DNA Synthesis in Heterokaryons Formed by Fusion of X-Irradiated Mouse Cells

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Abstract. The capacity of cell fusion and DNA synthesis in heterokaryons formed by fusion of two mouse somatic cells was studied. Prior to fusion, either one or both lines were X-irradiated (doses of 5 and 20 Gy). In both cases the frequency of heterokaryon formation in cultures with irradiated cells was higher than that in the non-irradiated control populations. Autoradiographic analysis based on ^{3}H-thymidine incorporation showed pronounced inhibition of DNA synthesis in the control irradiated cells cultured separately, and a significantly higher number of nuclei capable of DNA synthesis in the heterokaryons. Heterokaryons had also more nuclei of irradiated parental cells with unscheduled DNA synthesis. Association of these two phenomena is apparent and they may represent one of the possible mechanisms enabling repair of cells damaged by ionizing radiation.

Key words: DNA-synthesis — Heterokaryons — X-irradiation — Cell fusion

Introduction

The inhibitory effect of ionizing radiation on DNA synthesis in mammalian somatic cells has been studied both on the molecular (Lukášová and Paleček 1971; Cramp et al. 1981; Saenko and Gotlib 1981) and cellular level (Leeper et al. 1973; König et al. 1975; Williams 1976; König and Baisch 1980; Houldsworth and Lavin 1980; de Wit et al. 1981). Radiation damage to mammalian cells is mainly due to the effect of radiation on the DNA metabolism and disturbances of the synthesis of other vitally important macromolecules. The most striking biochemical effect of radiation is a reduction in the incorporation of DNA synthesis precursors into the cell (Weise 1971; Walters and Hildebrand 1975; Hawkins et al. 1981; Kapp and Painter 1982). The present work involves a study of fusion of mouse somatic cells and the possibility of induction of DNA synthesis in the nuclei of heterokaryons with one or both of the parental cells having been irradiated.

Material and Methods

Parental cells: L cells cultivated on a monolayer in minimal Eagle's medium with 10 % inactive bovine serum and antibiotics (MEM), and LS/BL mouse lymphosarcoma cells which were cultured in the peritoneal cavity of C57BL mice were used. Cultivation of the parental lines and the fusion technique have been described elsewhere (Hofmanová and Spurná 1981).

X-irradiation: Prior to fusion, the parental cells were exposed to X-rays in doses of 5 or 20 Gy (TUR, 180 kV, 15 mA, filter 0,5 Cu plus 0,5 Al). The irradiation technique has been described elsewhere (Hofmanová and Spurná 1981).

Following removal from the peritoneal cavity of C57BL mice the LS/BL cells were washed twice in balanced salt solution (BSS), centrifuged, and the cell suspension (1×10^6/ml) was exposed to the same doses of ionizing radiation as the L cells.

The effect of the radiation dose on the proliferation of L cells (proliferative capacity, colony-forming ability and survival fraction) exposed to doses of 5—50 Gy has already been described (Hofmanová and Spurná 1981).

The viability of LS/BL cells was studied at one-hour intervals during a 6-hour incubation following removal from the peritoneal cavity and after irradiation (doses 5—20 Gy) using vital staining with 0.2 % eosin solution or determining the number of DNA synthesizing cells. For these experiments a suspension of non-irradiated or irradiated cells (2×10^6/ml) was incubated in twice-concentrated MEM medium in a roller at 37°C and pulse labelled 20 min before each withdrawal using ^{3}H-thymidine (4×10^4 Bq/ml). Smears of the cell suspension were fixed and processed by autoradiographical techniques as described below.

DNA synthesis: DNA synthesis was evaluated auroradiographically based on the incorporation of ^{3}H-thymidine (1.2×10^4 Bq/ml, specific activity 6.03×10^{11} Bq/mmol, Praha Řež), added to the cultivation medium 24 h before each sampling. Cell samplings were performed 24, 48, and 72 h after fusion. The preparations were fixed and processed autoradiographically (Ilford K2 emulsion, 34 h exposure, staining with methyl green-pyronin) (Hofmanová and Spurná 1981).

The unscheduled DNA synthesis (UDS) was evaluated autoradiographically; after the fusion the irradiated cultures were radioactively labelled for 60 min with ^{3}H-thymidine at a concentration of 4×10^5 Bq/ml (Hofmanová and Spurná 1981).

