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FROM THE LABORATORY OF DOSIMETRY AND BIOPHYSICS, ENEA CASACCIA, I-00060 ROME, AND THE ISTITUTO DI RICERCA CESARE SERONO, I-00040 ROME, ITALY.

ORGOTEIN AS A RADIOPROTECTOR IN NORMAL TISSUES

Experiments on mouse skin and a murine adenocarcinoma

A. CIVIDALLI, M. ADAMI, F. DE TOMASI, L. PALMISANO, M. C. PARDINI, M. SPANÒ and F. MAURO

Abstract

The effects of Orgotein (a superoxide dismutase) on the radiation response of normal and malignant murine tissue in vivo were evaluated. The observations were made on the mouse hind leg bearing, in some cases, an adenocarcinoma. The following irradiation protocols were tested: 1) single dose (e.g., 35 and 53 Gy), 2) conventional fractionation (3 Gy/day, 5 days a week) and 3) multiple fractions per day (2×3 Gy/day, 3 h fractionation interval, 5 days a week). Radiation was either delivered alone or preceded by a subcutaneous injection of 20, 100 or 400 mg/kg Orgotein administered 1 or 2 h before the beginning of irradiation. No effects of Orgotein on tumor radiation response were detected. A protective effect on normal tissue was observed when radiation was delivered according to aggressive protocols and a relatively high dosage of Orgotein was administered. Furthermore, an accelerated trend of recovery of normal tissue was observed.

A clinical group (4, 5, 8, 9) has reported that the side effects, and in particular acute cystitis, after irradiation of bladder or prostate carcinoma are reduced when each fraction of radiation is followed by an injection of Orgotein—a superoxide dismutase (SOD). Furthermore, supposedly radioprotective effects of the drug have been reported for several biologic end-points in the mouse (13–15).

On the contrary, OVERGAARD et coll. (12) observed no variation of cell survival in clonogenic systems, and no effect on tumor growth and acute skin reaction in the mouse after treatment with Orgotein and radiation.

At present, two alternative main hypotheses are still open: 1) the reduced reaction to irradiation after Orgotein treatment is only an indirect effect of the anti-inflammatory action of the drug; 2) Orgotein induces indeed a direct radioprotective effect with an efficiency related to the natural concentration of SOD in the target cells; that is, in cells with high SOD content, further enzyme activity will not be needed and cannot be exploited to avoid radical superoxide injury (12).

Several authors (2, 11, 16) have observed that SOD activity is often lower in malignant than in normal cells. Apart from the implications on the role of SOD in cancer (10), such an observation suggests the possibility of a differential use of the drug in cancer therapy.

The present investigation has been designed to gather further evidence on the possible action of Orgotein as a radioprotector of normal tissue in an experimental system treated in a way similar to that of patients in radiotherapeutic oncology.

Material and Methods

Animals. For all experiments, female hybrid (C3H/Ri female × DBA male) mice, 10 to 12 weeks old, were used.

Tumor system. A spontaneous, moderately differentiated C3H/tif mammary carcinoma (kindly ob-

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tained from Dr J. Overgaard, Aarhus, Denmark) was kept by serial transplantation in the animal flank. For experimental purposes, microscopically viable tumor tissue was minced with scissors, and transplanted by trocar into the foot of the right hind limb. Under these conditions, 29/30 tumor takes are usually observed, and the tumor becomes palpable by 9 to 10 days after transplantation. The tumor grows according to a Gompertzian trend (Fig. 1). The quasi-plateau of tumor growth observable after day 20 may be partially due to the particular anatomy of the transplantation area. Untreated animals start to die around day 30. Treatments were initiated on tumors with a volume of 50 to 120 mm^3, as calculated on the basis of 3 orthogonal diameters measured by caliper.

Drug. Orgotein is a metal protein in the form of a Cu-Zn chelate with SOD activity of not less than 3500 units/mg. The Orgotein used in the present work was prepared from both bovine erythrocytes and liver in the Istituto di Ricerca Cesare Serono, Rome, Italy.

The drug was dissolved in physiologic solution immediately before use. For all experiments, the drug was injected subcutaneously 1 to 2 h before irradiation. This was decided on the basis of pharmacologic data (7) indicating that, in the mouse, after subcutaneous administration, a certain serum level of the drug is reached by 1 to 2 h and maintained for up to about 4 h.

Irradiation. Irradiation was given with a roentgen equipment (Stabilipan, Siemens, Erlangen, FRG) operated at 250 kV, 15 mA, 0.5 mm Cu equivalent filter, with a dose rate of 2.1 Gy/min, as measured by lithium fluoride thermoluminescence calibrated against an ionizing chamber.

