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2-Deoxy-D-glucose induced enhancement of radiation damage in 5-bromo-2′-deoxy-uridine sensitized mammalian cells

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The effect of 2-deoxy-D-glucose (2-DG) and 5-bromo-2′-deoxyuridine (BUdR) on Co 60 gamma ray induced damage has been studied in mammalian cells (BHK 21) in culture. Frequencies of cells with micronuclei were measured after 30 h of growth to assay radiation damage. Incorporation of BUdR (0·25 μg/ml for 24 h) and presence of 2-DG (5 m*M*) for 4 h after irradiation significantly increased the radiation damage. If BUdR and 2-DG treatments were combined, the enhancement in radiation damage was observed to be more than additive. These results provide important leads for improving radiotherapy of tumours.

To improve radiotherapy of tumours, it is necessary to reduce radiation damage to normal tissues and to enhance the damage in cancer cells. A number of biophysical and radiobiological approaches to achieve this are being investigated. Our earlier experiments[1-5] have shown that post-irradiation presence of 2-deoxy-D-glucose (2-DG), an inhibitor of glycolytic pathway for the supply of metabolic energy (ATP), can differentially enhance the radiation damage in cell systems with high rates of glycolysis (such as tumour tissue) and reduce the same in normal cells. These differential effects are achieved, possibly, by 2-DG induced modifications of energy linked processes of repair and fixation of repairable DNA lesions produced by irradiation[4]. We undertook, therefore, to explore the possibility of enhancing these 2-DG induced differential effects further, by increasing the number of repairable lesions in DNA of tumour cells, with the help of suitable radiosensitizers.

Halogenated analogues of thymidine like 5-bromo-2′-deoxy-uridine (BUdR) can be incorporated into the DNA of proliferating cells during the phase of DNA synthesis. Radiosensitizing effects due to the incorporated BUdR have been observed in various cell systems[6,7]. We have, therefore, carried out experiments to study the effects of 2-DG on radiation damage in BUdR incorporated cells. Frequency of micronuclei formation was measured as an index of DNA damage since this index has been shown to correlate well with cell death[8]. The results of some of our preliminary studies are reported in the present communication.

Material & Methods

A transformed cell line of Baby Hamster Kidney (BHK-21) cells initially obtained from the Indian Veterinary Research Institute, Bangalore, was used in these experiments. The cells were grown as monolayer in 5 per cent CO_2 atmosphere at 37°C in 55 mm plastic petri dishes (Laxbro, India). Growth medium consisted of Dulbecco's modified minimum essential medium (DMEM; Hi-Media, Bombay) supplemented with 10 per cent bovine serum, 10 per cent tryptose phosphate broth and antibiotics (50 units/ml penicillin, 35 μg/ml streptomycin and 2·2 μg/ml nystatin). The cell cycle time under our experimental conditions was 13±1 h. BUdR (Sigma, USA, final concentration 0·25 μg/ml) was added to the cultures 24 h after the inoculation of the cells. Twenty four hours later, the cells were washed with Hank's balanced salt solution (HBSS) and prewarmed HBSS with or without 2-DG (5 m*M* final concentration, equimolar with glucose) was added. Cells were irradiated at room temperature with Co-60 γ-rays (absorbed dose 2·5 Gy; dose rate 0·8 Gy/min) and subsequently incubated at 37°C. Four hours after irradiation, 2-DG was removed by washing with DMEM and the cultures were incubated further at 37°C in fresh growth medium. Thirty hours after irradiation, the cells were released by incubating with 0·5 per cent neutral protease (Dispase, Boehringer, West Germany) in phosphate buffered saline (*p*H 7·4), resuspended in DMEM and were fixed in methanol : acetic acid (3 : 1). Slides were prepared by air drying and stained in 5 μ*M* diamidino-2-phenyl-indole hydrochloride (DAPI, Serva, Heidelberg), a DNA specific fluorochrome, in phosphate buffer (*p*H 7·4) containing 0·05 per cent Tween-20[5]. The radiation damage to cells was assayed by scoring of micronuclei according to well established criteria[9]. The frequency of cells with micronuclei in the cell population (M-fraction) was determined by analyzing at least 1000 cells per sample.

Results & Discussion

Values of M-fraction obtained after different treatments are shown in the Table. It can be seen that BUdR and 2-DG given alone or in combination had no effect on micronuclei formation in un-irradiated cells. However, incorporation of BUdR before irradiation significantly enhanced the micronuclei formation. Presence of 2-DG also resulted in a slight increase in the γ-ray induced micronuclei. It is interesting to note that 2-DG treatment after γ-irradiation of the BUdR sensitized cells enhanced the formation of micronuclei by a factor of 1·8, which shows more than additive effect with respect to the treatment with BUdR or 2-DG alone. Lower concentration (1·25 m*M*) of 2-DG, however, did not show any significant change in the M-fraction in the BUdR incorporated γ-irradiated cells (data not shown).

Our observations offer interesting leads for improving tumour radiotherapy, as administration of BUdR and 2-DG may differentially enhance radiation damage in proliferating as well as quiescent and hypoxic tumour cells. Malignant brain tumours would be very appropriate for initiating clinical trials to verify this approach, because BUdR is not incorporated into the normal brain cells which

Table. Effects of BUdR and 2-DG on gamma-ray induced micronuclei formation in BHK-21 cells, measured 30 h after irradiation

Treatment				
BUdR (μg/ml)	Gamma rays (Gy)	2-DG (mM)	M-fraction (%)	Enhancement ratio
—	—	—	1·2±0·4	—
0·25	—	—	1·7±0·3	—
—	—	5	2·0±0·5	—
0·25	—	5	1·9±0·6	—
—	2·5	—	16·0±1·0	1·0
0·25	2·5	—	20·4±0·7	1·3
—	2·5	5	19·9±1·6	1·2
0·25	2·5	5	28·0±2·5	1·8

BUdR was given for 24 h and 2-DG for 4 h. Values represent mean±SD of 4 experiments. Irradiated-petri dishes were taken in duplicate in each experiment

are usually non-proliferating. Initial clinical trials using intra-arterial or intravenous infusions of BUdR have suggested radiosensitization of tumour cells in patients with glioblastoma[10,11]. However, considerable cytotoxicity in the bone marrow and skin has been observed. The combination of BUdR and 2-DG may reduce the concentrations of BUdR necessary for manifesting radiosensitization and consequently reduce the side effects. Further studies to test this approach are in progress.

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