Results

Frequency of heterokaryon formation: The frequency of heterokaryon formation was significantly higher (by 7—20 %) in the mixed culture of LS/BL lymphosarcoma cells and irradiated L cells than in the non-irradiated controls; the highest numbers of heterokaryons appeared in cultures after 72 h following cell fusion: up to 16 % following the exposure of L cells to 5 Gy, and up to 25 % after the exposure to 20 Gy. Following the exposure of both parental lines to 5 or 20 Gy the numbers of heterokaryons in the culture were by 3—5 % higher than those in the non-irradiated control culture as well (Fig. 1).

DNA synthesis: In one series of experiments irradiated L cells and non-irradiated LS/BL cells were used, in another both parental lines were X-irradiated prior to fusion. Non-irradiated and irradiated L cells or mixed culture of non-irra-

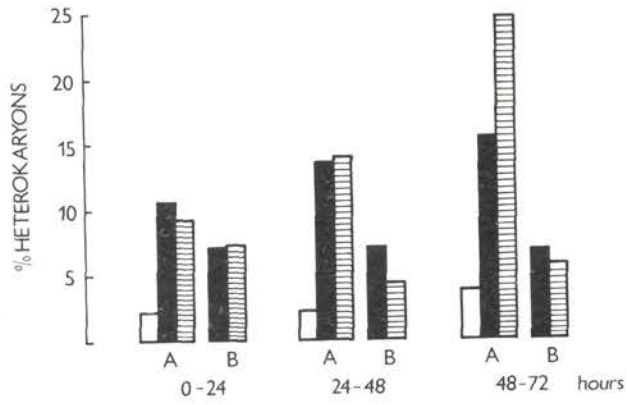

Fig. 1. Numbers of heterokaryons arising after fusion of L and LS/BL cells. Prior to fusion, L cells (*A*) or L and LS/BL cells (*B*) were irradiated with X-rays in doses of 5 Gy (■) or 20 Gy (▤). Non-irradiated cultures (□) were used as controls. 1000 cells were evaluated in each sample.

diated LS/BL and L cells were used as controls. In the experimental and control series the number of cells capable of incorporating ^{3}H-thymidine was established in 1. L cells cultivated separately, 2. mixed population of L and LS/BL cells, and 3. heterokaryons originating from the fusion of L and LS/BL cells.

DNA synthesis in L and LS/BL cells exposed to ionizing radiation: The number of DNA synthesizing independently cultured L cells changed in the non-irradiated population over the 72 h of cultivation as expected (84—91 % of cells able to incorporate radioactive thymidine). The number of L-cells capable of incorporating ^{3}H-thymidine after 5 or 20 Gy was by 35—62 % lower as compared with that in non-irradiated L cells; this drop was dose- and time-dependent (duration of the postirradiation interval).

The DNA synthesis in LS/BL cells themselves during a three-day cultivation was not established, since the cells can only be cultured in the peritoneal cavity of mice and their transfer to a permanent suspension culture requires a long adaptation period (Hill et al. 1970). The viability and DNA synthesis of LS/BL cells was studied during a short-term incubation only (1—6 h), immediately following the withdrawal of cells from the peritoneal cavity of mice, X-irradiation, washing, and centrifugation. The results of these experiments showed that the viability of the cells and the number of cells, capable of incorporating ^{3}H-thymidine decreased with the radiation dose and the duration of incubation (Table 1). In the initial stages of incubation, following 20 min of pulse labelling approx. 30 % of non-irradiated LS/BL cells and cells exposed to 5 Gy were in the S-phase; after three hours of incubation this number decreased to 20 % in cells exposed to 5 Gy, and to only 11 % in those exposed to 20 Gy as compared to non-irradiated controls. At the same time, the viability, evaluated on the basis of vital staining with eosin, decreased by 4 % as compared with non-irradiated controls. For this reason

Table 1. Viability of LS/BL cells and their DNA synthesis during short-term incubation in vitro. 1. % of living cells in the population (stained with 0.2 % eosin) 2. % of cells in S phase (population of cells pulse-labelled with ^{3}H-thymidine (4×10^4 Bq/ml) 20 min prior to withdrawal)

Incubations (hours)	Gy					
	0		5		20	
	1)	2)	1)	2)	1)	2)
0	—	27	—	34	—	11
1	91.6	32	92.2	30	87.2	26
3	94.9	30	92.8	20	90.7	11
5	—	10	—	12	—	4.5
6	77.6	11.5	68.0	9.5	61.6	5

Note: In each sample 500 cells were evaluated.