For all treatments, unanesthetized animals were placed in a specially designed perspex cage, with the leg to be irradiated extruding from the cage and maintained in position with Velcro cloth tape without affecting the integrity of the skin or impairing the blood flow. During irradiation, the animal body, except for the leg to be treated, was shielded with lead.

Experimental end-points. The effects on the tumor volume were monitored twice a week during treatments. At the beginning of each experiment, randomized groups of at least 8 mice were formed. In the graphs, the tumor volume is reported as the average volume of the group ± standard error of the mean. The repeatability of experimental results was

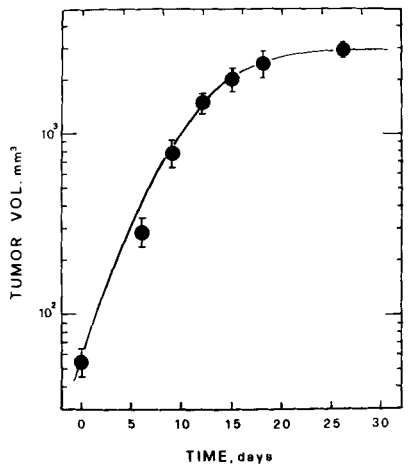

Fig. 1. Tumor growth curve for the murine adenocarcinoma used. The growth is described in terms of variation of the tumor volume as a function of time. In this and following figures, the bars indicate the standard errors of the means.

always controlled. Tumor 'cure' was defined as 'non evident disease' (NED); when possible, the curability was measured in terms of TDC_{50-120} (tumor control dose 50% at 120 days from the beginning of the treatment).

The effects on the normal tissue of the foot skin were examined. Because of the difficulty of a more precise damage recognition in an organ such as the mouse foot, the effects were evaluated according to a simpler version of the method proposed by DENEKAMP (1) and by DOUGLAS & FOWLER (3). The scoring system for acute skin reactions was the following: 1.0 erythema, 2.0 dry desquamation, 3.0 moist desquamation and 4.0 tissue breakdown. For the acute reactions, the score was assigned at response peak.

The scoring system for the so-called permanent reactions was actually an arbitrary system concerning the occurrence of deformity in the feet of rodents. The version used with the present work was stretched towards higher damage levels in order to cover total dose higher than in the original version. The system used was the following: 1.0 permanent depilation, 2.0 tissue hardening, 3.0 loss or fusion of toes and 4.0 foot loss.

Results

Results on the tumor. Single dose (roentgen) treatments were carried out. The TCD_{50-120} for tumors treated with radiation only was 52.4±4.6 Gy. Similar results were obtained in tumor-bearing

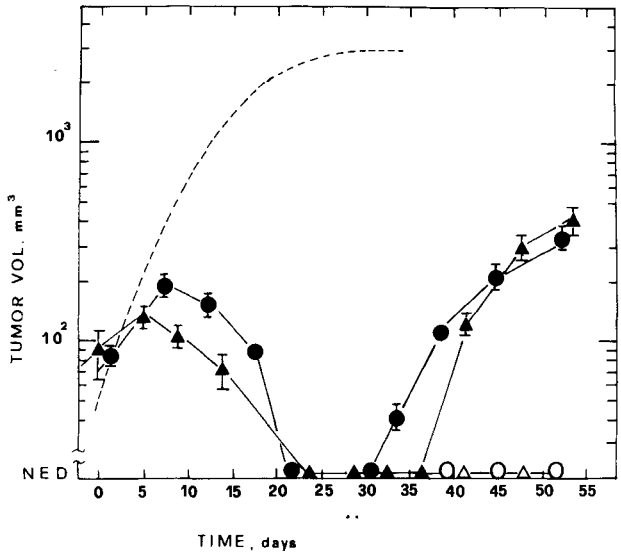

Fig. 2. Effects of single dose roentgen irradiation on tumor growth. Irradiation alone (▲). 100 mg/kg Orgotein administered 1 h before irradiation (●). On the right part of the graph, the open symbols refer to cures as opposed to recurrences. In the present case, the recurrence rate was 50 per cent for both experimental protocols. In this and in the following figure, the dashed line indicates the untreated tumor growth curve.

