an alkaline digest of yeast RNA, indicated a protective response in radiated human amnion cells in vitro. The purpose of this study was to compare the biologic responses to the RNA hydrolysate, with the characteristic responses of the same cell system to the polymerized form of the nucleic acid, as previously described (4).

MATERIALS AND METHODS

Preparation of the hydrolysate

Commercially obtained yeast RNA (0.6 percent solution) was incubated at 37° C. in 0.1 normal NaOH for 2 hours. The pH was adjusted to 5 with HCl. The hydrolysate was precipitated by the addition of 2 volumes of absolute alcohol and collected by filtration. The filtrate was washed with alcohol-ether,

ether, and dried to a light-colored powder. The hydrolysate was considered to be a mixture of nucleotides probably ranging from 4 to 19 units in length.

Toxicity test

Thirty T-30 flasks in replicate experiments were inoculated with 250,000 cells from the Fernandes strain of human amnion. An initial incubation of 48 hours insured that the cells were in the log phase of growth. Ten flasks were treated with 0.2 mg. yeast RNA hydrolysate per milliliter of Eagle's medium for 24 hours, then incubated in Eagle's medium alone for an additional 4 days. Ten flasks were continuously treated for 5 days with the same concentration of depolymerized RNA dissolved in the nutrient fluid. Ten flasks were maintained throughout the 5-day experimental period in control medium. The number of cells was evaluated after trypsinization and suspension in saline with the aid of a Coulter cell counter.

Effect of survival of radiated cells

Three replicate experiments, using a total of 75 uniformly seeded flasks of amnion cells, were performed to determine the antiradiation effect of depolymerized RNA. The hydrolysate was dissolved in Eagle's medium and added to flask cultures at a concentration range from 0 to 0.5 mg./ml. for 24 hours prior to radiation. At the end of the treatment period, all flasks were given 700 r gamma radiation with a cobalt-60 therapy unit (32.8 r per minute) in an air phase. Fresh medium was added and

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TABLE I
Rate of growth of amnion cells treated with depolymerized yeast RNA

Experiment No.	Concentration of hydrolysate (mg./ml.)	Treated/control ratio	
		24 hours	5 days
1, 2	0	1.00	1.00
1	0.2	1.02	1.81
2	0.2	1.08	2.09

TABLE II
Effect of various concentrations of depolymerized RNA administered for 24 hours prior to 700 r gamma radiation

Concentration of hydrolysate (mg./ml.)	Av. number of cells per flask ($\times 10^5$)	Treated/control ratio
0	23.1	1.00
0.1	30.1	1.30
0.2	35.2	1.52
0.3	47.3	2.04
0.4	37.5	1.62

TABLE III
Relative survival of treated and untreated irradiated cell strains of different origin

Cell type	Treated/control ratio
Amnion	1.63
HeLa	1.19
KB	1.06

the cultures were incubated for 4 days before being evaluated with the Coulter counter.

Response of three different cell types

Twenty T-30 flasks each of amnion and KB (nasopharyngeal carcinoma) cells and 15 flasks of HeLa cells were treated with 0.2 mg./ml. depolymerized yeast RNA for 24 hours prior

to 700 r gamma radiation. An equal number of culture vessels of each cell type served as untreated, irradiated controls. Fresh normal Eagle's medium was added to all flasks immediately following radiation and the cultures were incubated for 4 days prior to analysis with the cell counter.

Effect of a variable treatment period on radiated cells

Thirty flask cultures of amnion cells were treated with 0.2 mg./ml. of the alkaline digest of yeast RNA for periods ranging from 2 to 6 hours. An additional 10 flasks served as untreated controls. All cultures were given 700 r gamma radiation immediately following the treatment period. As with the other experiments, the number of cells per flask was determined with a Coulter counter 4 days post-irradiation.

RESULTS

The data given in table I indicate that depolymerized yeast RNA is nontoxic. In fact, the mixture of nucleotides appeared to be stimulatory when amnion cells were continuously treated for 5 days. However, cells which were treated for the first 24 hours of the 5-day interval showed an average of 5 percent more cells than control cultures, which probably was not significant.

Preirradiation treatment of amnion cells with depolymerized RNA appeared to increase survival of radiated cells, as indicated in table II. The average number of surviving cells increased at a rate proportional to the amount of nucleotide mixture used in the 24-hour treatment period, up to a maximum of 0.3 mg./ml.

The 3 tested cell strains responded differently to a 24-hour treatment with 0.2 mg./ml. yeast RNA hydrolysate prior to 700 r gamma radiation. Amnion cells showed the greatest protective response in comparison with HeLa and KB cell lines (table III).

