FROM RADIUMHEMMET AND THE DEPARTMENT OF HOSPITAL PHYSICS, KAROLINSKA SJUKHUSET, S-104 01 STOCKHOLM, SWEDEN.

EFFECT OF RADIATION THERAPY ON THE MITOGENIC RESPONSE OF IN VITRO IRRADIATED HUMAN LYMPHOCYTES TO PHYTOHAEMAGGLUTININ

EDWARD BARAL, HENRIC BLOMGREN, NINA EINHORN, INGMAR LAX and INGVAR JUHLIN

Radiation therapy of patients with malignant tumours may result in a peripheral lymphopenia lasting for several years (HEIER et coll. 1975, BARAL et coll. 1977). Most extensive reductions of lymphocyte numbers are observed when large blood vessels are included within the irradiated region indicating that lymphopenia is due to killing of cells by radiation (CHEE et coll. 1974). Recently it was observed that radiation therapy decreases both the number of T- and non-T-lymphocytes (BLOMGREN et coll. 1974 a, b, HEIER et coll., RABEN et coll. 1976).

Previous in vitro experiments indicated that the peripheral T-lymphocyte population in the human is composed of two subpopulations whose PHA reactivity differs in radiation sensitivity. The aim of the present report is to present an investigation on whether irradiation induces a shift of the ratio of these two subpopulations.

Material and Methods

The material comprised 16 patients ranging in age from 45 to 80 years, 14 of whom were admitted for carcinoma of the cervix uteri, one for carcinoma of the uterine body stage II and one for carcinoma of the vagina stage IV. In carcinoma of the cervix, 7 patients belonged to stage II A. All the other stages had one or two patients in each group.

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Radiation therapy. The intracavitary irradiation was given as two local radium applications at an interval of three weeks. For the patients with carcinoma of the cervix, the treatment usually consisted of the insertion of the tandem in the cervix and centrally in the uterine cavity, and a vaginal applicator against the portio uteri. The amount of radium in the uterus varied, with the length of the uterus, between 40 and 70 mg, and in the vagina between 60 and 90 mg. The treatment time varied between 20 and 30 hours for each application. The total dose at the surface of the uterine mucosa was calculated to be 200 to 300 Gy and at a depth of 2 cm 50 to 70 Gy (KOTTMEIER 1964, FORSBERG et coll. 1977). The integral dose was calculated to be approximately 100 kg Gy in each application. All patients received after an interval of 3 to 4 weeks external irradiation to the pelvis with 42 MV roentgen radiation from a betatron to a dose of 40 to 45 Gy during 5 to 6 weeks. Shielding was applied towards the bladder and the rectum, to a degree depending on the dose given previously to this region by the ntracavitary treatments. All of the patients were treated with anterior and posterior beams covering an area from the height of L4 to L5 down to the vagina with a good margin for covering the tumour. The lateral limit of the beams covered the lateral pelvic walls with a margin of at least 1 cm. Totally the irradiated area was approximately 16 cm × 18 cm. The integral dose in the external treatment was calculated to be approximately 400 kg Gy. The calculated integral doses are estimated mean values for the patient group. The treatment of the two patients with carcinoma of the vagina and uterine body, respectively, was similar to the one described.

Preparation of cell suspensions. Nucleated cells were separated from heparinized venous blood by centrifugation on Ficoll-Isopaque (JONDAL et coll. 1972). The cells were washed twice by centrifugation in Eagle's Minimal Essential Medium supplemented with Earle's salts (MEM). Approximately 90 to 95 per cent of the cells had the morphology of small lymphocytes, the remainder being classified as monocytic or granulocytic cells.

Irradiation of cells in vitro. Cell suspensions were exposed to various doses by a radiation quality of 140 kV, HVL 0.45 mm Cu, as detailed previously (BARAL & BLOMGREN 1976).

Lymphocyte stimulant. The stimulant used was phytohaemagglutinin (PHA, Bacto-Phytohaemagglutinin M, Difco Lab., Detroit, Mich., USA). The contents of commercially available vials were dissolved in 5 ml of MEM (100 % of PHA). The cells were exposed to this agent at a final concentration of 3 per cent which has previously been shown to yield optimum DNA-synthetic responses of human lymphocytes (BLOMGREN 1974).

