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LACK OF RADIATION PROTECTIVE EFFECT OF ORGOTEIN IN NORMAL AND MALIGNANT MAMMALIAN CELLS

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Recently EDSMYR et coll. (1976) observed that the symptoms of acute cystitis induced by irradiation of bladder carcinoma could be reduced if each radiation fraction was followed by an injection of orgotein. (Orgotein is the non-proprietary name assigned by the United States Adopted Names Council. The trade name in Scandinavia is Ontocin.)

The mechanism by which orgotein exhibits its cystitis-reducing effect is not known, although it is likely that it partly may be contributed to an anti-inflammatory effect of the drug (MARBERGER et coll. 1975, EDSMYR et coll.). However, a main component of orgotein is superoxide dismutase (SOD), an enzyme known to reduce the response to ionizing radiation under oxygenated conditions by rapid fusion of superoxide radicals (PETKAU & CHELACK 1974, OBERLEY et coll. 1976). Furthermore, it has been shown that SOD has a protective ability when given either before or after whole-body irradiation of mice (PETKAU et coll. 1975, 1976). Therefore, it might be that the reduced radiation reaction observed in the treatment of bladder carcinoma could partly be ascribed to a direct radiation protective effect of the drug. Since no differential response between tumour and normal tissue may be expected in that respect, a radiation protection of the bladder would also mean reduction of the effective dose

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to oxygenated tumour cells. No quantitative investigations appear to have been performed to estimate the direct effect of orgotein on radiation cell-killing of mammalian cells. Therefore, an evaluation of the potential protective effect of orgotein on the primary radiation response was performed before extensive clinical use of orgotein was started.

Material and Methods

The influence of orgotein on the primary radiation response was evaluated in three different experimental systems.

L_1A_2 tumour cells in vitro. A spontaneous transformed hypotetraploid cell line L_1A_2 developed in 1970 from C_3H mouse lung tissue was used in this experimental system. The cells were grown in Basal Medium Eagle (GIBCO G14) supplemented with 10 % foetal calf serum and 1 % streptomycin and penicillin and kept in a 37°C humidified incubator ($PCO_2 = 5\,\%$). In order to get asynchronous exponentially growing cells, 2 to 3×10^6 cells from a stock culture were plated out in Nunclon 80 cm^2 flasks 3 days before the experiment. At that time, cells were trypsinated, counted in a haemocytometer and diluted out in Nunclon 25 cm^2 flasks to get about 100 to 200 colonies. The cells were overlaid with 10 ml medium and incubated for 12 hours at 37°C for maximum cell attachment.

The flasks treated with orgotein were overlaid with 10 ml medium containing 20 μg orgotein per ml for a period of 3 hours. One group was irradiated in the last hour of this 3 h period while the other group was irradiated 30 min before addition of orgotein-containing medium. Controls, only irradiated, were treated similarly except that they were exposed to a medium without the drug.

After treatments, the flasks were incubated undisturbed for 8 to 10 days. Then the medium was discharged and the cells were fixed in methanol and stained with Toluidine Blue. Colonies containing more than 50 cells were counted and the relative survival was calculated from the data relative to the number of colonies in the controls. At least 3 culture flasks were used for each data point, and standard errors of the mean were calculated.

Jejunal crypt cells. Ten to 12 weeks old C_3H/Aa female mice were used. Unanaesthetized mice were whole-body irradiated after having been placed in a special lucite jig. This set-up allowed 2 mice to be irradiated at a time.

Orgotein was administered by intraperitoneal injection in a concentration of 50 μg/g body weight either 2 hours before irradiation or 30 min after.

The animals were killed 4 days after irradiation. The upper part of the jejunum was injected in situ intraluminally with formalin and removed for further formalin fixation for 24 hours. After dehydration and embedding in paraffin the gut was cut at the transverse diameter and stained with haematoxylin and eosin. The number of crypt colonies (more than 10 basophil cells in close connection) were counted under

the microscope. Calculation of survival curves was performed according to the principles described by WITHERS & ELKIND (1970). Each data point represents 6 to 8 individual observations.

Mouse mammary carcinoma in vivo. In this series a spontaneous C_3H mouse mammary carcinoma was used as a model. The tumour, which is propagated by serial transplantation, was transplanted to the feet of $C_3D_2F_1$ mice, a hybrid between isologous C_3H (female) and D_2BA (male) mice. Tumours at a size of approximately 200 mm^3 were treated 10 to 14 days after challenge.

