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The effect of evening primrose oil on the radiation response and blood flow of mouse normal and tumour tissue

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Abstract.
Purpose: To investigate the effect of the oral administration of evening primrose oil on the radiation response and the blood flow of normal tissue and a tumour in BALB/c mice.
Methods and materials: Aliquots of evening primrose oil were fed to BALB/c mice daily and the radiation response of the skin was assessed by the determination of ED_{50} values for the incidence of moist desquamation, using probit analysis. Tumour radiosensitivity was investigated by determining the growth delay caused by irradiation of a transplantable rhabdomyosarcoma. The ^{86}RbCl uptake technique was used to determine the blood flow in normal foot and tumour tissue. The fatty-acid content of red blood cells, plasma and tumour tissue was measured using gas chromatography.
Results: Daily evening primrose oil dietary supplementation reduced the sensitivity of skin to radiation-induced moist desquamation and prevented the radiation-associated increase in blood flow that was observed in this tissue. No modification of tumour blood flow or of tumour sensitivity to radiation resulted from evening primrose oil supplementation of mice. Evening primrose oil supplementation resulted in changes in plasma levels of linoleic acid (LA), gamma-linolenic acid (GLA), dihomo-gamma-linolenic acid (DGLA) and arachidonic acid (AA). These changes were contingent on whether the mice had been irradiated or not. In red blood cells evening primrose oil supplementation increased the GLA level of unirradiated mice and the LA level at 20 days after irradiation. There were no changes in tumour fatty-acid levels as a result of evening primrose oil treatment.
Conclusions: Daily evening primrose oil supplementation reduced the sensitivity of skin to radiation-induced moist desquamation but did not alter tumour sensitivity to radiation.

1. Introduction

A major pathophysiological pathway involved in the development of radiation damage to normal tissues appears to be related to eicosanoid metabolism (Eldor *et al.* 1987, Ramesh *et al.* 1992). The eicosanoids, which include the 1- and 2-series prostaglandins, are metabolites of essential fatty acids and are associated with numerous physiological responses of tissues and blood vessels (Wolfe 1982, Needleman *et al.* 1986, Smith 1989). Of relevance to clinical radiotherapy is the role of eicosanoids in the inflammatory response evoked by radiation. It has been proposed that modifications in essential fatty-acid levels may ameliorate the expression of radiation-induced damage by correcting for imbalances in eicosanoid metabolism (Hopewell *et al.* 1993). Essential fatty acids are not only precursors of the eicosanoids but also play an important role in the structure of cell membranes and consequently in the regulation of membrane functions such as permeability and transport (Poon *et al.* 1981, Spector and Yorek 1985, Hagve 1988). Manipulation of fatty acids themselves, therefore, may be a major factor affecting the cellular integrity and, hence, the response of tissues to radiation.

Evening primrose oil is a rich source of the essential fatty acids, LA and GLA (Hopewell *et al.* 1993). Dietary supplementation with evening primrose oil is hypothesized to alter not only the concentrations of LA and GLA but also the relative levels of the 1- and 2-series prostaglandins, the latter group having strong pro-inflammatory properties (Hopewell *et al.* 1993). Tumours obtain fatty acids preformed from the host and this is supplied primarily from circulating free fatty acids (Spector 1967). Alteration of the host fatty-acid intake via dietary manipulation should consequently alter tumour fatty-acid levels. As polyunsaturated fatty acids are readily oxidizable (Rice-Evans and Burdon 1993), they serve as substrates for free-radical species generated by ionizing radiation and in this way could increase tumour radiosensitivity. An increase in tumour radiosensitivity could be achieved if an increase in the 1-series prostaglandin, prostaglandin E_1, followed evening primrose oil supplementation. Prostaglandin E_1, by virtue of its vasodilatory action could result in an increase in tumour blood flow and hence a decrease in tumour hypoxia.

