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DNA DAMAGE IN CLINICAL RADIATION THERAPY STUDIED BY MICRO-ELECTROPHORESIS IN SINGLE TUMOUR CELLS

A preliminary report

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Abstract

Aspiration biopsy specimens were taken from malignant tumours—1 Hodgkin's lymphoma, 3 non-Hodgkin lymphomas, 1 squamous cell carcinoma and 1 adenocarcinoma—before and after irradiation. Individual cells were analysed by micro-electrophoresis, a new technique which estimates radiation-induced DNA strand breaks. The cells were embedded in agarose gel; after lysis of the cells in a neutral detergent solution, an electric field (5 V/cm) was applied for five minutes. DNA showed a tendency to migrate, some cell diameters, and was more pronounced in irradiated than in control cells. The DNA migration was evaluated by a microscope photometer which estimated the fluorescence in cells stained with acridine orange. This technique was found to be suitable for human material in vivo as only a few cells are needed and no radioactive prelabelling is necessary.

Key words: Radiation biology; DNA damage, tumor cells, mircoelectrophoresis.

Damage to DNA is thought to be the critical event which gives rise to cell death after irradiation. It appears that DNA double strand breaks or other DNA breaks which are not rejoined one to two hours after irradiation are somehow involved in the induction of chromosomal damage and cell death (1, 6). Single strand breaks seem to be rejoined, as shown by present methods (2, 4).

A correlation between the result of tumour therapy using DNA damaging agents, such as ionizing radiation and many cytostatics, and the amount of damage after a certain repair phase does probably exist.

In studies of DNA breaks after a radiation dose of 1 to 5 Gy the two most common methods used today are the DNA unwinding (9, 10) and the filter elution (5) methods. The first can be used in the study of cultured cells after irradiation using doses of 0.05 to 0.1 Gy as a lower limit (10). The method has been used also after doses of a few Gy given to cells in tissues (3, 11). Filter elution can be used following doses of 0.1 Gy or more (5). The number of cells used in both methods must be greater than 10000.

Our new method of studying DNA breaks in individual cells is based on embedding cells in agarose gel followed by lysis of the cells in a neutral detergent solution and study of the migration of DNA in an electric field (7). The DNA is stained with acridine orange and the intensity of fluorescence is measured by a microscope photometer using a small measuring diaphragm first in the centre of the 'cell', then at 50 μm in the direction of migration. The ratio between these two measurements is considered to quantify the amount of DNA breaks induced in the cell.

The present work was done during an extended period when developing the new technique. This preliminary report shows that the technique may be used to study the induction and repair of DNA strand breaks in human tumours during radiation therapy. The technique is relatively non-traumatic and requires only a few cells.

Material and Methods

Sampling. Biopsies from 6 patients with superficial tumours were examined: 3 malignant lymphomas of non-

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Table

The clinical and cytologic characteristics of the examined cells and patients

Case No.	Age and sex	Diagnosis	Localization	Cytology	Diameter
1	28 M	Hodgkin's disease (nodular sclerosis)	Cervical lymph node	Immature lymphocytes, some Reed-Sternberg cells, high number of reticulo-endothelial cells	15 μm 20–30 μm 40–50 μm
2	63 F	Non-Hodgkin lymphoma (immunocytoma)	Skin manifestation on the lower part of the abdomen	Numerous uniform lymphocyte-like highly differentiated cells	10–15 μm
3	57 M	Non-Hodgkin lymphoma (immunoblastic)	Cervical lymph node	Numerous fragmented histiocyte-like cells	5–35 μm
4	72 M	Non-Hodgkin lymphoma (centrocytic-centroblastic)	Cervical lymph node	Numerous, partially fragmented poorly differentiated lymphocytes	15–30 μm
5	70 M	Metastatic nodule of poorly differentiated squamous cell carcinoma	Left thigh	Numerous polymorphic squamous carcinoma cells	15–50 μm
6	68 M	Poorly differentiated adenocarcinoma	Cervical lymph node	Abundant cytoplasm, adenocarcinoma cells	30 μm

Hodgkin type, and 1 each of Hodgkin's disease, squamous cell carcinoma and adenocarcinoma (Table). The diagnosis had been confirmed by surgical biopsy before the initiation of treatment.

The tumour cells were obtained by the aspiration technique (12) using a needle 30 mm long and 0.7 mm in diameter. Two punctures were made immediately before radiation treatment. The first aspirate was used to prepare a smear and was stained by the May-Grünwald Giemsa method and examined by light microscopy. The second aspirate was ejected into a tube containing 1 ml ice-cold phosphate buffered saline (PBS) and stored on ice until analysis of the DNA damage was made. A new puncture was made 1 to 2 minutes after irradiation and a further 15 to 45 minutes later. They were used for analysis of DNA damage.

