Induction of Unscheduled DNA Synthesis in LS/BL Cells Exposed to Fast Neutrons and Gamma Rays

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Abstract. Induction of unscheduled DNA synthesis (UDS) in mouse lymphosarcoma cells (LS/BL) was studied in vitro after exposure to fast neutrons (6.2 MeV; 92.5 % neutrons + 7.5 % gamma rays), and to gamma rays of ^{60}Co. UDS was detected by means of autoradiography after incubation of the cells in the medium supplemented with 7.4 kBq ^{3}H-dTh/ml. In the autoradiographs either cells out of the S-phase, or all cells in the sample after block of DNA replication with 0.01 mol/l hydroxyurea were determined. The following observations were made: (1) according to UDS induction in LS/BL cells, the RBE of fast neutrons amounted to about 2.8; (2) after exposure to fast neutrons UDS was directly proportional to doses of up to 50 Gy. A dose of 200 Gy inhibited UDS; (3) during the observed time interval of 150 min UDS was running at an almost constant rate; (4) the quantity of DNA material released during the excision process and the amount of UDS were in good agreement.

Key words: Unscheduled DNA synthesis — Neutrons — Gamma rays

Introduction

Rasmussen and Painter (1964) described ^{3}H-dTh incorporation into DNA in UV-irradiated HeLa cells out of the S-phase. They called this kind of ^{3}H-dTh incorporation into DNA unscheduled DNA synthesis (UDS). Two years later UDS was demonstrated also in X-irradiated cells (Rasmussen and Painter 1966).

The lowest doses of ionizing radiation that are reflected in a change of DNA sedimentation velocity range between 1 and 2Gy (Drášil et al. 1972). This may be demonstrated by centrifugation both in neutral and alkaline sucrose gradients. However, for a quantitative evaluation of DNA single- and double-strand breaks in dependence on doses, the suitable dose range begins from a few tenths of Gy (Elkind and Kamper 1970).

Regarding the irradiation doses, a similar situation is observed when studying UDS induction. Although after 2 Gy exposure UDS induction has been observed (Sega et al. 1978), doses of tenths of Gy are usually used for these studies. Yet, the data

on dose response vary in a wide range even in the case of a given laboratory and one cell kind; e.g., in the papers by Painter's group on HeLa cells doses of 10 to 1000 Gy were applied (Rasmussen and Painter 1966; Painter and Cleaver 1967; Painter 1970).

In the present paper four groups of experiments were described: induction of UDS with fast neutrons in comparison with gamma rays, dose dependence of UDS, time-course of UDS, and an attempt to estimate loss of DNA material during the excision process.

Material and Methods

The experiments were carried out on mouse lymphosarcoma cells (LS/BL) in vitro. The cell suspension was prepared in Hanks' solution (without bivalent ions; pH 7.4) and inactivated calf serum (2:1) containing 10^7 cells per ml. The cells were labelled during 1h incubation in the medium supplemented with 74 kBq ^{3}H-dTh/ml (specific activity 0.73 TBq per mmol, ÚVVVR Prague). The ^{3}H-activity of DNA in the cells was detected by means of high-resolution autoradiography (standard procedure with stripping film Kodak AR.10) and, in some experiments, with the β-spectrometer Nuclear Chicago Mark 2. Either cells out of the S-phase, or all cells in the specimen after the block of DNA replication with 0.01 mol.l^{-1} hydroxyurea (HU) were checked in autoradiographs. The latter cells were also used for scintillation counting after their solubilization in an NCS-solubilizer (Nuclear Chicago).

UDS was induced after the exposure of the cells either to fast neutrons (6.2 MeV, 92.5 % neutrons + 7.5 % gamma rays) using cyclotron with a Be-target (Institute of Nuclear Physics, Academy of Sciences of the GDR, Rossendorf), or to gamma rays of a Chisostat ^{60}Co-unit (Chirana Prague), with the doses ranging from 10 to 200 Gy.

Results

Induction of UDS in LS/BL cells after the exposure to fast neutrons was compared with that induced by gamma rays of ^{60}Co. UDS was detected by means of high resolution autoradiography as ^{3}H-dTh incorporation into DNA of cells out of the S-phase. In the autoradiographs the S-phase cells were indicated by heavy labelled nuclei. Cells out of the S-phase labelled with few grains only were considered as UDS cells. Since a small proportion of weak labelled cells could also be observed in the autoradiogaphs of the non-irradiated cell population, this cell count was used to correct the number of UDS cells. The results summarized in Fig. 1 show that, on the average, fast neutrons were 2.8-times more effective as for UDS induction in LS/BL cells than were gamma rays.