2. Methods and materials

2.1. *Mice and tumours*

Female, specific-pathogen-free BALB/c mice, aged from 6 to 8 weeks and weighing 20 ± 1 g at the

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beginning of the experiments, were used throughout the study. A total of 700 mice were used. The transplantable rhabdomyosarcoma used in these experiments was grown in BALB/c mice by the inoculation of 2×10^6 viable cells into the right gastrocnemius muscle. The rhabdomyosarcoma was originally induced in our laboratory by the injection of 0.1 ml of a 1 mg/ml solution of 3-methylcholanthrene in arachis oil into the flanks of BALB/c mice.

2.2. *Evening primrose oil administration*

Evening primrose oil was kindly supplied by Scotia Pharmaceuticals, South Africa. 10 μl of the oil was fed to mice daily by gavage. The constituent fatty acids of the evening primrose oil mixture used in these studies were linoleic acid (70.2%), gamma-linolenic acid (8.7%), oleic acid (12.7%), palmitic acid (5.8%), stearic acid (1.8%) and others (0.8%) (Hopewell *et al.* 1993).

2.3. *Irradiation*

All irradiations were carried out in air at room temperature. Mice were restrained without anaesthesia in perspex jigs such that their right hind legs protruded into the radiation field. The feet were gently taped to the base of the jig with thin strips of masking tape. Mice were irradiated from above with ^{60}Co γ-rays (SSD = 80 cm, dose rate ± 90 cGy/min). Lead blocks, 10 cm thick, shielded the mice so that only the leg was irradiated. Build-up was provided using a 0.5-cm-thick acrylic plastic plate.

2.4. *Normal tissue radiation response*

In addition to a standard diet, mice received 10 μl of either evening primrose oil or water daily for 2 weeks prior to and 4 weeks post-irradiation. The right hind foot was exposed to single doses of radiation ranging from 33 Gy to 43 Gy. The irradiated foot was assessed daily and the percentage of mice that developed moist desquamation was plotted as a function of radiation dose. A sigmoidal dose–response model with variable slope was fitted to the data and the ED_{50} (the dose of radiation that results in moist desquamation of 50% of the mice) determined. Significance of difference between the ED_{50} values was assessed using the Student's t-test.

2.5. *Tumour radiation response*

Aliquots of 10 μl of evening primrose oil or water were administered daily for 2 weeks prior to irradiation and the treatment continued until tumours reached their end-point. Mice were inoculated with tumour cells one week after commencing evening primrose oil or water treatment, and tumours ranged in diameter from 7.25 to 8.75 mm after another week, i.e. at the time of irradiation. The response of tumours to radiation was assessed by a growth delay assay (Thomlinson 1980). Tumour diameter was determined by fitting the tumour-bearing leg into apertures of known diameter, which had been cut into a perspex plate (Hendrikse *et al.* 1995). Tumours were locally irradiated with a single dose of 30 Gy. After irradiation, mice were kept under ambient conditions and the tumours measured daily until a diameter 1.5 times the initial diameter was reached. The growth delay caused by irradiation was determined as the time for the irradiated tumours to grow to 1.5 times their initial diameter minus the time for the unirradiated controls to do likewise. Significance of difference was assessed by the Student's t-test.

2.6. *Blood flow estimation*

The ^{86}RbCl extraction method was used to determine blood flow (Sapirstein 1958). Mice received 10 μl aliquots of either evening primrose oil or water daily for 2 weeks. The normal foot and tumour were then given a single dose of 38 Gy and blood flow of these tissues was determined at various times after irradiation. The tumour size at the time of irradiation ranged from a diameter of 7.25 mm to 8.75 mm. ^{86}RbCl, specific activity 37–296 MBq/mg, was obtained from Amersham International plc, Buckinghamshire, UK. The isotope (0.185 MBq) was injected in 0.1 ml of saline into the tail vein of mice. The mice were killed 1 min. later by cervical dislocation and tumour, as well as normal foot tissue, excised. The tissues were weighed and subsequently counted in a gamma counter. Tissue samples were excluded from the analysis if more than 10% of the injected activity was measured in the tail. A standard of 0.1 ml of the injected solution was also counted.

