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RADIOPROTECTIVE AND METABOLIC EFFECTS OF AET ON RAT EMBRYONIC CELLS IN CULTURE

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SUMMARY

The experimental results confirm the literary data on the radioprotective activity of AET. This radiation protection has been demonstrated in the increase of survival of rat embryonic cells exposed to various doses of ionizing radiation in culture.

Concerning the mechanisms of action, it seems important that a correlation exists between the radioprotective and metabolic effects of the compound, the latter manifesting itself in depression of DNA, RNA, and protein syntheses of pretreated cells. It is suggested that slowing of the metabolic activity and mitotic cycle in cells lead to a state of their enhanced radioresistance or repair capability.

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INTRODUCTION

Much evidence exists that the chemical radioprotectors protect the organism against lethal and harmful effects of ionizing radiations, when administered prior to irradiation. Even though these substances are chemically unrelated to each other, they protect the organisms against a lethal dose of ionizing radiations. Since a scant chemical relationship between these compounds occurs, their classification is difficult. Sulphydryl compounds, cyanides, nitriles, amines, and antibiotics are examples to some of the protective compounds mentioned in the literature. One of the most common characteristics of these compounds is their necessary presence in the organism that is exposed to ionizing radiations.

Cystein and cyanide were the first radioprotectors to be discovered in 1949. Since then, numerous compounds were found to have protective effects. In 1957, in the search for a new effective radioprotective agent, a sulphydryl compound, namely β-aminoethylisothioronium (AET) was discovered by DOHERTY and his friends. This compound changes to mercaptoethylguanidine (MEG) at neutral pH values. In fact, MEG is the essential protective molecule. The radioprotective effects of AET have been confirmed by other investigators also in many organisms.

The exact mechanism of action of chemical radioprotectors is not specifically known. Among the numerous radioprotectors, the aminothiols seem to be the most effective ones. For this reason, most of the work dealing with the action of radioprotective agents has been done with these compounds. The most effective aminothiols are those whose structures are closely related with cysteamine. Three main theories involving the mechanism of action of radioprotectors are present: 1) the anoxia theory, 2) the mixed disulphide theory, and 3) the free radical hypothesis. Besides these theories, other suggestions about the mechanisms of action of these compounds have been put forward. It is known that the sulphydryl compounds by decreasing DNA synthesis, inhibiting the mitosis, and thus affecting the metabolic activity of the cells exposed to ionizing radiations, exert their protective effect.

Besides decreasing the vitality of the cells, ionizing radiations also induce changes in the chemical and structural elements of the cells. In several cell systems, it was confirmed that the synthesis of nucleic acids was depressed especially several hours after exposure to moderate X-ray doses.

Deficiency in DNA synthesis seemd to result from lesions in this molecule, persisting from the time of irradiation and related to permanent mitotic arrest.

The purpose of this investigation was to determine the effects of AET at its highest non-toxic concentration, on the vitality of rat embryonic cells in culture exposed to different dose-rates of ionizing radiation. Its effects on the synthesis of DNA, RNA, and proteins of irradiated and unirradiated cells in culture were also examined.

EXPERIMENTAL METHODS

The metabolic effects of AET in the highest non-toxic concentration (20 mM) for rat embryonic cells, were studied by comparing the nucleic acid and protein synthesizing activity of the cells with that of untreated ones. In the experiments, rat embryonic cells in secondary passage 48 hours after their inoculation on cover-slips were used. AET was dissolved in a tissue culture medium (TC-199) prior to its usage. The pH of the medium was adjusted to 7.4 with a few drops of NaOH and 10 % calf serum was added to the final mixture (Vos et al). Following their incubation in AET for 30 minutes, the cells were washed with BSS (Balanced Salt Solution) and fresh medium was added. Tritiated precursors of DNA (thymidine), RNA (uridine), and proteins (glycine) was added to the culture medium of pretreated and untreated cells for 30 minutes. Following this second incubation, the cells were fixed Carnoy's fixative (3:1 ethanol: glaciel acetic acid), and washed with 70 % ethanol. Their autoradiograms were prepared by the use of film-stripping technic, Kodak AR. 10 plates. At appropriate times, the films were fixed with Kodak

fixative and stained with freshly prepared Giemsa stain. The rate of DNA, RNA, and protein syntheses was established from the number of labelled and unlabelled nuclei, the grains over the whole cell area, and nucleolus, respectively and compared with that of the cells untreated with AET.

