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CELL SURVIVAL AND GROWTH DELAY IN RAT R-1 TUMOURS AFTER RADIATION AND VINBLASTINE TREATMENT

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Summary.—The rat R-1 rhabdomyosarcoma with a capacity for colony growth *in vitro* after excision of the tumour and dissociation by a trypsin method has been used to investigate the effectiveness of radio-chemotherapy. Growth delay data were compared with data on survival of cells derived from tumours treated *in situ*.

An excess in growth delay was observed when vinblastine (1·5 mg/kg) was given at intervals of 0·3 to 2 d after or 4 d before a dose of 20 Gy of X-rays.

Cell survival data indicate that the maximum effectiveness of the drug treatment and the combined treatment (vinblastine and a dose of 10 Gy) can be assessed 2 to 3 d after treatment. The fractions of surviving cells determined after combined therapy at 0, 1 and 2 d intervals were not significantly different from the fractions expected on the basis of simple multiplication of the fractions surviving individual treatments. The data suggest that the excess in tumour growth delay observed cannot be accounted for by co-operative interaction of the doses of radiation and drug.

COMBINED treatments of cancer with radiation and chemotherapeutic drugs, in principle, offer the potential of increasing probability of the control of local and regional disease and possibly also of distant subclinical disease. A rational approach to the selection of specific combinations and treatment schedules requires experimental studies of tumour responses and the tolerance of normal tissues. We describe here experiments aimed at obtaining information on tumour responses after treatment with doses of radiation, vinblastine and combination of the 2 using 2 endpoints: tumour growth delay and *in vitro* survival of tumour cells isolated from the treated tumour.

METHODS AND MATERIALS

A rat rhabdomyosarcoma, designated as the R-1 tumour, was used in this study. Detailed information on cell proliferation kinetics and responses of the tumour to single and fractionated doses of radiation have been published (Barendsen & Broerse, 1969; 1970; Hermens and Barendsen, 1967). Tumours of 0·5 to 1·0 cm^3 have a volume doubling time of $3{\cdot}5 \pm 1{\cdot}0$ d. The growth fraction of the tumour cells is about 0·4. In culture, R-1 cells have a cell doubling time of about 20 h.

Volume changes in R-1 tumours growing in the flanks of WAG/Rij rats were derived from measurements of 3 perpendicular dimensions with vernier calipers. Growth delay in the treated tumour is defined as the time required to reach a volume twice as large as the volume at the time of treatment, subtracting the volume doubling time of the control tumour. Excess growth delay is defined as the difference between growth delay due to combined treatment and the sum of delays induced by the individual treatments.

The cells of the R-1 tumour can be directly cultured *in vitro* after dissociation of the excised tumour by a trypsin method (Reinhold, 1965); the plating efficiency of control tumours is about 30%. The cell yield, *i.e.* the number of cells per gram of untreated tumour, is about 2×10^7 cells/g. This cell yield may decrease after treatment (Barendsen & Broerse, 1969). Therefore, since tumour volume changes also occur after treatment, the fraction of surviving cells in a treated tumour must be corrected for cell yield and tumour volume changes to determine the fraction of clonogenic cells relative to the

number of cells present at the start of the treatment.

Doses of 10 or 20 Gy of 300 kV X-rays were administered locally at a dose rate of 3·4 Gy/min. Vinblastine was given intraperitoneally at a dose of 1·5 mg/kg rat body weight.

RESULTS

Vinblastine causes a growth delay of 2 d and the dose of 20 Gy results in a delay of about 16 d. When combined treatment for a specific time interval resulted in a growth delay of 23 d, an excess growth delay of 5 d was calculated. In a large fraction of treated R-1 tumours, the time required to double the volume was longer than the doubling time of control tumours. The difference between the doubling times of the treated and the control tumour was applied as a correction for the excess growth delay values derived from the caliper measurements. This was done to eliminate influences due to the choice of the reference volume level from which growth delay data are derived. Therefore, the corrected excess growth delays discussed here may show deviations from earlier reported values (Barendsen, 1977; Kal *et al.*, 1979).

The results of the combined treatments with 20 Gy of X-rays and vinblastine are shown in Fig. 1. They are presented as excess growth delay as a function of time interval. The data show that, for the combination at intervals of 0·3 to 2 d (X-rays first), an excess growth delay of about 5 to 6 d is observed. This decreases to zero for longer or for very short time intervals. When vinblastine is administered before the radiation treatment, the excess growth delay increases to about 2 d at an interval of 4 d and decreases again for longer intervals.