Culture conditions. DNA-synthetic responses of lymphocytes exposed to PHA was determined as described previously (BARAL & BLOMGREN). Briefly, 1.0×10^5 lymphoid

Table 1

Lymphocyte counts and PHA responses obtained before and following radiation therapy

	Before irradiation (I)	After intracavitary irradiation (II)*	After external irradiation (III)
Number of lymphocytes/μl blood mean values $\pm$ 95 % confidence intervals	1.674 $\pm$ 335	1.112 $\pm$ 236 $p_{I-II} < 0.01$**	568 $\pm$ 168 $p_{I-III} < 0.001$
PHA response mean cpm $\times 10^3$ $\pm$ 95 % confidence intervals	93.2 $\pm$ 19.0	87.1 $\pm$ 21.4 NS***	87.5 $\pm$ 12.3 NS

* The values were obtained after the first intracavitary irradiation.
** Statistical significance of the difference between values obtained before and after irradiation.
*** The mean PHA responses obtained at tests I, II, and III did not differ significantly.

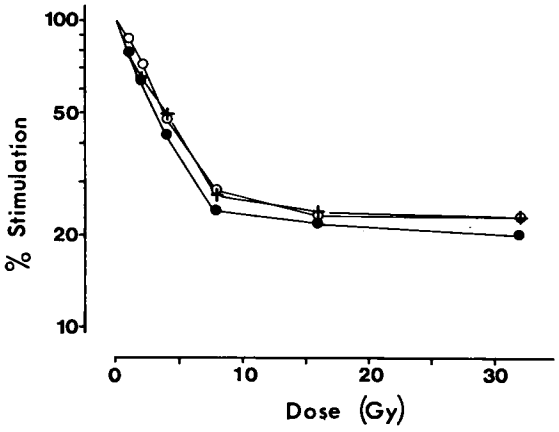
cells were cultured in the wells of plastic microtest plates containing 0.2 ml of MEM, penicillin, streptomycin and 10 per cent of heat-inactivated human serum. Cultures were set up in quadruplicate; half of them received PHA and the others served as controls. After four days of incubation at 37°C in a humidified 5% CO_2-air atmosphere each culture received 1.0 μCi of ^{3}H-thymidine (5 Ci/mM, The Radiochemical Center, Amersham, England). Twenty-four hours later the cultures were terminated and incorporated activity was determined (LILLIEHÖÖK & BLOMGREN 1974) and expressed as counts per min (cpm).

Experimental design. The number of lymphocytes per μl of blood and their relative responses to PHA was determined at three occasions: immediately before the first intracavitary application (Test number I), after three weeks and just before the second intracavitary irradiation (Test number II), and after an additional 8 to 10 weeks at the end of the external irradiation (Test number III). At all occasions the lymphocytes were exposed to varying radiation doses in vitro and within 1 to 1½ h after irradiation cultured with or without PHA.

The PHA responses of the irradiated cells, after deduction of the ^{3}H-thymidine uptakes of the corresponding control cultures, were expressed as cpm and related to the values obtained in cultures of non-irradiated, PHA-stimulated cells from the same donor. The latter values are expressed as per cent of ^{3}H-thymidine incorporations (Figure).

Results

Number of lymphocytes and their PHA responses following radiation therapy. The results are summarized in Table 1. Intracavitary treatment reduced the number of lymphocytes to approximately 66 per cent ($p < 0.01$) and there was a further reduction to 34 per cent ($p < 0.001$) after external irradiation. The response of 1.0×10^5 cells to PHA did not change after irradiation.



Relative ^{3}H-thymidine uptakes of blood lymphocyte prepatations incubated with 3 % of PHA after exposure to various doses of radiation in vitro before and following radiation therapy. Test number I ○—○, Test number II +—+, Test number III ●—●.

PHA responses of in vitro irradiated lymphocytes obtained before and after radiation therapy. Exposure of lymphocytes to doses ranging between 1 and 8 Gy caused a sharp reduction of the PHA reactivity (Figure). Further increase of the radiation dose did not cause any further reduction of PHA reactivity. Thus, the dose-response profiles were biphasic, both before and after radiation therapy. An analysis of variance was performed to test whether there was any significant difference in the mean levels of PHA response of the lymphocytes irradiated with doses of 8 to 32 Gy between test I (before treatment), and tests II (after the first intracavitary irradiation) and III (after external irradiation), respectively. The results revealed no significant differences following intracavitary and external radiation therapy (Table 2).

