

Effect of n-3 Fatty Acids on the Antitumour Effects of Cytotoxic Drugs

MICHAEL P. WYNTER, STEVEN T. RUSSELL and MICHAEL J. TISDALE

Pharmaceutical Sciences Research Institute, Aston University, Birmingham, B4 7ET, U.K.

Abstract. *Background:* n-3 fatty acids are increasingly being administered to cancer patients for the treatment of cachexia, and it is thus important to know of any potential interactions with ongoing cytotoxic drug therapy. *Materials and Methods:* For this reason eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were administered to mice bearing the cachexia-inducing MAC16 colon adenocarcinoma, and the effect of epothilone, gemcitabine, 5-fluorouracil and cyclophosphamide on tumour growth and body weight determined. *Results:* Epothilone alone had a minimal effect on tumour growth rate, but this was potentiated by DHA, while for 5-fluorouracil and cyclophosphamide tumour growth inhibition was enhanced by EPA. The antitumour effect of gemcitabine was not altered by either fatty acid. EPA arrested the development of cachexia, while DHA had no effect and the same was true for their effect on tumour growth rate. The anticachectic effect of EPA was only seen in combination with 5-fluorouracil. *Conclusion:* These results suggest that n-3 fatty acids do not interfere with the action of chemotherapy and may potentiate the effect of certain agents.

Polyunsaturated fatty acids (PUFA) of the n-3 series, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), have important roles in inhibiting tumour growth and metastasis (1). EPA has been shown to attenuate the development of weight loss in patients with advanced pancreatic cancer (2), and a fish oil enriched nutritional supplement, high in protein and energy, has been shown to promote weight gain in cachectic cancer patients through an increase in lean body mass (3). In addition, n-3 PUFA have

a considerable immunomodulating effect in patients with solid tumours, increasing the ratio of T-helper cells to T-suppressor cells, prolonging the survival of both cachectic and non-cachectic subjects (4).

Incorporation of n-3 PUFAs into membrane lipids would alter membrane fluidity and membrane-related functions and could influence the uptake of cytotoxic chemicals. Modification of tumour cell membranes with DHA has been shown to significantly enhance the sensitivity of L1210 murine leukaemic cells to the antineoplastic agent adriamycin (5), as well as chemoresistant human small cell lung cancer cell lines, concomitant with a 10-30% increase in adriamycin concentration (6). Fish oil has also been shown to enhance the antitumour effect of optimal doses of doxorubicin, as well as reducing severe heart damage caused by high doses of this agent (7), and DHA has been shown to increase the sensitivity of a human small cell carcinoma cell line to cisplatin (8).

Fish oil supplements are now marketed in high-energy and high-protein, calorically dense nutritional supplements as Resource[®] Support and ProSure for the treatment of cancer patients. It is thus important to understand how these n-3 PUFAs interact with chemotherapy. This study examines the effect of EPA and DHA alone and in combination with selected chemotherapeutic agents on tumour growth and cachexia using the MAC16 murine cachexia model (9). The MAC16, like most human tumours producing cachexia, is highly chemoresistant (10) and is thus considered a good model to study chemotherapeutic interactions.

Materials and Methods

Materials. EPA and DHA were obtained as their triglycerides Omegavie 90-TG from Polaris, France and contained 93.2% EPA and 93.5% DHA, respectively. Epothilone (EP0906) was a gift from Novartis Pharmaceuticals, Switzerland, and all other materials were purchased from Sigma Aldridge, Dorset, UK.

Animals. Pure strain male NMRI mice were bred in our own colony and were transplanted with fragments (1-2mm³) of the MAC16 tumour *s.c.* into the flank, by means of a trochar, as described (9).

Abbreviations: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; 5-FU, 5-fluorouracil; PUFA, polyunsaturated fatty acid.

Correspondence to: Michael J. Tisdale, Pharmaceutical Sciences Research Institute, Aston University, Birmingham, B4 7ET, U.K. Fax: +44 121 333 3172, e-mail: m.j.tisdale@aston.ac.uk