The data presented in table IV suggested that preirradiation treatment for 2 hours with the mixture of nucleotides does not elicit a protective response. Similar treatment for 4- and 6-hour intervals results in small, but significant increases in surviving cell populations.

DISCUSSION

The alkaline hydrolysate of yeast RNA appeared to stimulate the growth rate of amnion cells when added to the culture medium for a 5-day period. This finding may be consistent with the report of Kutsky(7) and Maganini et al. (8) that nucleoproteins (but not RNA alone) can stimulate growth in tissue cultures. However, Rounds (3) has indicated that the commercial yeast RNA used in this study produced an inhibition of the growth rate during continuous treatment for a 6-day interval. Digestion with sodium hydroxide may have removed a toxic contaminant from the starting material. Neither the polymerized (3) nor the depolymerized form of the nucleic acid produced a significant effect on growth during a 24-hour treatment.

As with yeast RNA (3, 4), preirradiation treatment with the alkaline hydrolysate resulted in increased numbers of surviving cells. However, while the maximum cell survival resulting from yeast RNA treatment was observed to be approximately three times the number of surviving cells in radiated control cultures, the depolymerized form of the molecule showed a maximum of two times the cell population of control vessels. The optimum nucleic acid dosage was reported to be 0.2 mg./ml. for the larger molecule, while the data in table II suggest that the optimum concentration for the polynucleotide mixture was 0.3 mg./ml. This variation might be due to the presence of inactive nucleotide forms in the hydrolysate. Maisin et al. (5) have reported that injection of radiated rats with a predominance of oligonucleotides results in a 70 percent survival 30 days after 500 r, while another mixture containing 87 percent mononucleotides showed 50 percent survival under the same experimental conditions.

TABLE IV

Effect of short-term treatment of amnion cells with yeast RNA hydrolysate prior to 700 r gamma radiation

Treatment period	Av. number of cells per flask ($\times 10^5$)	Treated/control ratio
0	36.0	1.00
2	32.8	0.91
4	40.2	1.12
6	43.6	1.21

In previous studies, cell types varied in their survival response to RNA treatment followed by irradiation. Yeast RNA treatment had no significant effect on the degree of radiosensitivity of HeLa and KB strains, while amnion cells appeared to become more radio-resistant following this procedure. The RNA hydrolysate showed a similar, though less marked, effect, with the most notable variation being the result of a 19 percent increase in the surviving HeLa cell population. The specificity of action may be due to the degree of cellular potentiality to metabolize the larger molecular forms.

It is interesting to note that amnion cells were not "conditioned" after 6 hours of treatment with yeast RNA, but showed a protective response only after 8 to 10 hours of contact with the nucleic acid. The depolymerized form of the extract produced a small, but significant effect after 4 to 6 hours of treatment. The data suggest that either the smaller molecular form can penetrate the cell membrane more rapidly, or the protective agent may be the result of RNA digestion and resynthesis into another molecular structure with antiradiation characteristics. These possibilities are currently being investigated.

It can be concluded that the alkaline hydrolysate of yeast RNA retains the potential to increase survival of radiated cells in vitro. Such an effect cannot be attributed to an increase in the cell population via growth stimulation. Treatment periods up to 24 hours, which produce a significant radioprotective response,

have been shown to lack growth-stimulating potentialities. The data suggest that depolymerized RNA, although probably having an analogous action to that of yeast RNA, shows certain differences of cellular response which may lead to a better understanding of the mode of action of RNA on radiated cells.

SUMMARY

Yeast ribonucleic acid was hydrolyzed with 0.1 normal NaOH to produce a mixture of polynucleotides and oligonucleotides which were tested for potential radioprotective activity on cells in vitro. It was found that the depolymerized RNA stimulated the growth rate of amnion cells when treatment periods were extended to 5 days, but had no effect on growth

when the cells were treated for a 24-hour period. A 24-hour treatment of amnion cells with the nucleotide mixture prior to 700 r gamma radiation resulted in an increase in the surviving cell populations. This effect appeared to reach a maximum with the use of a polynucleotide concentration of 0.3 mg./ml., with a resulting treated/control ratio of 2.04. The RNA hydrolysate had no significant effect on radiated KB cells, but surviving HeLa and amnion cell populations were increased by 19 percent and 63 percent, respectively, over controls. This radioprotective response was detected after a minimum of 4 hours of treatment of amnion cells with the test material. Variations of effects of yeast RNA and its hydrolyzed form on radiated cells were discussed.

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