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TOCOPHEROL IN IRRADIATION OF TEMPORARY HYPOXIC TUMOURS

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The sensitivity of malignant tumours to radiation is largely affected by intracellular tumour oxygenation. In solid tumours a considerable amount of cells has been found to be hypoxic and radiation resistant (VAN PUTTEN & KALLMAN 1968). This is one reason for utilizing fractionated irradiation, where hypoxic cells are supposed to be reoxygenated during the interval between the fractions. By eliminating dead cells, hypoxic cells might more easily come into closer contact with capillaries. This hypothesis has also support from in vivo experiments (THOMLINSON 1967). However, respiratory hyperoxia at the time of irradiation will not induce an effective oxygenation of hypoxic tumour cells. The reason for this is absence of adequate tumour vascularization (VAUPEL 1977).

In recent years many sensitizing compounds have been introduced. Much interest has been focused upon the nitroimidazoles (FOWLER et coll. 1976, 1979), and these substances have been tested in humans (URTASUN et coll. 1976). An ideal hypoxic sensitizer should sensitize hypoxic tumour tissue but not normal, well oxygenated tissue. It should be non-toxic to normal tissue and easy to administer, even to poorly vascularized tumour tissue.

Previously (KÅGERUD et coll. 1978a, b), it was found that tocopherol significantly enhanced the effect of local irradiation of two experimental tumours in the rat. However, tocopherol did not seem to directly influence the tumour oxygenation.

The aims of the investigation now reported were: (1) to investigate the influence of tocopherol on the effect of local irradiation of two transplanted rat tumours under tumour ischaemia, and (2) to assess the tumour oxygen pressure fields after tocopherol treatment and during induced tumour ischaemia.

Material and Methods

Inbred rats from a Lister strain of animals with a mean body weight of 150 g were used. The animals were fed ad libitum a diet of water and rat pellets (Astra-Ewos, Södertälje, Sweden; tocopherol added in a concentration of 40 mg/1 000 g pellets).

A 20-methylcholanthrene-induced sarcoma and a hepatoma were transplanted intramuscularly by trochar technique (about 1 mm^3 tumour tissue) into one hindleg of the animals. These tumours have recently been described in detail by KJARTANSSON (1976). Tumour ischaemia was induced by temporary tourniquet of the tumour-bearing hindleg during irradiation. The duration of the tourniquet was about 5 min, including one min before irradiation and the period of irradiation (3.75 min).

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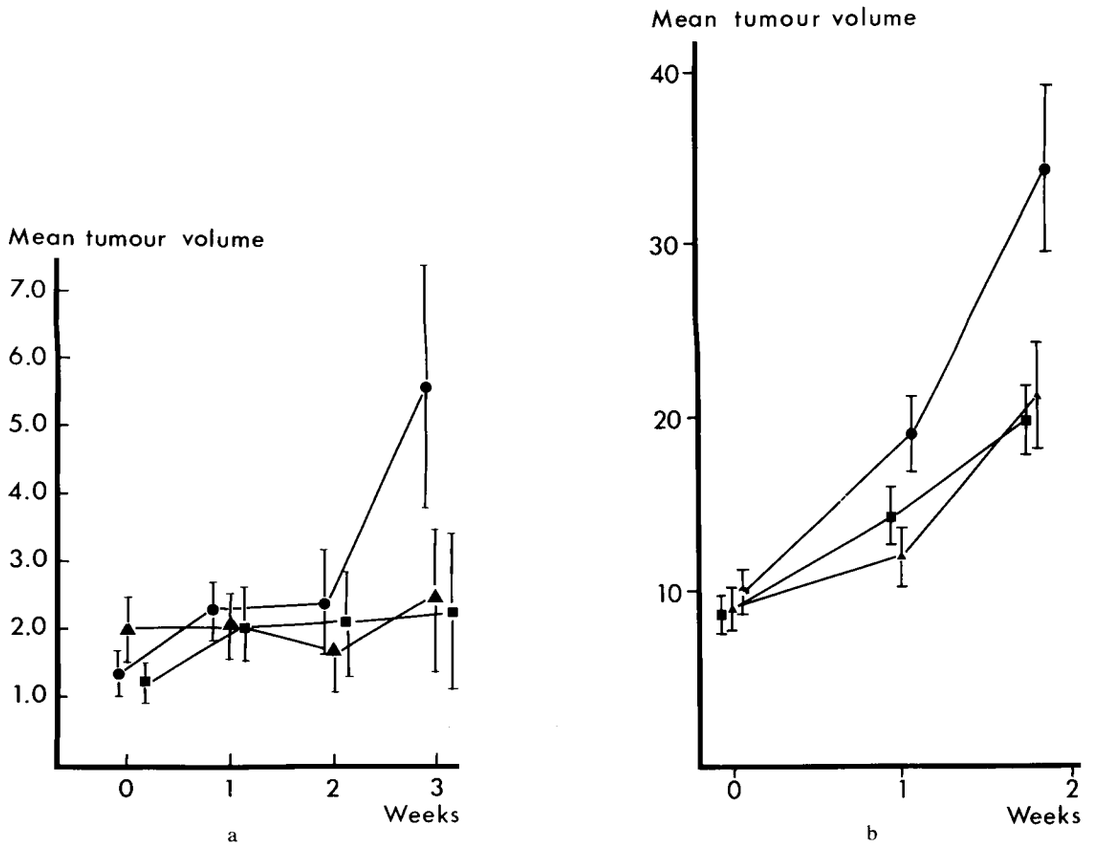