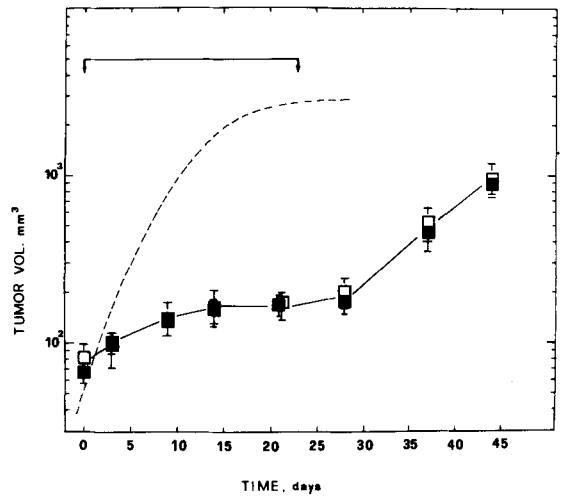

Fig. 3. Effect of 'conventional' radiation therapy on tumor growth. Irradiation alone (■). 400 mg/kg Orgotein administered 1 h before each daily fractional dose (□). The period of time marked by the arrows indicates the duration of the radiation therapy protocol.

Table

Effect on normal tissue

Schedule	Total dose (Gy)	Early damage		Permanent damage
		Peak score	Day of full recovery	Final score
SD (no Org.)	35	2.13	20	1.00
100 mg/kg Org. + 1 h + SD	35	1.67	20	0.50
400 mg/kg Org. + 1 h + SD	35	1.25	18	0.25
SD (no Org.)	53	3.19	28	2.25
20 mg/kg Org. + 1 h + SD	53	2.75	28	2.50
100 mg/kg Org. + 1 h + SD	53	3.00	23	1.50
100 mg/kg Org. + 2 h + SD	53	2.75	22	1.80
400 mg/kg Org. + 1 h + SD	53	2.25	18	1.30
MFD (no Org.)	90	3.75	50	2.50
100 mg/kg Org. + 2 h + 1st fract. MFD	90	3.10	45	1.87
400 mg/kg Org. + 2 h + 1st & 2nd fract. MFD	90	2.75	36	1.25

SD: Single dose. MFD: Multiple fractions per day.

animals treated with 20, 100 or 400 mg/kg Orgotein 1 h before irradiation. The TCD_{50-120} for all Orgotein doses pooled together was 53.7+7.3 Gy.

In Fig. 2, the variations of tumor volumes after irradiation of 53 Gy with and without 100 mg/kg Orgotein are given as an example. In both cases, the tumor volume started to decrease by day 5 and reached the stage of NED by day 20, some tumor recurrences being observed at a later time.

In other experiments, multifraction schedules similar to those used in clinical practice were selected. In the instance of 'conventional' radiation therapy (3 Gy/day, 5 days/week, up to a total dose of 54 Gy), the results are reported in Fig. 3 for radiation alone and for 400 mg/kg Orgotein administered 1 h before irradiation. For both treatments, only some tumor growth delay was observed, with a complete overlapping of the experimental points. Neither was

any protective effect observed in the instance of a protocol with multiple fractions per day (3×3 Gy/day, 3 h fractionation interval, up to a total dose of 90 Gy) in which the animals were treated with 100 mg/kg 1 h before the first and second daily radiation doses.

Results on normal tissue. The results on normal tissue are reported in the Table. They indicate a protective effect for a single dose irradiation of 35 or 53 Gy and a multiple fractions per day schedule up to a total dose of 90 Gy in terms of both peak score and day of full recovery for early skin damage and final score for permanent foot damage if 100 or 400 mg/kg Orgotein were administered. Furthermore, a certain dose dependence of the protective effect was evident.

The data reported in the Table refer to animals without tumors so that a precise appreciation of the damage could be made. However, in other experiments, no relevant differences were found for normal tissues of animals with and without tumors.

Discussion

The data obtained from our experimental system clearly confirm that no radioprotective effect of Orgotein can be observed on the irradiated tumor. This is an important observation in consideration of the fact that some of the irradiation protocols used in the present investigation are of a clinical type.

In the case of normal tissue, a protective effect was observed in a consistent way for both early and permanent damage.

It is of interest that, in the instance of early damage, a radioprotective effect in form of a more rapid tissue healing could also be observed. It should be noted that a protective effect in terms of acceleration of cell population recovery has already been reported by other authors (14) for the erythrocytes of irradiated mice. Actually, the present data are not in contrast with those reported by OVERGAARD et coll. (12). As a matter of fact, they are in very good agreement as far as the lack of effect on mouse mammary carcinoma in vivo is concerned. The other data reported by these authors refer to clonogenic systems in vitro (L1A2 cells) and in vivo (jejunal crypt cells); in the latter case, a dose of 50 mg/kg was used. In the present investigation, normal tissue of the mouse foot was used, where a clear relation between single cell viability and overall tissue effects is not easy to establish, especially with parameters such as the day of full recovery or end-points such as permanent tissue damage.