LS/BL cells were irradiated and used for co-cultivation or fusion with L cells no later then 1/2—1 h after their removal from the peritoneal cavity of mice; although DNA synthesis of the cells was partly inhibited at this moment, they were capable of interacting and fusing with the other parental line.

DNA synthesis in L and LS/BL cells cultured together or in heterokaryons arising from their fusion: The number of L cells replicating DNA, exposed to 5 Gy and cultured together with irradiated or non-irradiated LS/BL cells was approximately the same or lower than in the control cells that were exposed to radiation alone (Fig. 2). Following the exposure of L cells to 20 Gy, however, enhancement of the initiation of DNA synthesis in L cells cultured in the mixed population with non-irradiated or irradiated LS/BL cells was apparent. This is illustrated on Fig. 2. The numbers of L cells replicating DNA increased when cultured together with non-irradiated LS/BL cells, amounting to 11 % after 48 h, and to 14 % after 72 h when cultured with irradiated LS/BL cells.

However, statistically significant increase in the number of L cells capable of DNA synthesis after irradiation was found particularly in heterokaryons arising from the fusion of irradiated L and non-irradiated or irradiated LS/BL cells ($P = 0.01$). In these heterokaryons the number of L cell nuclei able to incorporate radioactive thymidine increased by as much as 21 % as compared to the control population of irradiated L cells cultured separately, and up to 20 % when both parental cells were irradiated.

In evaluating the number of LS/BL cells synthesizing DNA after irradiation when cultivated together with L cells or in their heterokaryons after fusion, the number of non-irradiated LS/BL cells incorporating radioactive thymidine and cultured together with non-irradiated L cells was used as a control; the results of these experiments showed that after irradiation the number of LS/BL cells capable of DNA replication was higher than that in the controls (Fig. 3).

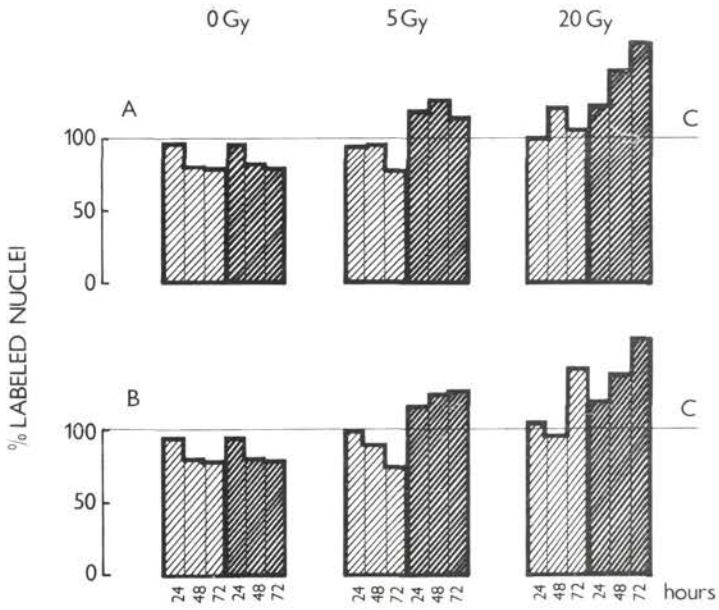

Fig. 2. Numbers of DNA synthesizing L cells (%) cultured together with LS/BL cells (▨) or in heterokaryons arising from their fusion (▩). L cells (*A*) or L and LS/BL cells (*B*) were exposed to 5 or 20 Gy. The cultures were labelled with ^{3}H-thymidine (1.2×10^4 Bq/ml) 24 h prior to each withdrawal. The resulting values were related to the number of radioactive L cell nuclei exposed to 5 and 20 Gy and cultivated separately (= 100%). 1000 cells were evaluated in each sample.

