FAST NEUTRON IRRADIATION EFFECTS ON LIVER CHROMATIN STRUCTURE

B. Constantinescu[1] and Liliana Radu[2]

[1] Cyclotron Laboratory, Institute of Atomic Physics, P.O.Box MG-6, Bucharest, Romania.
[2] Department of Molecular Genetics, Victor Babes Institute, Spl. Independentei, 99-101, Bucharest 76201, Romania

EXPERIMENTAL

The growing interest in neutron therapy requires complex studies on the mechanisms of neutron action on biological systems, especially on chromatin (the complex of deoxyribonucleic acid-DNA with proteins in eukaryotic cells).

The chromatin was extracted from a normal tissue-livers of Wistar rats-and from a tumoral tissue-Walker tumour maintained on Wistar rats. For chromatin separation, the method indicated in (1) was used, with a supplementary nuclei's purification through a passage on 1M sucrose, and the obtained purity verified by an absorption test (2). Suspension of cells-5×10^6 cells/ml concentration-from Wistar rat livers and from Walker tumours were also isolated. Irradiation doses from 5Gy to 100 Gy by fast neutron intense beams produced via d(13.5MeV)+Be (thick target) reaction at Bucharest U-120 Classical Cyclotron were used (3).

To study the post-irradiation effects, various methods were employed. So, the variation in the 260nm absorbency in chromatin thermal transition was pursuit (E_0-before and E-after transition). The chromatin-ethidium bromide complexes fluorescence with λ_{ex}=480nm and λ_{em}=600nm was analysed. To determine chromatin DNA strand breaks a fluorimetric method (4), with cells' suspensions as starting material was used. This method requires a partial treatment with alkali, producing three components: T-estimating the total fluorescence of DNA double helix, P-assigning the untwisting rate and B-the blank, where DNA is completely unfolded. The percentage of DNA double strand,-D-, remaining after this treatment, is:

$$\%D=100\times(P-B)/(T-B)$$

The intrinsic chromatin fluorescence was determined for tyrosine (λ_{ex}=280nm, λ_{em}=305nm), specific for basic chromatin proteins, and for tryptophane (λ_{ex}=290nm, λ_{em}=345nm), specific for acid chromatin proteins. Polyacrylamide gel electrophoresis was performed as indicated in (5).

The double fluorescent labelling of chromatin was realised with acridine orange for DNA and with dansyl chloride for chromatin proteins (6). Fluorescence intensity determinations were done with λ_{ex}=505nm, λ_{em}=530nm for acridine orange and with λ_{ex}=323nm, λ_{em}=505nm for dansyl chloride.

A Pye Unicam SP 1800 spectrophotometer and a Aminco SPF 500 spectrofluorimeter were employed.

RESULTS AND DISCUSSION

For normal chromatin, a diminution of the negative fluorescence intensity for chromatin-ethidium bromide complexes with the increasing of neutron dose (from 0.98 at 5Gy to 0.85 at 100Gy) was observed. This fact reflects chromatin DNA injuries, with the decrease of double helix DNA proportion. As is known, single and double strand breaks are produced under fast neutron action. The quantity of the remaining DNA double strand, determined by fluorimetric analysis of the unwinding DNA, also decrease with neutron dose.

A reduction of intrinsic fluorescence intensities with neutron dose was also observed (approximately 1.5 times from 5 to 100Gy), denoting a destruction of chromatin proteins' structure. The energy transfer efficiency also decrease under neutron action (from 0.224 at 5Gy to 0.135 at 100Gy), indicating a more unstable tertiary structure of chromatin.

For tumour chromatin, some results are presented in figures 1, 2, 3. So, in figure 1 are represented the relative absorbencies (E/E_0) obtained for the thermal transitions of chromatin samples versus fast neutron dose, lower values indicating a damaged chromatin DNA (with a smaller proportion of double helix).

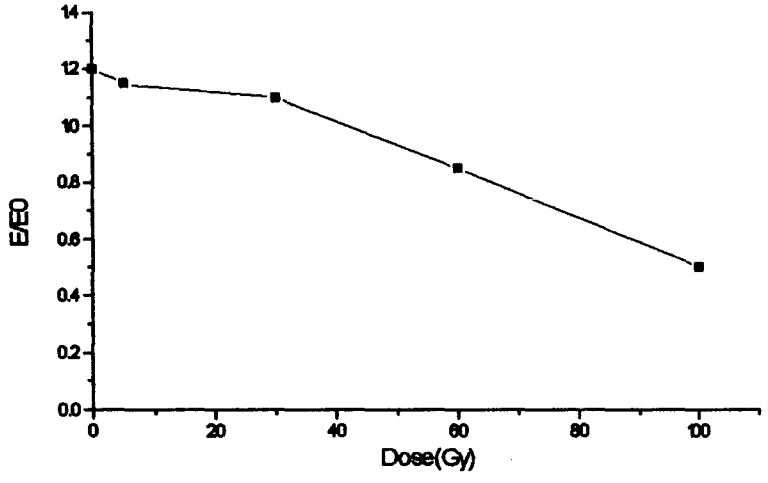

Figure 1Thermal transition of chromatin samples exposed to fast neutrons.