In further experiments the dose effect of fast neutrons on UDS rate in single cells was studied (Fig. 2). A dose range of 10 to 200 Gy was used. UDS was detected autoradiographically in cells after blocking DNA replication with 0.01 mol.l^{-1} HU. Grain counts above labelled cell nuclei were determined in the autoradiograph. However, the so-called residual DNA synthesis was maintained in HU-treated control cells. This residual DNA synthesis was subtracted from the grain counts used for UDS characterization. As shown in Fig. 2 UDS was directly

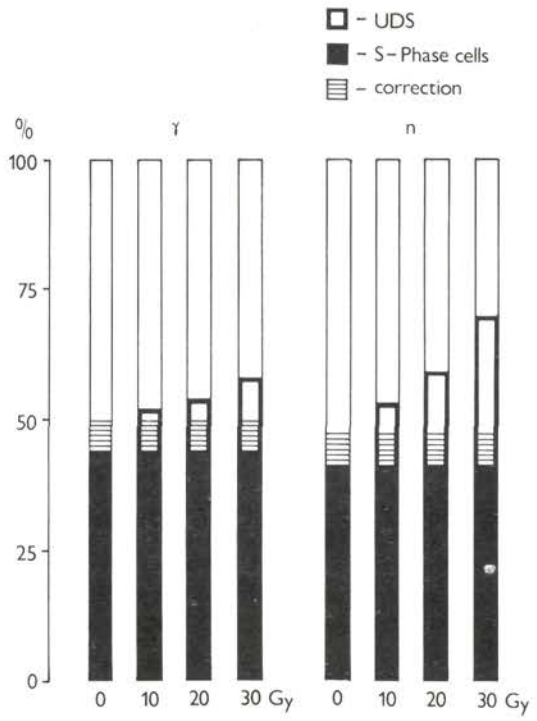

Fig. 1. UDS cell number/gamma ray (γ) and fast neutron (n) dose dependence. Black and hatched columns: S-phase cells; white columns: cells out of the S-phase. Bold columns represent cell numbers labelled with few grains only, white segments represent UDS cells, and hatched segments those which were only shortly in the S-phase at the labelling interval (Late entering or early leaving S-phase).

proportional to doses of up to 50 Gy. Further raising of the doses resulted in no further increase in UDS. UDS was inhibited by doses of 200 Gy.

Fig. 3 illustrates the time-course of UDS in LS/BL cells treated with HU after the exposure to gamma rays. The most probable graphical expression of the experimental data is given by the bold curve, calculated according to the equation: $y = 53.8599 - 3.067839x + 0.05848x^2 - 0.000197839x^3$. No significant changes in the time-course of UDS could be found over the 150 min of observation.

An attempt was made to estimate DNA material released during the excision process. Repeated application of ^{3}H-dTh (3 times, 8 hr intervals) resulted in a high labelling of the cell DNA. Free ^{3}H-activity was washed out (3 subsequent washings) using a medium supplemented with cold dTh (30 μg/ml) and the cells were exposed to 50 Gy gamma rays with subsequent determinations of the viability and cell counts. ^{3}H-activity in the cells and ^{3}H-activity released to the medium were estimated (Table 1). Approx. 1.9 % and 2.39 % of ^{3}H-activity originally bound in

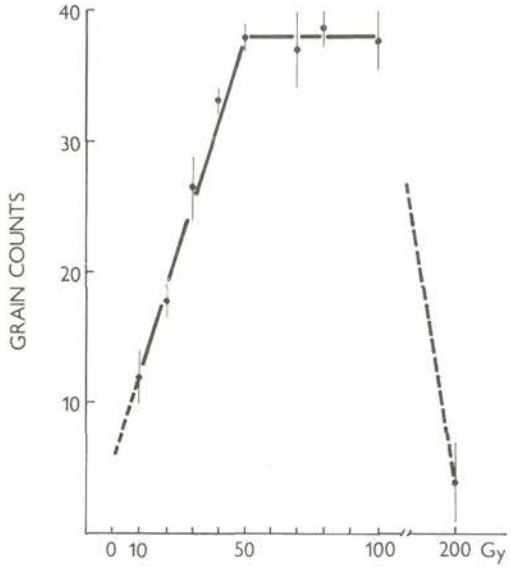

Fig. 2. UDS rate in HU-treated cells plotted against the dose of 6.2 MeV neutrons.

DNA was lost during 60 min and 120 min of incubation after cell irradiation, respectively.

Discussion

Autoradiography enables the detection of UDS in asynchronous proliferating cell populations as measured by ^{3}H-dTh incorporation into DNA using two modifications of the procedure.

The first procedure allows UDS evaluation in cells out of the S-phase only. This method was used for the comparison of the efficiency of fast neutrons (6.2 MeV) and gamma rays in inducing UDS in LS/BL cells. The cells in the autoradiographs were divided into three groups: cells with heavy labelled nuclei, cells with the nuclei labelled with few grains only, and unlabelled cells.

The cells with heavy labelled nuclei were considered as S-phase cells and were not taken for evaluation, even though UDS could have occurred. The cells with weak labelled nuclei were studied from two different aspects: i) cells which were only very shortly in the S-phase due to its late entering or early leaving (these cells were determined in a control autoradiograph); ii) cells in which UDS occurred. The numbers of UDS cells increased with the increasing radiation dose and the comparison of their counts between both experimental series revealed that fast neutrons were 2.8-times more effective in UDS induction than gamma rays.

