INFLUENCE OF FREE 5-BROMODEOXYURIDINE ON SCE INDUCTION IN HUMAN LYMPHOCYTES IRRADIATED AT THE PRESYNTHETIC STAGE

T. Kondrashova, N. Luchnik

Medical Radiology Research Centre, Obninsk, Russia

INTRODUCTION

Published data on induction of sister chromatid exchange (SCE) in mammalian cells by gamma-irradiation at the G_1 stage contain a contradiction: irradiation at the G_1 of the first replication cycle in the presence of 5-bromodeoxyuridine (BrdU) did not induce SCE (1, 2), while irradiation at the G_1 of the second cycle enhanced its frequency significantly (3, 4). Explanations of this controversy suggested either that chromosomes containing no BrdU were insensitive to gamma-rays, contrary to those BrdU-substituted; or that DNA lesions formed in the first cycle induced isolocus SCE both in the first and in the second cycle, these exchanges annihilating each other. However, an attentive analysis of the published works shows that in all cases BrdU was added to the culture just before the onset of the S stage of the first cell cycle; thus, the cells were exposed in absence of BrdU at the G_1 of the first cycle, and in its presence at the G_1 of the second cycle. In an earlier paper (5) we have shown that this difference in the experimental protocols, which seemed to escape notice in interpretations of the results, was very likely the reason of the above discrepancies. Here we describe the results of experiments directly addressed to this problem.

SCE INDUCTION BY IRRADIATION AT THE G_1 STAGE OF THE FIRST CYCLE

Fig. 1 shows the data on SCE frequencies induced in cultured human lymphocytes at the G_1 stage of the first post stimulation cycle by 2 Gy ^{60}Co gamma-rays for different schedules of BrdU addition to the culture. The cells were irradiated either immediately after PHA-stimulation (early G_1), or after 10 (middle G_1) or 20 h (late G_1) incubation. BrdU (final concentration 9×10^{-5} M) was added either at 23 h after PHA-stimulation (i.e. just before the onset of the S phase), or simultaneously with PHA, or immediately after irradiation. The cells were harvested at 59.5 h after PHA-stimulation. The comparison of the left (grey) columns between four groups displayed in the diagram shows that irradiation in the absence of BrdU did not induce SCE. From the comparison of the dark columns it is evident that, provided BrdU was present in the culture from the very beginning, irradiation enhanced SCE frequency reliably. Moreover, the control frequency was significantly higher in the latter case

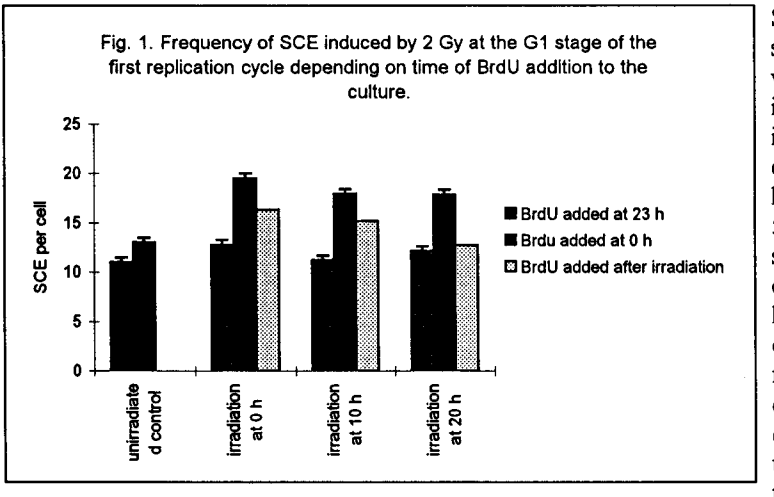

Fig. 1. Frequency of SCE induced by 2 Gy at the G1 stage of the first replication cycle depending on time of BrdU addition to the culture.

than in the former (see the first pair of columns). Comparison between light-coloured columns in three groups and comparison between light and dark columns in each group implies that presence of BrdU in culture medium was essential for SCE formation both at the moment of irradiation and during postradiation incubation of cells.

SCE INDUCTION BY IRRADIATION AT THE G_1 STAGE OF THE SECOND CYCLE

The other set of experiments was aimed to study SCE induction by irradiation at the G_1 stage of the second replication cycle of lymphocytes. BrdU was added to the culture simultaneously with PHA. Three variations of experimental protocol were employed: (1) the cells were irradiated immediately after washing off BrdU; (2) quite the reverse, BrdU was washed off immediately after irradiation; (3) BrdU was washed off 1h after irradiation. It was important to choose the time of BrdU removal so that the bulk of cells would pass only one replication cycle in its presence and the subsequent cycle without it. Under this condition, the time of BrdU removal and that of irradiation being very close to each other, one can be sure that the metaphases with differentially stained sister chromatids, which are to be analysed, are those very cells which were irradiated at the G_1 stage of the second cycle. In preliminary experiments this time was determined as 40 h after PHA-stimulation. Cells were harvested at 62 h after stimulation.

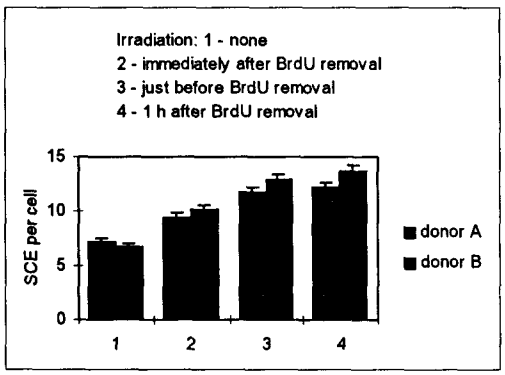

Fig. 2. Frequency of SCE induced by 2 Gy at the G_1 stage of the second cycle depending on time of BrdU removal from the culture.

The results obtained for two donors are illustrated in Fig. 2. Unlike the data for cells irradiated in the first replication cycle, exposure at the G_1 of the second cycle induced SCE even in the absence of BrdU (comparison of the first pair of columns to the second). This may be associated either with the different composition of chromatids (they did not contain BrdU at the G1 of the first cycle, but contained it at the G1 of the second one) or with rather prolonged presence of BrdU in the culture medium before irradiation in the second cycle. However, consideration of all the four groups shown in Fig. 2 evidences that, like in the case of irradiation in the first cycle, the presence of BrdU during the irradiation in the second cycle (and during an hour post irradiation) reliably increased the frequency of radiation induced SCE.

CONCLUSIONS

Irrespective of the number of replication cycle (the first or the second) and of the extent of thymidine substitution with BrdU in chromosomal DNA, irradiation in the presynthetic stage induced significantly more SCE in the presence of BrdU in culture medium than without it. Presence of BrdU was essential for SCE formation both in the moment of irradiation and during postradiation incubation of the cells. It should be underlined that this effect cannot be associated with a direct modification of chromosome radiosensitivity by BrdU, because we are dealing with the presynthetic stage. The influence of free (not incorporated into chromosomes) BrdU can probably be explained by its interplay with mechanisms of repair of initial genetic changes (or lesions). The biochemical possibility of such an interplay had been described (6). Evidences indicating a regular repair process taking place in the G_1 stage were accumulating

over a period of years (see (7) for references) and seem to find a further substantiation recently (8).

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