Table 2

PHA stimulations of 1.0 × 10^5 lymphocytes after exposure to various doses of radiation in vitro *obtained before and following radiation therapy*

Dose (Gy)	Before irradiation (I) (mean cpm × 10^3 ± 95 % confidence intervals)	After intracavitary irradiation (II) (mean cpm × 10^3 ± 95 % confidence intervals)	After external irradiation (III) (mean cpm × 10^3 ± 95 % confidence intervals)
Non-irr. controls	93.2 ± 19.0	87.1 ± 21.4	87.5 ± 12.3
1	82.2 ± 11.2	68.0 ± 18.1	69.0 ± 10.3
2	67.3 ± 16.7	56.6 ± 17.3	56.0 ± 11.8
4	44.6 ± 11.5	43.3 ± 12.0	37.0 ± 8.3
8	25.8 ± 6.6	23.6 ± 5.6	21.4 ± 3.6
16	22.4 ± 4.8	20.1 ± 5.1	19.4 ± 4.0
32	21.0 ± 4.3	19.8 ± 4.7	17.6 ± 3.7

Discussion

External radiation therapy may severely reduce the circulating pool of lymphocytes including both T- and non-T-cells (BLOMGREN et coll. 1974 a, b, HEIER et coll., RABEN et coll.). Recent experiments have suggested that peripheral phytomitogen responsive lymphoid cells of healthy subjects can be divided into two populations differing in their sensitivity to irradiation in vitro (CIRKOVIC 1969, BRAEMAN & MOORE 1974, BARAL & BLOMGREN). The PHA response of lymphocytes decreases sharply, in a fairly linear fashion, by exposing them to doses within 1 to 8 Gy. Further increase of the dose causes little or no further reduction of the phytomitogen reactivity (BARAL & BLOMGREN). Thus, the first decreasing segment of the dose-response line may reflect the existence of relatively sensitive cells and the second horizontal segment of the curve indicates the presence of relatively resistant cells.

This investigation was performed to determine whether the lymphopenia which follows radiation therapy is associated with a shift in the proportion of sensitive and resistant cells. Theoretically, a relative increase of the latter cell population would be expected, since lymphopenia which follows radiation therapy is most likely due to a direct killing of lymphocytes by radiation. The results have shown that the number of lymphocytes decreases both after intracavitary and external irradiation for carcinoma of the uterus and vagina resulting in a total reduction of 66 per cent. This cell depletion was not associated with any change of the PHA reactivity of the cells, which is in agreement with previous results (BLOMGREN et coll. 1976). The reduction of the lymphocyte number did not significantly change the ratio of sensitive : resistant cells.

One explanation of this finding is that peripheral lymphocytes cannot be divided into two distinct subpopulations differing in radiation sensitivity. It is possible that the biphasic dose-response curve observed in vitro rather reflects the existence of two groups of lymphocytes which differ in their lengths of survival after irradiation. For instance, the sensitive population may die at interphase stage before having been triggered by PHA and the resistant one may respond to PHA at an earlier stage and may disintegrate after one or several mitotic divisions.

In conclusion, the results indicate that the loss of peripheral lymphocytes following radiation therapy does not affect the sensitive subpopulation to a higher extent than the resistant one.

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SUMMARY

Irradiation of human peripheral lymphocytes in vitro reduces their capacity to be triggered to DNA-synthesis by PHA in a two-dose shaped fashion suggesting the presence of one relatively radiation sensitive and one relatively resistant cell population. Intracavitary and external radiation therapy for carcinoma of the uterus and vagina, which reduced the lymphocyte counts by approximately 66 per cent, did not significantly change the ratio of these subpopulations, indicating that PHA-reactive cells cannot be grouped into radiation sensitive and resistant subpopulations.

ZUSAMMENFASSUNG

Die Bestrahlung von humanen peripheren Lymphozyten in vitro vermindert deren Kapazität durch PHA in einer zwei-Dosis-artigen Weise in die DNA-Synthese stimuliert zu werden, was das Vorkommen einer relativ strahlensensiblen und einer relativ strahlenrezistenten Zellpopulation vermuten lässt. Intrakavitäre und externe Strahlentherapie von Karzinomen des Uterus und der Vagina, die die Lymphozytenzahl um etwa 66 Prozent herabsetzt, verändert nicht signifikant das Verhältnis dieser Subpopulationen, was darauf hindeutet, das PHA-reaktive Zellen nicht in strahlensensible und resistente Subpopulationen aufgeteilt werden können.

RÉSUMÉ

L'irradiation in vitro de lymphocytes périphériques humains réduit leur capacité d'être stimulés à synthétiser du DNA sous l'effet de PHA suivant un mode à deux doses, faisant penser qu'il y a une population cellulaire relativement sensible aux radiations et une relativement résistante. Le traitement par les radiations intracavitaire et externe pour le cancer de l'utérus et du vagin, qui réduit la numération lymphocytaire d'approximativement 66%, ne modifie pas de façon significative le rapport de ces sous-populations, ce qui indique que les cellules réagissant à PHA ne peuvent pas être groupées en sous-populations radio-sensibles et radio-résistantes.

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