Irradiation was given to unanaesthetized animals placed in a special jig with the tumour-bearing feet immersed into a water-bath with a 5 cm layer of water between the radiation source and the tumour; thus a uniform local tumour irradiation was obtained.

The results were evaluated as the dose required for 50 per cent local tumour control at 120 days (TCD_{50}). The data were computed by a logit analysis (SUIT et coll. 1965).

Irradiation was given with a clinical roentgen ray unit at 250 kV at 15 mA and 2 mm Al filtration with 36 cm SSD. The dose rate was 5 Gy/min for the in vitro and whole-body irradiation and 2 Gy/min for the local tumour treatment (1 Gy = 100 rad).

Dosimetry was accomplished by lithium fluoride thermoluminescence calibrated against a Baldwin Farmer ionizing chamber.

Orgotein. The drug is a metalloprotein in the form of a Cu–Zn chelate with superoxide dismutate activity at approximately 3 300 units/mg.

The drug was dissolved with isotonic saline to a concentration of 2 mg/ml. For all animal experiments 50 μg/g body weight was given intraperitoneally either 2 h before irradiation or 30 min after. Control animals given irradiation alone were treated with an equal amount of isotonic saline only.

For the in vitro cell experiments orgotein was added to the medium at times and concentrations described.

Results

In vitro cell survival. Exposure to orgotein alone for 3 h at a concentration of 20 μg/ml did not influence the survival of L_1A_2 tumour cells as compared with controls. The survival of L_1A_2 cells exposed to graded doses of radiation in air and treated with orgotein before or after irradiation appears in Fig. 1. Orgotein exposure did not cause any change in the sensitivity to radiation as indicated by the value of D_0, which was found to be 1.09, 1.09 and 1.10 Gy for cells treated with orgotein before or after irradiation and for the controls, respectively. Neither did the orgotein treatment influence the repair of sublethal damage as the extrapolation number was the same for all 3 survival curves.

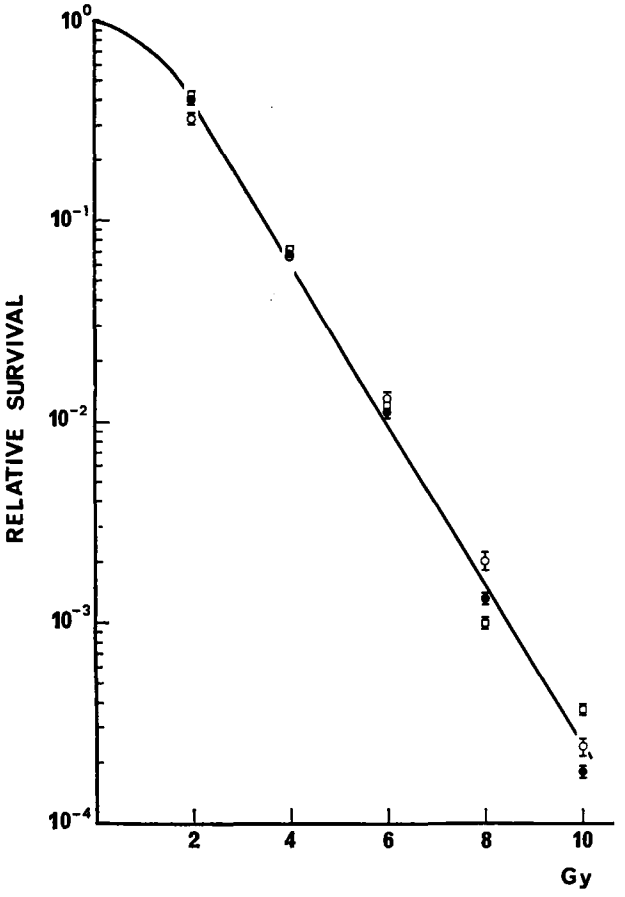

Fig. 1. Survival of L_1A_2 cells treated with orgotein before or after a single dose of irradiation. ● orgotein 2 h before and □ orgotein 30 min after irradiation. ○ controls (irradiation alone). As no significant difference was observed between the 3 groups, the data were pooled and are represented by the curve. This is characterized with a D_0 of 1.09 Gy and an extrapolation number n of 2.70.

Jejunal crypt cell survival. Similar results were found by estimating the injury to the mouse jejunum irradiated in vivo. Fig. 2 shows the number of surviving crypt cells per circumference in controls and after orgotein given before and after a single dose of radiation between 10 and 18 Gy. The value of D_0 calculated by linear regression was found to be 1.25, 1.21 and 1.18 in the 3 groups, indicating no significant difference in sensitivity. Similarly no difference was found in the value of D_{10} (the dose to produce 10 surviving crypt cell colonies on average), which in the same groups were 13.3, 13.2 and 13.1 Gy, respectively.