Unscheduled DNA synthesis (UDS): In order to establish the possible interference with unscheduled DNA synthesis induced by high doses of ionizing radiation (Painter and Young 1972), L cells were exposed to doses of 5 or 20 Gy, and subsequently fused with LS/BL cells, both non-irradiated and irradiated with the same doses (see under Methods). Irradiated L cells cultured separately were used as controls. The numbers of heavily labelled L cells replicating DNA (those in the S-phase) varied from 67 to 71 % in all groups; the number of lightly labelled cells, i.e. those with non-replicative DNA synthesis (UDS), was on average by 10—15 % higher in heterokaryons than in irradiated L cells, or a mixed population of L and LS/BL cells (Fig. 4). The unscheduled DNA synthesis expressed as the number of lightly labelled cells or the number of grains over their nuclei depended on radiation dose and occurred respectless of whether one or both parental lines had been irradiated prior to fusion (Table 2).

Discussion

Interaction and induction of DNA synthesis in heterokaryons formed by fusion of

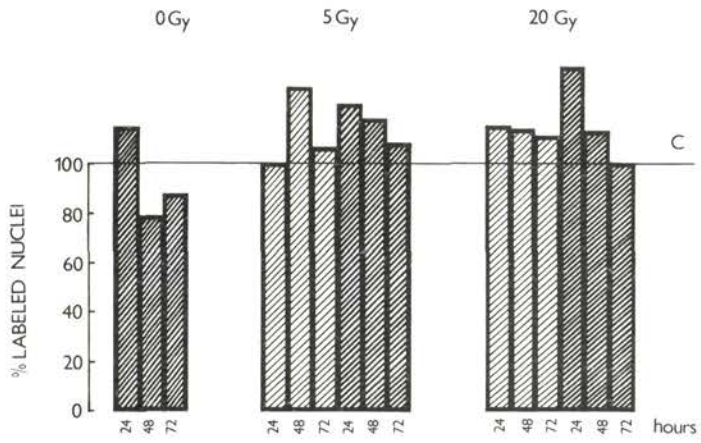

Fig. 3. Numbers of LS/BL cells (%) synthesizing DNA; (▨) cultured in a mixed population with L cells; (▨) heterokaryons arising from their fusion. Both parental lines were exposed to 5 or 20 Gy. The resulting values were related to the number of radioactive LS/BL cells in a non-irradiated L cell culture (= 100%). 500 cells were evaluated in each sample.

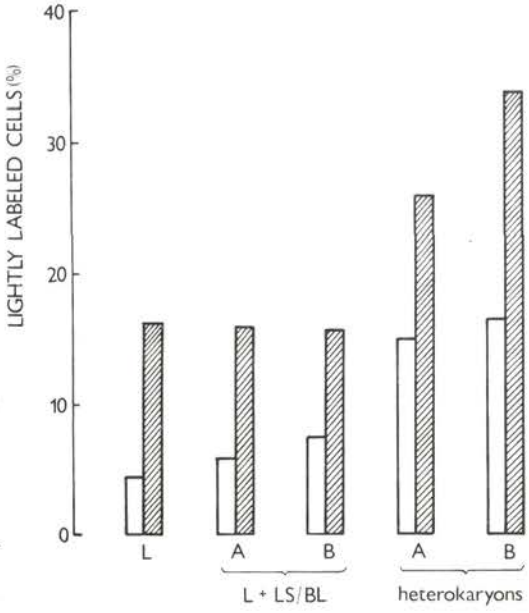

Fig. 4. Unscheduled DNA synthesis in L cells cultured separately (L); in mixed population with LS/BL cells (L + LS/BL); or in heterokaryons arising from their fusion (lightly labelled non-irradiated control L cells were substracted). Prior to fusion, L cells (*A*) or L and LS/BL cells (*B*) were exposed to 5 (□) or 20 (▨) Gy. The cultures were labelled with ^{3}H-thymidine (4×10^5 Bq/ml) and 1000 cells were evaluated in each sample.

Table 2. Unscheduled DNA synthesis in L cells 1 h after fusion expressed as the number of grains above their nuclei. L cells cultured separately (1), co-cultivated with LS/BL cells (2), or heterokaryons after fusion (3).