The clonogenic capacity of cells from R-1 tumours treated *in situ* was assessed by using an *in vitro* assay for clone formation. Fractions of clonogenic cells relative to the number of cells present at the start of treatment is plotted as a function of time after treatment with either vinblastine, a lower dose of 10 Gy of X-rays or 10 Gy followed after 1 d by vinblastine in Fig. 2. The treatment with vinblastine reduced the fraction of clonogenic cells to 0·2 as determined at 2 and 3 d after treatment. After 10 Gy, a minimum value of 0·033 was calculated. This value does not depend significantly on the time interval after treatment, provided that the assay is performed within 4 d. After the combined

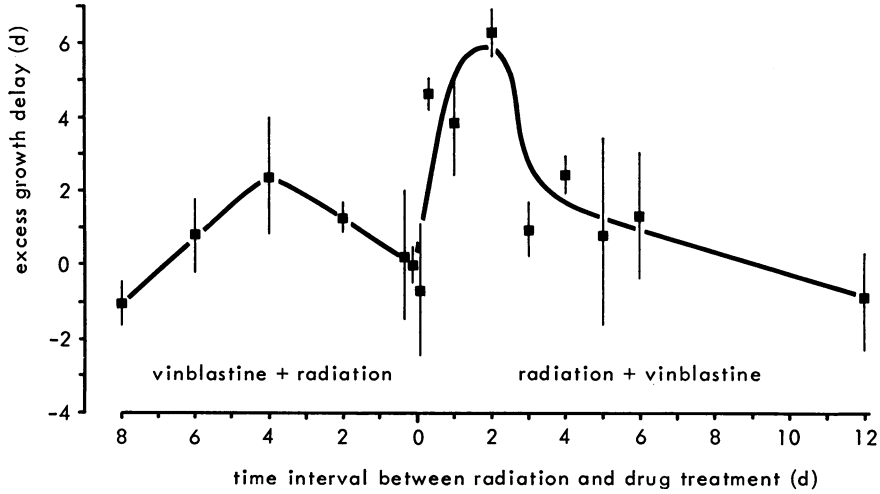

FIG. 1.—Excess growth delay after combined treatment of R-1 tumours with 20 Gy of X-rays and vinblastine, 1·5 mg/kg, as a function of time interval between radiation and vinblastine treatment.

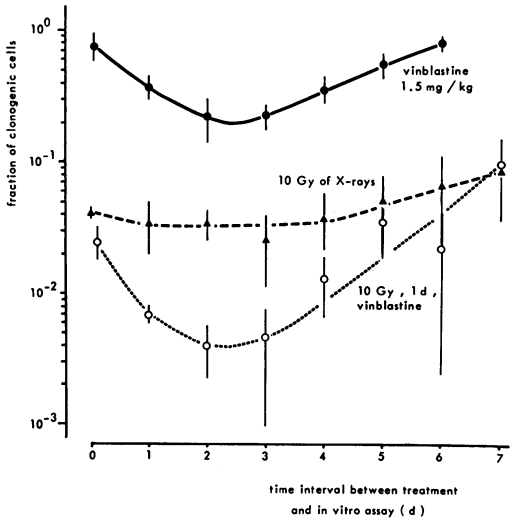

FIG. 2.—Fractions of clonogenic R-1 cells relative to the number of cells present at the time of irradiation, after treatment of R-1 tumours *in vivo*, as a function of time interval between treatment and *in vitro* assay. The curves represent fractions of clonogenic cells after treatments with vinblastine, 1·5 mg/kg, a dose of 10 Gy of X-rays and a dose of 10 Gy followed after 1 day with a dose of vinblastine.

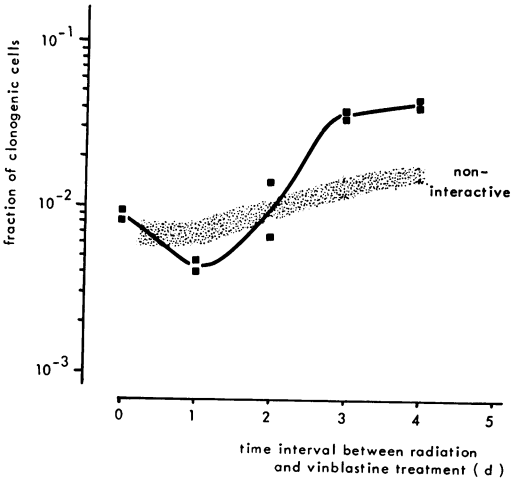

FIG. 3.—Fractions of clonogenic R-1 cells of R-1 tumours determined on days 2 and 3 after treatment as a function of time interval between radiation and vinblastine treatment. The squares represent the measured values, the shaded area the values calculated on the basis of noninteraction.

treatment, the minimum fraction of clonogenic cells is 0·004 as assessed on days 2 and 3 after treatment. Fractions of surviving cells for combined treatments with intervals between radiation and drug dose of 0 to 4 d (the range in which the largest excess growth delays were observed) were also assessed on days 2 and 3 after the administration of the drug dose; they are shown in Fig. 3 by the closed squares.