Effect on mouse mammary carcinoma in vivo. The last experiment was an assay of the radiation dose needed to control a mouse mammary carcinoma with a single dose given in air. The results (Table) indicate that also in this system there was no influence on pre- or post-exposure to orgotein on the radiation response when evaluated as a TCD_{50}.

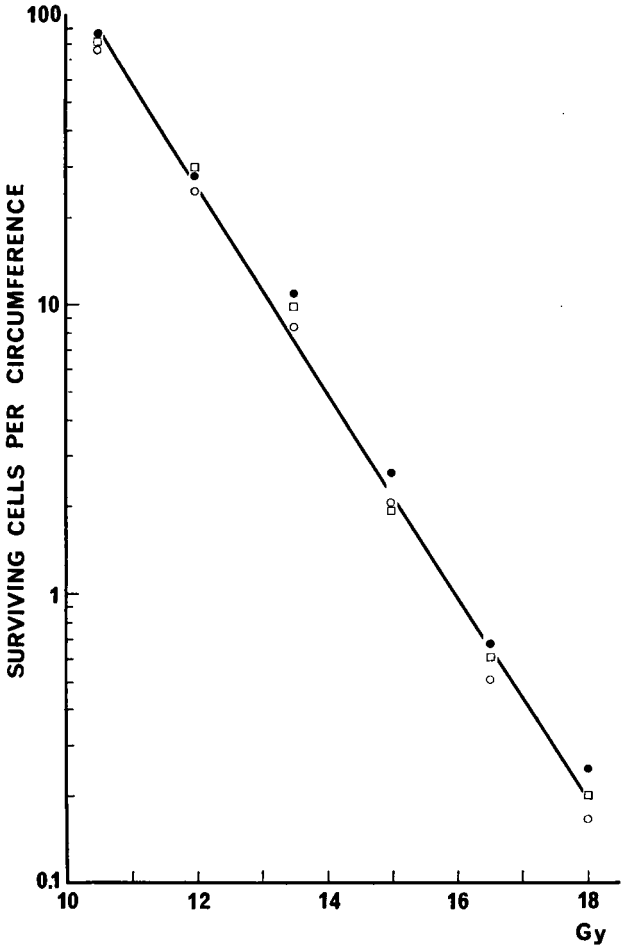

Fig. 2. Survival of jejunal crypt cells when given orgotein before or after a single dose of irradiation between 10 and 18 Gy. ● orgotein 2 h before and □ orgotein 30 min after irradiation. ○ controls (irradiation alone). Each point is the mean of 6 to 8 measurements. No significant difference was observed between any of the 3 groups. The pooled data of the 3 experiments are indicated by the curve and is characterized with a D_0 of 1.21 Gy and a D_{10} of 13.2 Gy.

Although not fully investigated, there appeared to be no variation in the acute radiation skin reaction on the tumour-bearing leg found in the 3 groups where a moist desquamation reaction seemed to occur at the same dose level in all 3 groups.

Discussion

An attempt was made to evaluate a possible protective effect of orgotein on the radiation response in different mammalian cell and tumour systems.

In none of the experimental systems used was any significant difference observed between the controls (i.e. irradiation alone) and the groups given orgotein before or after irradiation, respectively. Since the systems represent both malignant and normal cells treated under in vitro or in vivo conditions, it seems justified to conclude that orgotein given in combination with a single irradiation may not alter the quantitative

Table

Effect of pre- or post-treatment with orgotein on the radiation response in a C_3H mouse mammary carcinoma

Treatment	Dose (Gy) for TCD_{50}
Irradiation alone	55.52 (50.61–60.92)
Orgotein 2 h before irradiation	54.04 (47.66–61.28)
Orgotein 30 min after irradiation	55.18 (48.24–63.10)

95 % confidence limit in parentheses.

response to radiation. Thus, any reduced radiation reaction should be regarded as an indirect effect rather than a direct reduction of the primary radiation response. As discussed by others the influence of orgotein on leukocyte activity and migration may be a likely possibility (MARBERGER et coll. 1975, EDSMYR et coll.); this is also supported by the anti-inflammatory activity observed after administration of the drug under other circumstances (LUND-OLESEN & MENANDER 1974, MARBERGER et coll. 1974).