2.7. *Fatty acid analysis*

Mice were administered 10 μl aliquots of either evening primrose oil or water daily for 1 week. Mice were then inoculated with tumour cells and fed for a further week. At the end of this period blood was obtained from the inferior vena cava and centrifuged to separate the plasma and red blood cell fractions, and tumour tissue was excised. The fatty-acid composition of these tissues was then determined. In a further experiment, groups of BALB/c mice were

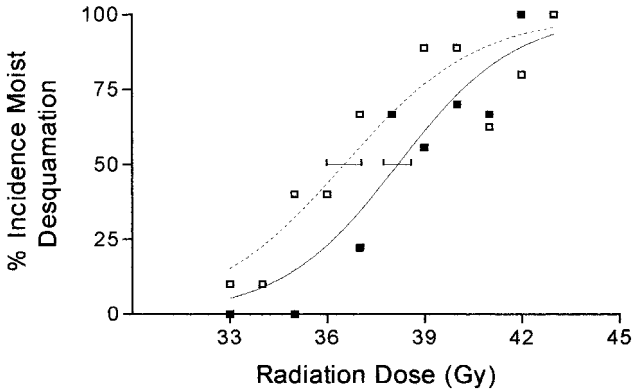


Figure 1. Radiation dose-related changes in the percentage incidence of moist desquamation in BALB/c mice administered water (–□–) or evening primrose oil (–■–) for 2 weeks prior to irradiation and a further 4 weeks post-irradiation. The curves drawn are best fits of the data. At least eight mice were used for each data point. Error bars indicate SEM.

fed 10 μl aliquots of evening primrose oil or water for 2 weeks and then given a 38 Gy dose of radiation to their right hind foot. Fatty-acid composition of plasma and red blood cell fractions was then determined at 5 h and at 20 days (mice continued to receive evening primrose oil daily after irradiation) after irradiation. In brief, the method for the determination of the fatty-acid composition of tumour tissue, plasma and red blood cells was as follows: tumour tissue (50 mg) was pulverized in liquid nitrogen and the total fatty-acid fraction was extracted with chloroform:methanol (2:1; v/v) containing 0.01% butylated hydroxytoluene (Smuts and Tichelaar 1991). The total fatty-acid fraction from the red blood cells and plasma was similarly extracted from 100 μl aliquots (Rose and Oklander 1965, Smuts and Tichelaar 1991). Total fatty-acid methyl esters were analysed on a gas chromatograph. Fatty-acid composition was calculated and results expressed as % weight/weight. Fatty-acid methyl esters were identified by comparison of the retention times with those of the standard mixture of fatty acids. Significance of difference was assessed by the Student's t-test.

3. Results

3.1. *Normal tissue radiation response*

The incidence of moist desquamation as a function of radiation dose for mice given either the evening primrose oil or water-supplemented diet is illustrated in figure 1. The $ED_{50} \pm$ S.E.M. for mice fed evening primrose oil was 38.17 ± 0.43 Gy whilst that for mice fed water was 36.52 ± 0.53 Gy, yielding a dose modification factor of 1.05 ± 0.02. The difference between the two ED_{50} values was statistically significant ($p < 0.05$).

3.2. *Tumour tissue radiation response*

The administration of evening primrose oil without radiation had no effect on tumour growth (table 1). Dietary evening primrose oil supplementation did not modify the response of the tumour tissue to radiation (table 1).

3.3. *Blood flow*

3.3.1. *Normal tissue.* Dietary evening primrose oil supplementation did not change the blood flow of unirradiated normal tissue. A significant increase in blood flow was evident at 5 h after irradiation in mice supplemented with water ($p < 0.05$). In mice receiving evening primrose oil supplementation, blood flow at 5 h after irradiation was not statistically significantly different from that of unirradiated mice (figure 2). No change in blood flow was observed at 10 days (i.e. the time when the main erythema reaction peaked) or 20 days (i.e. the time when moist desquamation peaked) after irradiation in mice supplemented with either water or evening primrose oil.