DISCUSSION

The data presented in Fig. 1 show that different excess growth delays are found and that these depend on the sequence of the administration of the doses of radiation and drug. This indicates that, depending on the sequence of administration, different processes may occur in the R-1 tumour. Experiments with combined treatments of a dose of 10 Gy and vinblastine resulted in excess growth delay similar to the values for 20 Gy and vinblastine. Therefore, the *in vitro* survival and growth delay data can be compared and conclusions can be made as to whether the combination of 2 agents may result in a positive interaction, *i.e.* whether there is evidence for one agent influencing the response to another.

The survival data obtained for the vinblastine treatment (Fig. 2) indicate that the maximum effect, *i.e.* the minimum survival of 0·2, can be assessed on day 2 or 3 after treatment. After day 3, repopulation of the tumour becomes important and results in a larger surviving fraction. After 10 Gy, a minimum value of 0·033 was calculated. This value does not depend on the time interval after treatment, provided that the assay is performed within 4 d. Repopulation of the tumour by surviving cells becomes manifest about 5 d after radiation treatment. Cells affected by the radiation treatment will probably suffer from a mitotic delay and may then undergo one or 2 division cycles before they lyse and are subsequently removed from the tumour. These processes probably require more time than when tumours are treated with vinblastine, when cells lysis may occur

earlier. After the combined treatment, the minimum fraction of clonogenic cells is 0·004 as assessed on day 2 or 3 after treatment. A combination effect of the 2 individual treatments is seen. Two different populations of cells doomed to die are probably present in the tumour: one population affected by the drug treatment which will be removed from the tumour population within 2 to 3 d and another sterilized by the radiation that will persist for up to 5 or 6 d. This indicates that radiation and vinblastine affect different populations of cells or affect the cells differently. Vinblastine administered 1 d after radiation might selectively kill the surviving cells and not interfere with repair processes after the radiation treatment. Earlier studies have shown that repair of potentially lethal damage after radiation treatment cannot be demonstrated in the R-1 tumour (Barendsen & Broerse, 1969).

Fractions of surviving cells were assessed on days 2 and 3 after combined treatments with intervals of 0, 2, 3 and 4 d (Fig. 3, closed squares). Calculations were made on the basis of noninteraction, *i.e.* the situation in which the agents are assumed to act independently. For instance, for the combined treatment with an interval of 2 d, the fraction of surviving cells was assessed on day 2, *i.e.* 4 d after irradiation which is also 2 d after vinblastine. The calculated surviving fraction on the basis of noninteraction is then derived from the product of the fractions of surviving cells assessed on day 4 after radiation and on day 2 after vinblastine, respectively, from Fig. 2. The shaded area in Fig. 3 represents the values calculated on this basis of noninteraction for days 2 and 3 after the combined treatment. It is clear that, for the 0, 1 and 2 d intervals, the calculated values do not significantly differ from the experimental values. For the 3 and 4 d intervals between administration of the 2 agents, the assessed values seem to be larger than the calculated values, indicating that the combined treatment is less effective than expected on the basis of noninteraction.

The growth delay data indicate that response to the combined treatments w 1 and 2 d intervals is greater than addit as evidenced by the positive excess gro delays of up to 6 d, but the *in vitro* surv data suggest that the response is appr mately additive. The excess growth del of about 1 to 2 d observed for the combi tions with 3 and 4 d intervals betw administration of the 2 agents indic a slightly more than additive effect, the *in vitro* survival data are stron suggestive of a less than additive eff These discrepancies were also obser by Stephens & Peacock (this Supplem using B16 melanoma and Lewis l carcinoma treated with gamma rays cyclophosphamide or CCNU.

It is evidently of interest to accumu more data on cell survival, cell prolif tion and cell loss to interpret the differer observed among the responses of the tumour to the different treatments, si the differences observed are probably to differences in the rate of repopula of the tumour after different treatme

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REFERENCES

BARENDSEN, G. W. (1977) Effectiveness of bined radio and chemotherapy in the treatme experimental tumours and an evaluation of factors influencing response. In *Radiobiolo Research and Radiotherapy*, Vol. 1. Vie IAEA. p. 351.

BARENDSEN, G. W. & BROERSE, J. J. (1969) Ex mental radiotherapy of a rat rhabdomyosarc with 15 MeV neutrons and 300 kV X-ray Effects of single exposures. *Eur. J. Cancer*, **5**,

BARENDSEN, G. W. & BROERSE, J. J. (1970) Ex mental radiotherapy of a rat rhabdomyosarc with 15 MeV neutrons and 300 kV X-rays Effects of fractionated treatments applied times a week for several weeks. *Eur. J. Cancer*, **6**

HERMENS, A. F. & BARENDSEN, G. W. (1 Cellular proliferation patterns in an experim rhabdomyosarcoma in the rat. *Eur. J. Cance* 361.

KAL, H. B., BARENDSEN, G. W. & JANSE, H (1979) Tumour growth delay and cell sur after treatment with radiation and vinblas *Int. J. Radiat. Oncol. Biol. Phys.* (in press).

REINHOLD, H. S. (1965) A cell dispersion techn for use in quantitative transplantation st with solid tumours. *Eur. J. Cancer*, **1**, 67.