Fig. 1. a) 20-methylcholanthrene-induced sarcoma. Mean tumour volumes (cm^3) with SEM in 3 groups of animals with locally irradiated tumours on day 0. Temporary ischaemia during irradiation was induced in 2 groups and one of these groups was pretreated with a single dose of tocopherol. Temporary retardation of tumour growth in all groups, followed by a significantly enhanced regrowth in irradiated ischaemic tumours. This enhanced regrowth after irradiation under ischaemia seems to be eliminated by tocopherol pretreatment. b) Hepatoma. Same experimental design as in (a). Significantly increased growth rate in irradiated ischaemic tumours compared with irradiated controls and tocopherol-pretreated irradiated ischaemic tumours. Control (▲). Ischaemia (●). Ischaemia plus tocopherol (■).

Tocopherol. A dl-α-tocopherol preparation for veterinary use (Ido-E Aquosum Vet., Ferrosan, Sweden), containing 50 mg tocopherol/ml was injected intramuscularly in a single dose of 5 mg/100 g body weight 7 days before the irradiation.

Irradiation. The tumours were given a single, controlled dose of 30 Gy, the rest of the animal being protected by a lead shield. The radiation was generated by a therapy machine (Müller, RT 200), operated at 200 kV and 20 mA, and filter 0.2 mm Cu. The absorbed dose was controlled by a thermoluminescence system (LINDSKOUG et coll. 1967).

Recordings of tumour tissue oxygenation. For measurements of the oxygen pressure fields of tumour tissue the MDO (Mehrdraht Dortmund Oberfläche) electrode was used (KESSLER & GRÜNEWALD 1969). This electrode measures oxygen pressure at 8 points simultaneously. Wide variations in oxygen pressure in tissue, due to the relation of the measuring points to the capillary system, can thus be recorded (KESSLER 1968, LUND 1979).

The presentation of local tissue pO_2 (p_tO_2) by means of a frequency distribution histogram is a suitable method to describe the three-dimensional tissue oxygen pressure field (LÜBBERS 1977). The frequency distribution allows comparisons of different states of oxygen supply. (For a full description of methodology and technical equipment, cf. LUND.)

Experiments

Experiment I. Twenty-eight animals transplanted with the sarcoma and 26 animals transplanted with the hepatoma were used. For each tumour, animals were divided into 3 groups: (1) irradiated control

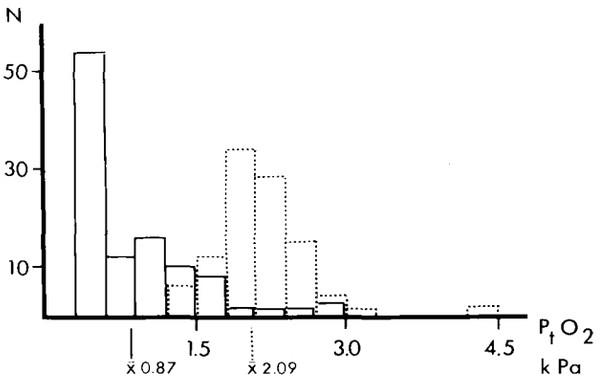

Fig. 2. Frequency distribution histograms for one tumour during temporary ischaemia (left) and one control tumour (right). Ischaemia by tourniquet did not only lower the mean pO_2 but also changed skewness and kurtosis of the distribution histogram, distorting it from a Gaussian type to a low-pressure type.

animals, (2) control animals irradiated under ischaemia, and (3) animals irradiated under ischaemia and pretreated with tocopherol. Induction of ischaemia and irradiation were made under light ether anaesthesia.

The tumour growth rate was followed by measuring once weekly 3 external tumour diameters and from these calculating the external tumour volumes according to the formula for a spherical body: $a/2 \times b/2 \times c/2 \times 4\pi/3$=tumour volume, where a, b, and c are external tumour diameters. This estimation of tumour volumes was previously found to be significantly correlated to exact tumour volumes and weights (KÅGERUD et coll. 1978a). The sarcomas were followed for 3 weeks and the hepatomas for 2 weeks. The animals were then killed.