3.3.2. *Tumour tissue.* Evening primrose oil supplementation did not alter the blood flow of unirradiated tumours (figure 3). Radiation did not alter the tumour blood flow of mice supplemented with either water or evening primrose oil.

Table 1. Tumour growth delay caused by 30 Gy γ-radiation in BALB/c mice supplemented with water or evening primrose oil.

Supplementation product	Radiation dose (Gy)	Time to reach end-point (days) ± SEM	Radiation-induced growth delay (days) ± SEM
Water	0	4.95 ± 0.19	—
Evening primrose oil	0	4.90 ± 0.30	—
Water	30	12.3 ± 0.33	7.35 ± 0.38
Evening primrose oil	30	13 ± 0.56	8.1 ± 0.64

Each treatment group consisted at least 18 mice.

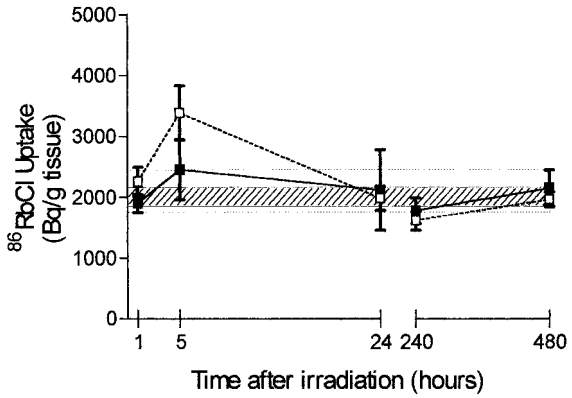


Figure 2. Changes in normal tissue blood flow after 38 Gy. BALB/c mice were administered water (–□–) or evening primrose oil (–■–) for 2 weeks prior to irradiation. Each data point represents the mean of 11 animals, and error bars indicate SEM. The hatched area indicates normal tissue blood flow (± 1 SEM) of unirradiated mice fed evening primrose oil. The area between the dashed lines indicates normal tissue blood flow (± 1 SEM) of unirradiated water-fed mice.

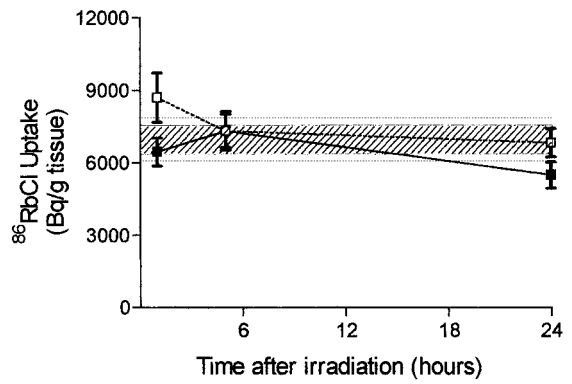


Figure 3. Changes in tumour tissue blood flow after 38 Gy. BALB/c mice were administered water (–□–) or evening primrose oil (–■–) for 2 weeks prior to irradiation. Each data point represents the mean of 11 animals, and error bars indicate SEM. The hatched area indicates tumour blood flow (± 1 SEM) of unirradiated mice fed evening primrose oil. The area between the dashed lines indicates tumour blood flow (± 1 SEM) of unirradiated water-fed mice.

3.4. *Fatty-acid analysis*

In figure 4 the LA, GLA, DGLA and AA levels of mice supplemented with evening primrose oil are compared with those of mice supplemented with water. Fatty acids were measured in plasma, red blood cells and tumour tissue of unirradiated mice. In addition, plasma and red–blood-cell fatty-acid levels were measured at 5 h (coinciding with the time at which blood-flow changes were measured) and 20 days (when moist desquamation peaked) after irradiation.

3.4.1. *Plasma.* Evening primrose oil supplementation increased the levels of LA and GLA in unirradiated mice ($p < 0.05$) (figures 4A and B). In groups of mice supplemented with evening primrose oil there were statistically significant increases in the LA, DGLA and AA levels at 5 h after irradiation ($p < 0.05$) (figures 4A, C and D). At 20 days after irradiation the GLA and AA levels were significantly higher ($p < 0.05$) in the evening primrose oil supplemented group than in the water supplemented group (figures 4B and D).