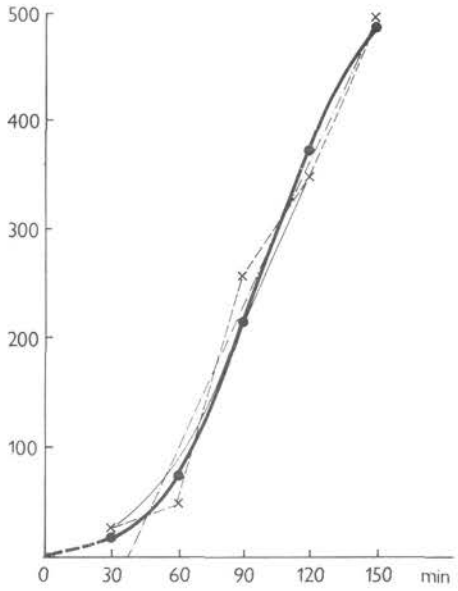

Fig. 3. UDS induced by 50 Gy as a function of time after gamma irradiation. The bold curve was calculated according to the equation: $y = 53.8599 - 3.067539\ x + 0.05848\ x^2 - 0.000197839\ x^3$.

The group of unlabelled cells behaved in a rather unexpected manner. They were considered as cells out of the S-phase without UDS. Regarding conditions of irradiation, and thus of UDS induction, the probability of DNA damage was approximately the same in all the cells. Either not all of the cells in the population were capable of UDS during the observation interval (this possibility remains still open), or the method used was not sufficiently sensitive. The rather low efficiency of the method is shown by the fact that at least 4 grains per nucleus were required in order that it might be evaluated as labelled at the 99.0 % probability limit.

The autoradiographic method allowing UDS estimation in the entire cell population is based on an experimental block of DNA replication. This procedure was employed for the study of the effect of fast neutron doses on UDS rate using HU block. This experiment showed that UDS was directly proportional to doses of up to 50 Gy. Further raising of the doses resulted in no further increase in UDS rate. The limited dose-proportionality is in good agreement with the findings by Sega et al. (1978), as well as with some data concerning DNA damage (Drášil and Jurášková 1979).

UDS was inhibited by doses of 200 Gy. As compared with doses used for UDS induction (e.g. Painter et al. 1972), a UDS inhibitory dose of 200 Gy seems to be rather low. However, the type of cells may play an important role since Sega et al. (1978) described UDS inhibition in spermatogonia after doses as low as 12 Gy,

Table 1. Loss of ^{3}H-activity from DNA of irradiated cells

Incubation	Cells exposed to 50 Gy			
	^{3}H-activity in	^{3}H [CPM]	^{3}H [%]	Loss [%]
60 min	Incub. medium	3,404	1.12	1.90
	5 % TCA	2,370	0.78	
	Cells	297,872	98.10	
	Total	303,545	100.00	
120 min	Incubation medium	5,962	1.91	2.39
	5 % TCA	1,162	0.48	
	Cells	304,272	97.61	
	Total	311,295	100.00	

while Krupnova and Zhestyanikov (1977) reported UDS inhibition in root cells of *Vicia faba* after doses as high as 1000 Gy.

Painter and Cleaver (1967) consider UDS and repair of DNA damage as signs of the same phenomenon. Correlation of UDS with some kind of DNA damage, e.g. with DNA single-strand breaks (ssb), would imply some relation in the time-course of UDS and ssb-repair. Fig. 3 shows that over the 150 min time interval UDS proceeded at an almost constant rate. On the other hand, about 80 % of ssb was repaired within approximately 30 min (Elkind and Kamper 1970). Also Koval et al. (1979) found a marked disproportion between the speed of UDS and ssb-repair; in TN-368 cells the UDS speed was 20-times higher than that in V—76—4 cells; however, there was only a threefold difference in the speed of ssb-repair.

Painter (1972), Painter and Young (1972) calculated that 2 to 3 deoxyribonucleotides are required for ssb-repair, while Roberts et al. (1971) reported about 100 deoxyribonucleotides. In our calculations with respect to ^{3}H-dTh utilization during UDS at 50 Gy exposure, some 10 000 deoxyribonucleotides are required for ssb in LS/BL cells (Beneš et al. 1979). This result may hardly be compared with the literary data. For this reason an attempt was made to estimate the quantity of DNA material released during the excision process. The loss of ^{3}H-activity from DNA of irradiated cell (Table 1) was of the same order as ^{3}H-dTh incorporation during UDS (Beneš et al. 1979). The increasing loss of ^{3}H-activity with time after

irradiation suggested that excision of damaged deoxyribonucleotides was almost parallel to the incorporation of new deoxyribonucleotides.

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