Gy		Average number of grains		
L	LS/BL	1	2	3
5	0	22.6	22.7	23.4
5	5		17.3	21.5
20	0	34.6	32.6	25.4
20	20		34.4	29.8

Note: In each sample 50 cells were evaluated

irradiated mouse somatic cells were studied. Cell populations with their DNA replication ability being impaired by ionizing radiation were used for fusion with another, irradiated or non-irradiated, genetically related cell line; we tried to establish whether the interaction between the nuclei in the common cytoplasm of the heterokaryon might lead to processes resulting in the induction of DNA replication. In many other systems of mammalian cells, interactions have been described resulting in reactivation of RNA and DNA synthesis (Harris et al. 1969; Ringertz and Bolund 1974) and expression of some enzymes (Harris and Cook 1969). Following fusion with cells with normal DNA replication DNA synthesis may also be induced in senescent cells (Norwood and Zeigler 1977), or in cells where DNA synthesis has been inhibited by other factors (Norwood et al. 1979; Mercer and Schlegel 1980).

In our experiments, X-irradiated (5 or 20 Gy) L and LS/BL mouse lymphosarcoma cells were used as the parental lines, and the DNA synthesis was measured using radioactive thymidine. As compared to the non-irradiated population of L cells, in irradiated cells the number of cells capable of DNA synthesis was decreased to 50 % after 48 h; following 20 Gy, the respective value was only 38 % of the control level after 72 h incubation. As expected, the proliferation capacity was diminished (Hofmanová and Spurná 1981), the cells were however able to fuse with other, irradiated or non-irradiated, lines of LS/BL cells; the fusion frequency was even higher than that with non-irradiated cell lines.

The second parental line, LS/BL cells, was irradiated immediately after the withdrawal from the peritoneal cavity of mice. The cells were used for experiments immediately after the irradiation, i.e. still in the period when the number of cells capable of DNA synthesis was limited, but their viability was sufficient to obtain the expected interaction with irradiated L cells.

The interaction of irradiated parental cells during 1—3 day cultivation in

mixed culture, or in heterokaryons arising from their fusion, was studied. In both cases there were more cells in the population capable of initiating DNA synthesis than in the irradiated L cells cultured seperately. The interaction of the cells was not only reflected in increased heterokaryon production, but in higher numbers of nuclei of irradiated cells capable of incorporating radioactive thymidine as well. Interactions occurred both in heterokaryons arising from the fusion of irradiated and non-irradiated cells and in those originating from the fusion of two different irradiated cells.

The possible causes of the increased heterokaryon production in the intraspecific system used in our experiments have already been discussed (Hofmanová and Spurná 1981); they have also been supported by findings of other authors who studied changes in the structure of the cell membrane produced by ionizing radiation. These changes could be one of the possible causes of the increased sensitivity to the formation of intercellular connections between neighbouring cells and their subsequent fusion (Sato et al. 1972; Baisch and Bluhm 1978; Baisch 1978; Fonck and Konings 1980).

The increased number of cells in the mixed population capable of DNA synthesis, especially heterokaryons, indicates that the interaction between their macromolecules resulted in the induction of some mechanisms enabling the repair of the incurred damage. These conclusions are supported by the results obtained by Paterson et al. (1974); they found that in heterokaryons arising from the fusion of human and chicken fibroblasts with one of the lines exposed to UV-radiation prior to fusion, the repair enzymes specific to each of the species were not functionally inhibited, and were capable of diffusing into the other nucleus and operating on the damaged DNA with the same effectiveness as in their own nucleus.

These conclusions are also supported by the fact that unscheduled DNA synthesis (UDS), studied in parallel experiments, occurred at much greater frequency in irradiated heterokaryons than in L cells irradiated separately. In our experimental system the interference of UDS with the cells'own DNA synthesis could be ruled out (Hofmanová and Spurná 1981).

The increased number of cells replicating DNA and the increased number of cells with UDS found in heterokaryons following fusion of mammalian cells injured by ionizing radiation can therefore be suggested to be two separate but parallel phenomena; they may be a result of certain mechanisms contributing to the repair processes following cell irradiation. The nature of these repair systems and the possibilities of their induction should be studied on the molecular level; factors acting on replicons or clusters of them designed to initiate DNA replication after cell irradiation may be involved (Painter and Young 1975; Makino and Okada 1975; Tolmach and Jones 1977), or factors inducing changes in the template activity for DNA polymerase or on DNA metabolism and changes in its structure (Walters and Enger 1976) may be operative.

The possible role of such mechanisms is also indicated by the survival and proliferation of hybrid cells following fusion of lethally irradiated parental cells (Jullien et al. 1978; Donald et al. 1981), and by increased radioresistance and repair in some hybrid clones (Robertson and Raju 1980; Fox 1979; Limbosch 1982).

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