3.4.2. *Red-blood cells.* Evening primrose oil supplementation increased the GLA level in unirradiated mice (figure 4B) and increased the LA level at 20 days after irradiation (figure 4A) ($p < 0.05$).

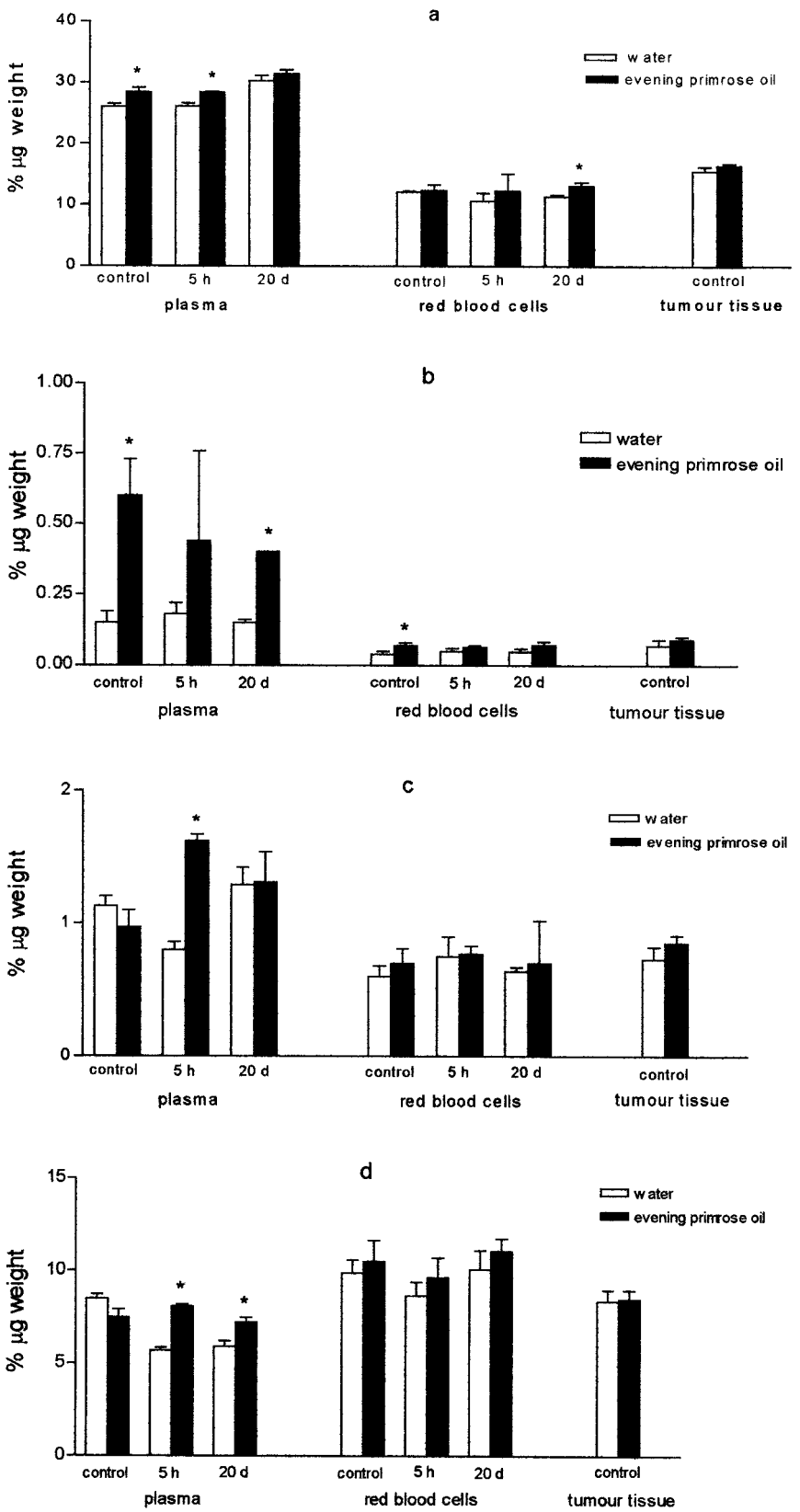
3.4.3. *Tumour tissue.* There were no changes in the fatty-acid levels of tumour tissue as a result of evening primrose oil supplementation.