The radioprotective activity of AET was examined by exposing the cells to different doses of gamma radiation in the presence and absence of AET as follows:

1) The amount of cells in well defined marked areas of cover-slips were counted prior to irradiation and addition of AET containing culture medium.

2) Whenever necessary, the medium of the cells was removed and AET containing one (prepared as mentioned before) was added.

3) The lid of the petri-dish, containing the cover-slips with cells attached on them, was paraffinized and the dish was immediately placed in the chamber containing the Co-60 source.

4) Depending on the dose-rate, 20 minutes after the exposure, the petri-dish was removed from the chamber. The medium was then replaced either with the fresh or AET containing one. The lid was paraffinized and the cells were irradiated for another 20 minutes.

5) At the end of irradiation, the petri-dish was immediately brought to the tissue culture laboratory. The cells were washed with BSS and incubated in fresh culture medium for 24 hours at 37°C.

6) Following this incubation, the number of cells in the same marked areas of the cover-slip was counted again. Thus, n/n_o values for dose-rates of 390, 580, 870, and 1060 R were calculated.

7) Due to the low dose rendering design of the source, at 1060 R level, the cells were not irradiated in AET containing culture medium.

8) The unirradiated control cells were incubated in culture medium during the same interval at 37°C. n/n_o values for them were also calculated.

Since AET becomes toxic for cells in 30 minutes, the exposure of the cells to this chemical, including the manipulations between irradiations, did not exceed this period.

A Cobalt-60 source of 52 Ci radiating 14.5 R/min from a distance of 28.5 cm, was employed in the experiments. The temperature of the chamber containing the source was kept at 37°C by the use of a contact thermometer.

The effect of AET on the alterations induced in DNA, RNA, and protein syntheses by radiation was also determined. Same tritiated precursors and methods mentioned above, were used. The tritiated precursors were added to the culture medium of the treated and untreated cells 24 hours after irradiation. The uniraadiated controls were labelled at the same time interval.

EXPERIMENTAL RESULTS

In our previously published work, under the same contract, we observed that 20 mM of AET proved to be its highest non-toxic concentration for rat embryonic cells incubated for a period of 30 minutes. 40 mM of this compound was found to be the lethal concentration (See, Üçer and Bölükbaşı).

Incubation of rat embryonic cells for 30 minutes in a culture medium containing 20 mM AET, resulted in a significant reduction in nucleic acids and protein syntheses. The extent of reduction in DNA, RNA, and protein syntheses was 13, 27, and 39 %, respectively, when the cells were labelled immediately after the removal of AET containing medium (Table I). The extent of reduction in DNA, RNA, and protein syntheses was 32, 37, and 31 %, respectively, when the cells were labelled 24 hours after the removal of AET (Table III).

Exposure of cells to 390, 580, 870, and 1060 R of gamma radiation decreased their survival to 87.0, 62.2, 57.7, and 50.0 %, respectively. At the same time of observation, the number of unirradiated control cells increased to 160 % of the initial value. Presence of AET in the medium during irradiation, abolished almost completely the decrease in cell survival irra-

diated with doses from 390 to 870 R (Table II).

The radiation induced depression in DNA synthesizing activity of cells at 580 R, was also diminished from 52.8 % to 27.4 % in the presence of AET. On the other hand, this compound did not influence significantly the the radiation induced alterations on RNA synthesis (38.2 % vs 36.9 %), and even aggravated the disturbance in protein synthesis reducing it by 49.6 % instead of 35.8 % caused by radiation in the absence of AET (Table III).

CONCLUSIONS DRAWN

Extensive studies have been done on the possible biochemical actions of sulphur containing radioprotectors in many laboratories. Since these substances delay mitosis and DNA synthesis, a hypothesis was submitted that these biochemical changes increase the efficacy of the repair system or protect the organism from radiation damage. It is believed that the adminstration of these compounds lead to temporal inhibition of metabolic processes, thus inducing a "biochemical shock". SH-protectors interact directly with subcellular structures and metabolites essential for life. As some investigators point out, the inhibition of nucleic acid synthesis result in slowing down of the mitotic cycle, quite likely a depressed state of radiosensitivity occurs.