It was expected that orgotein did not influence the tumour response following a single radiation dose. The used mammary carcinoma is known to include a significant proportion of hypoxic cells (OVERGAARD, unpublished observations). These cells are the most resistant to radiation, and in a single irradiation they will be responsible for failure of tumour control. Superoxide radicals are only formed in presence of oxygen, and any reduction of the radical formation will not influence the radiation response in hypoxic cells—and therefore neither the control probability.

More likely, an increased survival response of the cells treated in air under in vitro conditions or in the jejunum would have been expected, as these populations appear to be sufficiently oxygenated. The significant radiation injury occurs within the nucleus. It is the formation of superoxide radicals in this area which causes the increased radiation injury in the presence of oxygen. However, even though the SOD concentration is high in the extracellular environment, it may not be transported across the cell membrane in sufficient quantities (HUBER, unpublished observations) and therefore not be present in the nucleus at the time of irradiation. This may explain why bacterial systems show a protective effect when treated in presence of oxygen and SOD versus more differentiated mammalian cells which have a more complex membraneous system.

Another explanation may be that the cells observed have a sufficient concentration of SOD, and further enzyme activity will not be needed in order to avoid superoxide radical injury. If this was the case, an effect could first be observed during a fractionated irradiation as the enzyme is used during the first treatment fractions and is only slowly increased again. In order to exclude such a possibility, further experiments with fractionated irradiation schedules are needed. However, in the present

experimental systems, the radiation sensitivity was rather high (L_1A_2 cells, D_0 about 1.10 Gy, jejunal crypt cells, D_0 118). Besides, the L_1A_2 cells are known to have an oxygen enhancement ratio of about 3.0. Thus it seems unlikely that they should have an intracellular SOD concentration sufficient to partly protect them from a single dose of radiation. Furthermore, PETKAU et coll. (1975, 1976) showed a protective effect on LD_{50} following administration of SOD in mice treated with a single whole-body irradiation. Also the investigations on myoblast differentiation by MICHELSON & BUCKINGHAM (1974) were performed with single radiation exposure. In none of these reports the direct quantitative cell killing was estimated following the irradiation, and at least the LD_{50} data may well be related to a secondary anti-inflammatory effect. Neither is it likely that the SOD protection found in whole-body irradiated mice is due to a direct effect on superoxide radicals, as a protection was observed to about the same degree if the enzyme was administered following the irradiation.

The present data support the hypothesis that the reduced radiation induced complications observed following orgotein treatment are caused by a secondary (anti-inflammatory) mechanism in the irradiated normal tissue rather than by a reduced primary irradiation response. Consequently, it appears to be justified to introduce orgotein into clinical trials with therapeutic irradiation of pelvic malignancies.

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SUMMARY

The potential radiation protective effect of orgotein, a metalloprotein with superoxide dismutase activity, was investigated in L_1A_2 tumour cells in vitro, jejunal crypt cells and C_3H mouse mammary carcinoma in vivo. No effect of orgotein, given either 2 hours before irradiation or 30 min after, was observed compared to the effect of irradiation alone. Thus, it was concluded that orgotein did not influence the primary radiation response in air in mammalian cells.

ZUSAMMENFASSUNG

Der mögliche strahlenschützende Effekt von Orgotein, einem Metallprotein mit Superoxide-Dismutase-Aktivität, wurde bei L_1A_2 Tumoren in vitro und in vivo bei Kryptzellen des Jejunums und dem C_3H Maus-Mammakarzinom untersucht. Kein Effekt von Orgotein, gegeben entweder 2 Stunden vor der Bestrahlung oder 30 Minuten danach, wurde beobachtet verglichen mit dem Effekt von Bestrahlung alleine. Es wird daraus gefolgert, dass Orgotein nicht die primäre Strahlenrespons in Luft von Säugetier-Zellen beeinflusst.

RÉSUMÉ

L'effet radioprotecteur potentiel de l'orgotéine, une métalloprotéine ayant une activité superoxyde-dismutase, a été étudié sur des cellules tumorales L_1A_2 in vitro, sur des cellules

de cryptes jéjunales et sur un carcinome mammaire de souris C_3H in vivo. L'administration d'orgotéine soit 2 heures avant l'irradiation, soit 30 minutes après l'irradiation n'a pas permis d'observer d'effet, par comparaison avec l'effet de l'irradiation seule. Ainsi les auteurs concluent que l'orgotéine n'a pas d'effet sur la réponse primaire aux radiations sur les cellules de mammifères.

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