4. Discussion

Fatty acids are integral molecules of cell membranes and fatty-acid supplementation can lead to diverse biochemical and physiological effects. In this study, evening primrose oil supplementation was shown to prevent the increase in normal tissue blood flow that occurred after irradiation in mice receiving a standard diet only. Although this early radiation-induced increase in blood flow is unlikely to be involved in the main erythema, which occurs several days after irradiation, it is possible that preventing this blood flow increase may result in an amelioration of later sequelae of radiation. This is supported by our findings that evening primrose oil supplementation of mice reduced the sensitivity of normal tissue to radiation-induced moist desquamation, which also corroborates the pig skin data of Hopewell *et al.* (1993).

The increase in blood flow that occurs in response to an injurious agent, such as radiation, forms part

Figure 4. Changes in the level of LA (a), GLA (b), DGLA (c) and AA (d) in plasma, red blood cells and tumour tissue as a result of evening primrose oil supplementation. Fatty-acid levels of plasma and red blood cells were determined at 5 h and at 20 days, after a dose of 38 Gy, as well as in unirradiated mice. Tumour-tissue fatty-acid levels were determined in unirradiated mice. Each treatment group represents the mean of 4–6 animals, and error bars indicate ± 1 SEM. The asterisk denotes that the evening primrose oil supplemented group is significantly different to the water-supplemented group ($p < 0.05$).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of the inflammatory response and is mediated by the 2-series prostaglandins. It is proposed that evening primrose oil supplementation prevented the radiation-induced increase in blood flow by altering essential fatty-acid and prostaglandin levels. Evening primrose oil supplementation resulted in an increase in the GLA levels of both plasma and red blood cells at the time of irradiation of the normal tissue (figure 4b). GLA is metabolized within the cell to DGLA, which is then metabolized either to the 1-series prostaglandins or, via a rate-limiting step, to AA, which is the precursor of the 2-series prostaglandins. In water-supplemented mice, the decrease in plasma AA levels as a result of radiation may indicate an increased conversion of this fatty acid to the 2-series prostaglandins, which was possibly prevented by evening primrose oil supplementation (figure 4D). The production of the 1-series prostaglandins is dependent on the amount of DGLA that can undergo conversion and increased amounts of DGLA following evening primrose oil (figure 4C) supplementation is expected to elevate the 1-series prostaglandins, in particular prostaglandin E_1 (Kernoff *et al.* 1977, Horrobin *et al.* 1984, Horrobin 1990a,b). An increase in the amount of the 1-series relative to the 2-series prostaglandins in arterial and arteriolar smooth muscle cells following evening primrose oil supplementation may reduce, or even prevent the increase in blood flow that normally occurs in irradiated tissue (Sinzinger *et al.* 1984, Tate *et al.* 1988, 1989, Pullman-Mooar *et al.* 1990). As prostaglandins may be involved in regulating red blood cell deformability in passing through capillaries (Rasmussen *et al.* 1975, Oonishi *et al.* 1997, Oonishi *et al.* 1998), changes in prostaglandin levels in red blood cells following evening primrose oil supplementation could modify the blood flow changes that normally occur after irradiation.

Evening primrose oil supplementation did not modify the response of tumour tissue to radiation. This is compatible with the finding that evening primrose oil supplementation did not alter the fatty-acid profile or blood flow of tumour tissue.

In conclusion, this study has confirmed that evening primrose oil can ameliorate the damaging effects of radiation on normal tissue. No modification of the tumour radiation response as a result of evening primrose oil supplementation was observed. These findings suggest that evening primrose oil supplementation may be of benefit in clinical radiotherapy in improving the therapeutic ratio.

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