Experiment II. Seventeen animals with sarcoma transplanted into one hindleg were used. In 9 of these animals ischaemia in the tumours was induced by temporary tourniquet of the tumour-bearing leg for 5 min. The other 8 animals were controls. All animals were anaesthetized with ether at the time of tourniquet. The animals were followed for 2 weeks and then killed. Tumour volumes were recorded weekly.

Experiment III. Twelve animals with sarcoma were used. Five animals were given tocopherol; 7 animals were controls. All animals were given light ether anaesthesia. In 3 tocopherol-pretreated animals and 4 control animals ischaemia was induced by tourniquet of the tumour-bearing legs. Recordings of tumour oxygen pressures were made at 105 different points of the gently dissected and exposed surface of each tumour. A tumour oxygen pressure histogram was calculated for each animal (ÖDMAN & LUND 1980).

Results

In the first experiment (Fig. 1) a temporary retardation of the sarcoma growth rate was found in all groups after irradiation. At the end of the experiment a significantly ($p<0.05$, Wilcoxon test) enhanced regrowth was observed in the irradiated, temporary ischaemic group, compared with irradiated controls and tocopherol-pretreated ischaemic animals. The hepatomas were larger at the time of irradiation and the animals would not have survived for 2 more weeks without irradiation. The post-irradiation growth rate was significantly higher ($p<0.01$, Wilcoxon test) in hepatomas irradiated under ischaemia, compared with irradiated controls and tocopherol-pretreated ischaemic tumours.

In the second experiment mean tumour volumes (with SEM) increased from 3.9 (0.4) cm^3 to 31.5 (2.3) cm^3 in temporary ischaemic tumours and from 3.5 (0.3) cm^3 to 34.7 (1.8) cm^3 in control tumours. The 2 groups did not differ significantly ($p>0.1$, Student's t-test) in growth rate.

In the third experiment (Fig. 2) the mean oxygen pressure was 0.97 kPa in ligated tumours compared with 2.39 kPa in non-ligated tumours. Statistical evaluations of the frequency distributions confirmed this significant difference ($p<0.001$ for all differences, Kolmogorov-Smirnov test). Tocopherol-pretreated tumours with or without induced ischaemia did not differ from their corresponding control.

Discussion

The influence of pretreatment with tocopherol on the effect of local irradiation of 2 transplanted rat tumours, artificially made ischaemic by tourniquet during irradiation, was investigated. A retardation of tumour growth rate was found in all irradiated animals. A continuous growth of non-irradiated tumour was shown previously (KÅGERUD et coll. 1978a).

Local ischaemia during tumour irradiation can reduce the effect of irradiation (VAN PUTTEN & KALLMAN). According to these authors such an effect is found within 1 min after vascular occlusion. In the present experiment the retardation of tumour growth after irradiation was of short duration in ischaemic tumours and was followed by a significantly increased regrowth. Pretreatment with tocopherol seemed to eliminate the enhancing effect of ischaemia on tumour growth rate after irradiation.

The temporary ischaemia induced by tumour ligation did not per se influence the tumour growth rate.

In a separate experiment the tissue surface oxygen pressure fields of tumours with and without artificial ischaemia were determined. A significant distortion of the oxygen pressure histograms was found for ligated tumours, indicating that the method for induction of ischaemia did in fact induce a regional tumour hypoxia. Tocopherol-pretreatment did not influence the oxygen pressure and thus the enhancing effect by tocopherol on irradiation of hypoxic tumours could not be explained by an improved tumour oxygenation.

A protective effect of tocopherol to radiation has been found by some authors (PRINCE & LITTLE 1973, SAKAMOTO & SAKKA 1973, KONINGS & FONK 1979). On the other hand MALICK et coll. (1978), found no major effect of pretreatment with tocopherol on survival of mice after whole-body irradiation. However, tocopherol given after irradiation enhanced significantly the survival. Further investigations of timing, dosage and type of administration are needed to rule out the confusion regarding the influence of tocopherol on the effect of irradiation. The observations made in the present experiments support the conclusion of tocopherol being a sensitizer of hypoxic tumours, when given intramuscularly to rats in a dose of 5 mg/100 g body weight.

SUMMARY

The influence of tocopherol on the effect of local irradiation under induced ischaemia by temporary tourniquet of two rat tumours transplanted intramuscularly into one hindleg was evaluated. An impaired retardation of growth rate occurred in tumours irradiated under ischaemia. This effect was eliminated by pretreatment of animals with tocopherol. In separate experiments the method of inducing ischaemia was investigated by MDO-electrode measurements of tumour tissue oxygen pressure. A significant tumour hypoxia was found under tourniquet of the tumour-bearing leg of the animals. Pretreatment with tocopherol did not influence the tumour pO_2.

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