In conclusion, the present work indicates that some radioprotective effect of Orgotein is indeed present, especially if relatively high drug doses are used.

In the present investigation, an efficient dose range seemed to be 100 to 400 mg/kg. In clinical trials on humans, single doses around 0.2 mg/kg have been used. However, Orgotein is a remarkably safe drug and single doses of up to 10 or over 20000 times a clinical dose have been documented as acceptable in monkeys or rodents, respectively (6). Perhaps higher doses should be considered for oncologic patients, even if the processing capacity of the kidney for Orgotein has to be kept in mind. Further investigation should therefore be pursued, with particular reference to clinical use. The problem of anti-inflammatory versus/and radioprotective action also needs further investigation.

Request for reprints: Dr A. Cividalli, PAS/FIBI/DOSIBIO, ENEA Casaccia, Via Anguillarse 301, I-00060 Rome, Italy.

REFERENCES

1. DENEKAMP J.: Residual radiation damage in mouse skin 5 to 8 months after irradiation. Radiology 115 (1975), 191.
2. DIONISI D., GALEOTTI T., TERRANOVE T. and AZZI A.: Superoxide radicals and hydrogen peroxide formation in mitochondria for normal and neoplastic tissues. Biochim. Biophys. Acta 403 (1975), 292.
3. DOUGLAS B. G. and FOWLER J. F.: The effect of multiple small doses of X-rays on skin reactions in the mouse and a basic interpretation. Radiat. Res. 66 (1976), 401.
4. EDSMYR F., HUBER W. and MENANDER K. B.: Orgotein efficacy in ameliorating side effects due to radiation therapy. I. Double-blind placebo-controlled trial in patients with bladder tumors. Curr. Ther. Res. 19 (1976), 198.
5. — and MENANDER-HUBER K. B.: Orgotein efficacy in ameliorating side effects due to radiation therapy. Eur. J. Rheumatol. Inflammation 4 (1981), 228.
6. HUBER W.: Orgotein (bovine Cu-Zn superoxide dismutase), an anti-inflammatory protein drug. Discovery, toxicology and pharmacology. Eur. J. Rheumatol. Inflammation 4 (1981), 173.
7. — and SAIFER M. G. P.: Orgotein, the drug version of bovine Cu-Zn Superoxide Dismutase. I. A summary account of safety and pharmacology in laboratory animals. *In:* Superoxide and superoxide dismutases, p. 517. Edited by A. M. Michelson, J. M. McCord and I. Fridovich. Academic Press, New York 1977.
8. MARBERGER H., BARTSCH G., HUBER W., MENANDER D. B. and SCHULTE T. L.: Orgotein. A new drug for

the treatment of radiation cystitis. Curr. Ther. Res. 18 (1975), 466.

9. MENANDER-HUBER K. B., EDSMYR F. and HUBER W.: Orgotein (superoxide dismutase). A drug for the amelioration of radiation-induced side effects. A double-blind, placebo-controlled study in patients with bladder tumours. Urol. Res. 6 (1978), 255.
10. OBERLEY L. W. and BRETTNER G. R.: Role of superoxide dismutase in cancer. A review. Cancer Res. 38 (1979), 1141.
11. — BIZE I., SAHU S. K., LEUTHAUSER S. W. H. C. and GRUBER H. E.: Superoxide dismutase activity of normal murine liver, regenerating liver, and H6 hematoma. J. Natl. Cancer Inst. 61 (1978), 375.
12. OVERGAARD J., NIELSEN O. S., OVERGAARD M., STEENHOLDT S., JAKOBSEN A. and SELL A.: Lack of radiation protective effect of Orgotein in normal and malignant mammalian cells. Acta radiol. Oncology 18 (1979), 305.
13. PETKAU A., CHELACK W. S., PLESKACH S. D., MEEKER B. E. and BRADY C. H.: Radioprotection of mice by superoxide dismutase. Biochem. Biophys. Res. Comm. 65 (1975), 886.
14. — KELLY K., CHELACK W. S. and BAREFOOT C.: Protective effect of superoxide dismutase on erythrocytes of X-irradiated mice. Biochem. Biophys. Res. Comm. 70 (1976), 452.
15. — — — PLESKACH S. D., BAREFOOT C. and MEEKER B. E.: Radioprotection of bone marrow stem cells by superoxide dismutase. Biochem. Biophys. Res. Comm. 67 (1975), 1167.
16. YAMANAKA N., OTA K. and UTSUMI K.: Changes in superoxide dismutase activities during development, aging, and transformation. *In:* Biochemical and medical aspects of active oxygen, p. 183. Edited by O. Hayaishi and K. Adada. University Park Press, Baltimore 1978.