Figure 2 presents the fluorescence intensities of chromatin-ethidium bromide complexes versus fast neutron dose. Lower values also denote modifications in chromatin DNA structure, so that intercalation of ethidium bromide between pairs of superposed DNA bases takes place in a lesser degree.

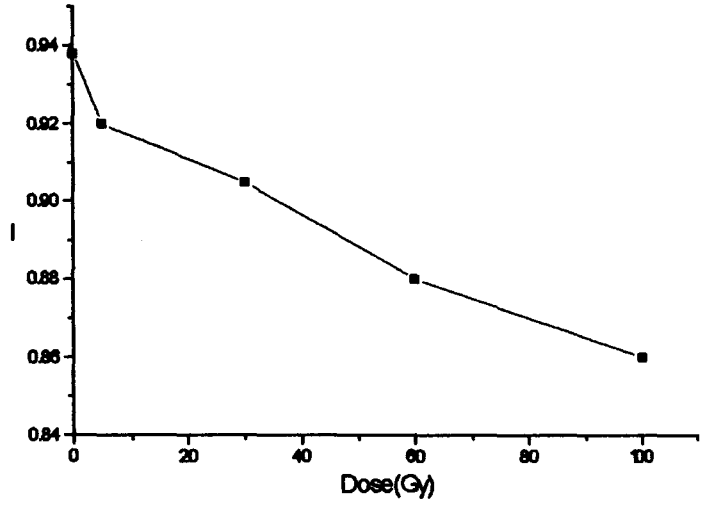

Figure 2. Chromatin-ethidium bromide complexes relative fluorescence versus fast neutron dose.

Intrinsic fluorescence of chromatin tyrosine and of chromatin tryphtofane versus fast neutron dose is presented in figure 3, the diminution of their values indicating a destruction of chromatin proteins.

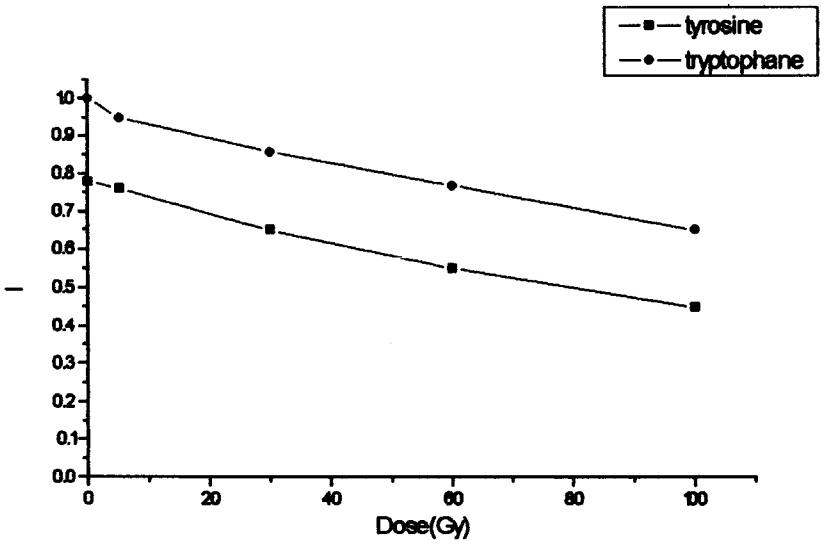

Figure 3. Chromatin tyrosine and tryptophane relative fluorescences versus fast neutron doses.

To study the influence of thyotepa, thyroxine and D_3 vitamin treatments on fast neutron radiolysis in tumour chromatin, 10mg/kg of anticancer drug thyotepa, 40μg/kg of hormonal compound thyroxine and 30,000 UI/kg of D_3 vitamin were administrated separately or associated to Wistar rats bearing Walker carcinosarooma. Similar studies on chromatin modifications as mentioned below were effectued. Preliminary results indicate that the association of thiotepa with thyroxine and D_3 vitamin produce a diminution of chromatin lesions induced by cytostatics and, consequently, differences in chromatin fast neutron radiolysis. Such results could constitute an indication for associated chemotherapy-radiotherapy scheduled in clinical applications. This research is in progress.

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