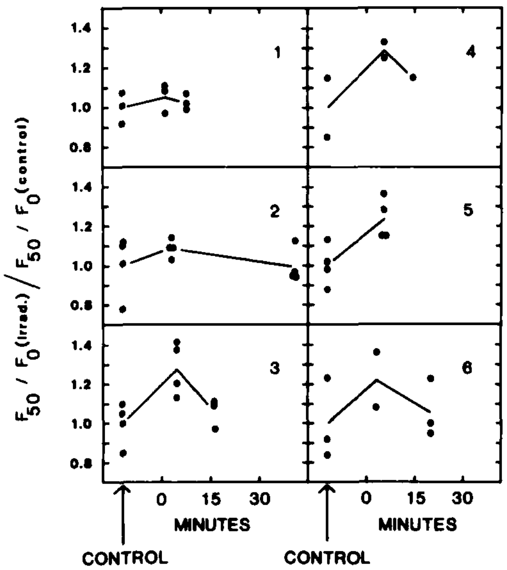
Irradiation. The treatment was in all cases given by a roentgen beam from a Philips 370 SL linear accelerator operated at 8 MV. The dose varied in each fraction between 1.8 and 3.5 Gy. The dose rate was 1 Gy per minute and the size of the field varied from 5×5 cm^2 to 10×10 cm^2. With the exception of cases 3 and 6 no previous irradiation or cytostatic treatment had been given.

Analysis of DNA damage. The details of the method have been given previously (7). Suspensions of cells were mixed with melted agarose (low gelling temperature; FMC Corp, Marine Colloids Div., USA) at 33°C and spread on microscopic glass slides which were chilled on ice to induce gel formation. Each cell was surrounded by agarose gel, making difficult the diffusion of DNA with its extremely high molecular weight. The cells were lysed in neutral 2.5% sodium dodecyl sulphate and then an electric field of 5 V/cm was applied for 5 min. The slides were then washed, dried and mounted in acridine orange solution (2 μg/ml). The fluorescence was measured in a microscope photometer (Leitz MPV II) at 525 nm, i.e. the fluorescence from acridine orange bound to double-stranded DNA. The fluorescence was measured at the center of what remained of the cell (F_0) and at a distance of 50 μm from that point in the direction of the migration of the DNA (F_{50}). The ration F_{50}/F_0 was considered to be a measure of the DNA damage in the cell studied. The cells analysed were chosen randomly, and no distinction between tumour and healthy cells was made.

Results

Cytologic analysis. The cytologic specimens were evaluated, special attention being paid to the number of cells, polymorphism and variation in cell diameter (Table).

Analysis of DNA damage. A summary of the results is shown in the Figure. After irradiation the DNA migrated in the electric field to a greater extent, giving a higher ratio (F_{50}/F_0). We interpret this as a measure of the number of radiation-induced DNA strand breaks (7). Irradiation induced breaks in the DNA and most of these breaks rejoined soon after irradiation. The method allows resolution of the DNA breaks in control cells and in tumour cells taken as soon as possible after irradiation with about 2 Gy. The repair period in these cells was 1 to 2 min plus the time needed for irradiation.



Case	Age	Sex	Diagnosis	Dose (Gy)
1	28	M	Mb Hodgkin	1.8
2	63	F		3.0
3	57	M	Non-Hodgkin lymphoma	2.0
4	72	M		2.0
5	70	M	Squamous cell carcinoma	3.5
6	68	M	Adenocarcinoma	2.5

DNA analysis of 6 cases. The results are expressed as the ratio of the green fluorescence 50 μm in the direction of migration from the centre of the cell cavity to that of the centre of the cell cavity. Each ratio is normalised to the average ratio obtained from control cells. Time is measured in minutes from the middle of the irradiation period until the cells are ice-chilled. Each point represents the average of measurements of 10 cells on one slide. Case number is marked at the upper right corner of the curves.

Discussion

Cells embedded in agarose gel form a cavity in it. After lysis of the cells the DNA seems to fill up the cavity. When an electric field is applied, DNA migrates partially into the gel; this migration is more pronounced for DNA of irradiated cells. A fibrous structure can be observed in the microscope, indicating DNA fibres in the direction of the migration. Whether a real migration of the DNA molecule occurs is however doubtful. The stretching of the DNA molecules fixed to the nuclear matrix would be an alternative explanation (7, 8).

We present here six cases of superficial tumours after radiation therapy. All the tumours were available for analysis of the induction and rejoining of the radiation-induced DNA strand breaks in cells obtained by fine needle aspiration. None of the patients had any obvious discomfort from the fine needle technique despite repeated aspirations before and after irradiation. The number of cells obtained was sufficient in each sample. There is a larger variation in the results than normally obtained in studies using cells in vitro. But the method seems to be semi-quantitative. Further development of the technique is in progress. It seems to be possible to discriminate cell populations of different sizes by comparing them with the simultaneous cytologic smears.

In some cells, the DNA was markedly degraded, which was indicated by an extended migration of fragmented pieces of DNA compared with the native DNA of living cells. This might indicate cell death.

The method described seems promising for the study of the effect of DNA-damaging agents. As the cells are collected by a fine needle non-traumatic aspiration technique, it is applicable clinically.

In the future, our approach may have a predicting value for the effects of radiation and other DNA-damaging agents such as cytostatics.

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