The most obvious result of irradiating a culture of cells and the most relevant to the subject of cancer therapy, is a significant growth inhibition. This growth inhibition may not exert itself immediately after irradiation. Cells may complete the subsequent division and 25 % are permanently arrested in the second post irradiation mitosis and suffer mitotic death. Radiation induced division and mitotic delay have been

extensively studied in many laboratories. From previous studies, it is known that continued progress around the mammalian cell cycle is dependent upon concomitant RNA and DNA and protein syntheses. Radiation might be damaging to the cell's ability to synthesize these molecules.

Our experimental results confirm the literaray data on the radioprotective and metabolic effects of AET, which is a sulphydryl protector. One of these effects has demonstrated itself by abolishing completely the decrease in cell survival even at a high dose of 870 R. The metabolic effects of the compound are manifested in the depression of DNA, RNA, and protein syntheses of pretreated cells. In the case of nucleic acid synthesis, this effect is more pronounced 24 hours from exposure to the compound. The slowing down of the metabolic activity and mitotic cycle in the cells is presumed to lead to a state of their repair capability and hence, enhanced radioresistancy.

It should be kept in mind that the effects of X-radiation on timing of cell lethality and DNA synthesis vary greatly with cell type. Protection can be obtained by the same substance in one system, at different concentration in another, and not at all in others.

TABLE I

THE EFFECT OF AET (20 mM) ON DNA, RNA, AND PROTEIN SYNTHESIS OF RAT EMBRYONIC CELLS IN CULTURE(1)

Sample	% labelling	No. of grains	Difference from control
Control, treated with H^3TDR(2), 3 µCi/ml, S.A. 5Ci/mM	40.65±0.44(3)		
Treated with AET and H^3TDR	35.24±0.28		13.3 %
Control, treated with H^3URD(4), 6 µCi/ml, S.A. 28.2 Ci/mM		110.00±1.57	
Treated with AET and H^3URD		80.00±1.19	27.2 %
Control, treated with H^3GLY(5), 12 µCi/ml, S.A. 1.05/mM		7.25±1.73	
Treated with AET and H^3GLY		4.39±1.41	39.4 %

(1) Tritiated precursors added immediately after removal of AET containing culture medium.
(2) DNA synthesis.
(3) Standard error from the mean.
(4) RNA synthesis.
(5) Protein synthesis.

TABLE II

THE EFFECT OF RADIATION, IN PRESENCE AND ABSENCE OF AET (20 mM) CONTAINING CULTURE MEDIUM, ON CELL SURVIVAL 24 HOURS AFTER THE EXPOSURE

Gamma radiation dose	% Survival in absence of AET	% Survival in presence of AET	% Survival of unirradiated Controls
390 R	87.05±2.12	105.0±1.26	
580 R	62.16±1.67	99.7±2.04	160.0 2.04
870 R	57.68±0.50	99.8±1.66	
1060 R	50.00±1.68	Not done	

TABLE III

THE EFFECTS OF IRRADIATION IN PRESENCE AND ABSENCE OF 20mM AET ON DNA, RNA, AND PROTEIN SYNTHESES OF RAT EMBRYONIC CELLS(1)

Sample	DNA SYNTHESIS		RNA SYNTHESIS		PROTEIN SYNTHESIS	
	% labelling	Diff. from control	No.of grains	Diff. from control	No. of grains	Diff.from control
Unirrad.,incubated in medium, Control.(2)	26.65±0.97		59.76±1.15		7.05±0.30	
Unirrad.,incubated in AET containing medium.	18.19±0.27	31.5%	37.53±0.92	37.1 %	4.86±0.19	31.0 %
Irrad.(3) in AET containing medium	19.36±0.10	27.5 %	37.60±0.80	36.9 %	3.55±0.13	49.6 %
Irrad. in medium only	12.57±0.55	52.8 %	36.91±0.79	38.2 %	4.52±0.19	35.8 %

(1) The cells were labelled 24 hours after each treatment.

(2) Same tritiated precursors as in Table I, were used.

(3) 580 R.

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