Chromosome Aberrations Induced in Human Lymphocytes by U-235 Fission Neutrons

Part III: Evaluation of the Effect of the Induced $\alpha$ and $\beta$ Activity on the Chromosomal Aberration Yield

A. Fajgelj$^1$, D. Horvat$^2$, J. Škrk$^3$

$^1$J. Stefan Institute, Ljubljana, Slovenia, $^2$Institute of Medical Research and Occupational Health, University of Zagreb, Croatia, $^3$Institute of Oncology, Ljubljana, Slovenia

Aim: Further experiments were performed to explain a difference in chromosomal aberration yield found between samples cultivated immediately after fission neutron irradiation and samples which were cultivated with 96 h delay after irradiation.

Material and Method: Human peripheral blood samples were irradiated in mixed fission neutron/gamma field (1800 s) and biological effect assessed in the mean of analysis of unstable chromosome aberrations with a time delay in culturing cells of 12, 24, 48, and 96 h. Additional measurements were performed on irradiated and blank blood samples with the aim to detect any increase in $\alpha$ and $\beta$ activity after fission neutron irradiation. No difference was found. Results were compared to theoretically calculated values of the $\alpha$ and $\beta$ activity released from natural radioactive isotopes.

Result and Conclusion: As a conclusion it is shown that in our experimental conditions the secondary effects resulting from nuclear transformations of natural or induced radioactive isotopes, recoil reactions and accompanying $\alpha$, $\beta$, and $\gamma$ radiation are not the reason for the increase observed in chromosomal aberration yield in blood samples cultured with a time delay of at least 24 hours.

Durch U-235-Spaltungsneutronen in menschlichen Lymphozyten induzierte Chromosomaberrationen. Teil III: Evaluation der Auswirkung der induzierten $\alpha$- und $\beta$-Aktivität auf Chromosomaberrationen

Ziel: Um Differenzen in der Chromosomaberration zu erkennen, wurden weitere Experimente durchgeführt.


Ergebnis und Schlußfolgerung: Das Ergebnis der Experimente zeigte, daß Sekundäreffekte, die aus der Kernumwandlung von natürlichen oder induzierten radioaktiven Stoffen resultieren, und die begleitende $\alpha$, $\beta$, und $\gamma$-Strahlung nicht die Ursache für das beobachtete Ansteigen der Chromosomaberration in Blutproben, die nach mindestens 24 Stunden kultiviert wurden, sind.

A significant difference in the incidence of chromosomal aberrations between blood samples cultivated immediately after “in vitro” irradiation by U-235 fission neutrons in the “dry cell” of the TRIGA Mark II nuclear of the J. Stefan Institute in Ljubljana, Slovenia, and samples which were cultivated after 96 h storage after irradiation was described in our previous work [9]. In further studies the separate effect of the induced Na-24 activity was evaluated, due to the pronounced gamma peak observed in gamma spectrometric measurements of the irradiated blood
samples at the 1386.5 keV, e.g., at the energy which belongs to Na-24 [8]. It was supposed and then experimentally proved that neutron activated sodium (Na-24), as an extracellular component of the human blood, cannot be the reason for double strand breaks and the corresponding unstable chromosomal aberrations at the level found in our investigations. The difference in the incidence of chromosomal aberrations between blood samples described above [9] was thus still not explained. Different models for evaluation of irradiation effects and estimation of fission neutron doses are described in literature [2, 3, 6, 11], but there are still gaps or controversies about the biological effects of fission neutrons. Beside many investigations of fission neutron irradiation effects, separate studies of possible influence of the radiotoxic and neutron irradiation induced radioactive isotopes have also been performed [1, 8]. Our interest in this research was mainly oriented to the radiochemical point of the problem. In general, to allow new theories and predictions of biological effects of fission neutrons to be accepted, all possible radiochemical effects should be clarified first.

According to the gamma spectra in the energy region between 30 and 1650 keV, obtained for fission neutron irradiated blood samples, where only a pronounced gamma peak of 24Na was found, the aim of the present work was to measure also α and β activity of blood samples after fission neutron irradiation. Comparison between α and β activity of blood samples after fission neutron irradiation with non-irradiated blood samples should provide evidence of the possible existence of radionuclides which are pure α or β emitters. The most interesting elements in this case are S and P, which are constitutional parts of DNA. Neutron activation of natural isotopes 32S, 34S and 31P results in production of the radioactive isotopes 35S (t_{1/2} = 87.5 days, β- 0.2 MeV, no γ), 37S (t_{1/2} = 5.0 min, β- 1.8 and 4.9 MeV, γ 3103 keV) and 32P (t_{1/2} = 14.3 days, β- 1.7 MeV, no γ) respectively; the nuclide 32P is also formed by the 32S(n, p)32P reaction [4, 7, 15]. Radioactive decay of the above mentioned nuclides leads to the formation of stable isotopes. In the case of sulphur 35Cl and 37Cl are produced, while in the case of 32P radioactive decay stable 32S is formed. The effect of chemical transformation of the above mentioned elements, recoil reactions, and accompanying α and/or β radiation have not yet been studied as a possible reason for double strand breaks and subsequent formation of chromosomal aberrations. Biological effects of fission neutron irradiation on the chromosomal aberration yield were in this work evaluated by analysis of unstable chromosomal aberrations, i.e., the formation of dicentrics and centric rings. At the same time a reaction kinetics for the development of radiation-induced chromosome aberrations was followed. For this reason the blood samples were cultivated at different times after neutron irradiation, e.g., 12 h, 24 h, 48 h, and 96 h respectively.

**Materials and Method**

**Irradiation Conditions**

Irradiation conditions in the “dry cell” of the TRIGA Mark II nuclear research reactor (250 kW) of the J. Stefan Institute in Ljubljana, Slovenia, applied for this type of study have been determined and described in our previous work [9]. In the present work all samples were irradiated for 30 min what is the longest irradiation time of our previous experiment, and where, as expected, the largest number of neutron-irradiation induced chromosomal aberrations was found. Samples were irradiated at the same irradiation position and under the same reactor power (250 kW), so that comparison with our previous data is possible. From our previously reported results and also from our additional measurements of the irradiation conditions [10], one can find out that samples were irradiated in the mixed neutron and gamma radiation field. Precisely, after 30 min of irradiation the samples have received a fission neutron dose of 3.96 Gy and a gamma dose of 1.09 Gy with an estimated error of ±10% in both cases.

**Blood Samples and Lymphocyte Cultures**

Whole blood samples were taken from the same healthy non-smoking female donor as in our both previous experiments [8, 9]. Blood samples were placed in 6 “Nunc” Cryotubes and heparinized. Six samples (samples 1 to 6) were irradiated at the fixed position in the “dry cell” of the reactor. Three blood samples (samples 0, 7 and 8) were prepared as a control samples for chromosomal aberration analysis and radioactivity measurements and were not irradiated. Into 8 cm³ F-10 medium (GIBCO) supplemented with 20% inactivated mycoplasma free calf serum (Biochrom KG), 10 μg/cm³ BrdU (Calbiochem) and 0.2 cm³ Phytohemagglutinin (Welcome) 0.7 cm³ whole blood was added. Lymphocyte cultures were incubated at 37 ± 0.5°C for 48 h in the dark. After 45 h colchicine was added. Two cm³ of colchicine (Calbiochem) stock solution (25 μg/cm³ in physiological saline) was added to each lymphocyte culture. Hypotonic treatment in 0.075 M KCl and fixation of cells