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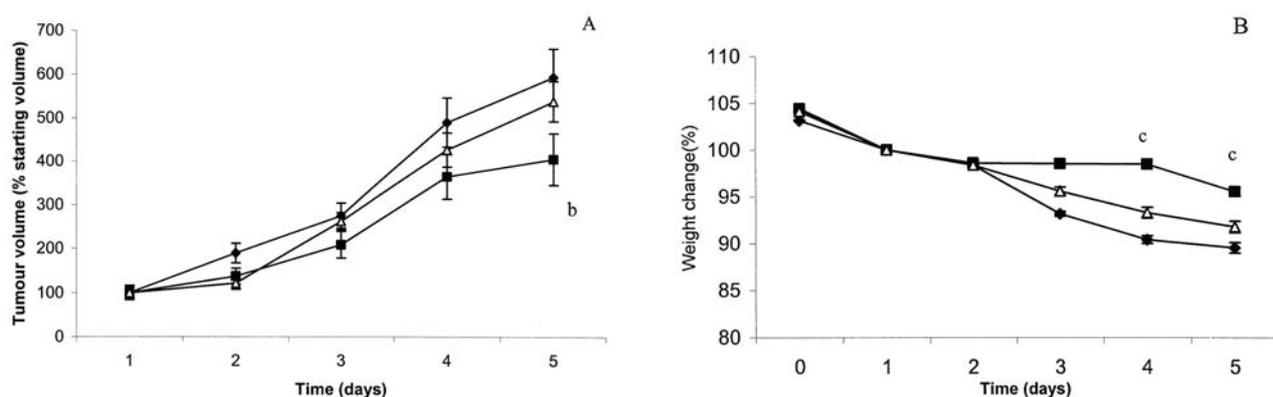


Figure 1. Effect of the synthetic triglycerides of EPA (2g/kg; ■) and DHA (2.25g/kg; △) administered p.o. daily in olive oil, compared with olive oil alone (◆) on tumour growth rate (A) and body weight loss (B) in mice bearing the MAC16 tumour. Differences from control are indicated as a, $p < 0.05$, b, $p < 0.01$ and c, $p < 0.001$.

All animal experiments followed a strict protocol, approved by the British Home Office. Transplanted animals were given free access to food and water and experiments were initiated when the tumour became palpable, at which time the average weight loss was about 5%. The fatty acids were administered as triglycerides in olive oil. The concentrations of EPA and DHA were based on those previously reported to exert an anticachectic and antitumour effect as the free acid (11). Control animals received olive oil alone. The solvents for the chemotherapeutic agents and the dose regime is indicated in the figure legends. Body weight was measured daily by means of a top loading balance, and tumour dimensions were measured by means of calipers. Tumour volume (T.V.) was calculated from the formula:

$$T.V. = \frac{\text{length} \times (\text{width})^2}{2}$$

Animals were terminated if the tumour volume exceeded 1000mm³, the tumour ulcerated, or if weight loss exceeded 25% of the starting body weight. All experiments have been carried out with groups of 10 mice (6-8 weeks of age, average weight 25g).

Statistical analysis. Results are expressed as means ± s.e.m. Differences were determined by one-way ANOVA followed by Tukey-Kramer Multiple Comparison Test.

Results

Effects of fatty acids alone. Treatment of mice bearing the MAC16 tumour with EPA alone (2g/kg) produced a small but significant inhibition of tumour growth rate (Figure 1A), while producing almost complete attenuation of the development of cachexia (Figure 1B). This shows that EPA as the triglyceride was as effective as the free acid (11). This contrasts with the ethyl ester, which has been shown to be devoid of antitumour and anticachectic activity (12). In contrast to EPA, DHA (2.25g/kg) had no significant effect on either the growth of the MAC16 tumour (Figure 1A) or on the development of weight loss (Figure 1B).

Effect of combination with epothilone. The epothilones are a new group of microtubule-stabilizing compounds, derived from the fermentation of the myxobacterium *Sorangium cellulosum*, which possess potent *in vivo* antitumour activity in some experimental animal models (13). The effect of epothilone alone or combined with either EPA or DHA on tumour growth and weight loss in mice bearing the MAC16 tumour is shown in Figure 2. Epothilone alone (2.5 mg/kg) had a minimal effect on tumour growth rate, which was not significant (Figure 2A). There was a significant reduction in tumour growth rate when epothilone was combined with DHA, but not EPA, nor the combination of EPA and DHA (Figure 2A). There was no increase in weight loss in mice treated with epothilone (Figure 2B), but epothilone appeared to attenuate the anticachectic effect of EPA.

Effect of combination with gemcitabine. Gemcitabine (2', 2'-difluoro-2'-deoxycytidine) is a fluorine-substituted cytarabine analogue, which is the agent of choice in the palliative treatment of pancreatic cancer (14). Gemcitabine alone (50mg/kg) had no significant effect on the growth of the MAC16 tumour (Figure 3A), and this did not appear to be enhanced or reduced by combination with either EPA or DHA. Gemcitabine had no effect on the development of cachexia in the MAC16 model (Figure 3B), and the anticachectic effect of EPA alone (Figure 1B) was not seen in combination with gemcitabine (Figure 3B).

Effect of combination with 5-fluorouracil (5-FU). 5-FU is an antimetabolite used to treat a number of solid tumours, including colon and breast cancer. 5-FU alone (80 mg/kg) inhibited growth of the MAC16 tumour and the combination with EPA or DHA gave a further small increase in tumour growth inhibition (Figure 4A). 5-FU did not produce a significant decrease in the loss of body weight (Figure 4B), but the combination with EPA was highly

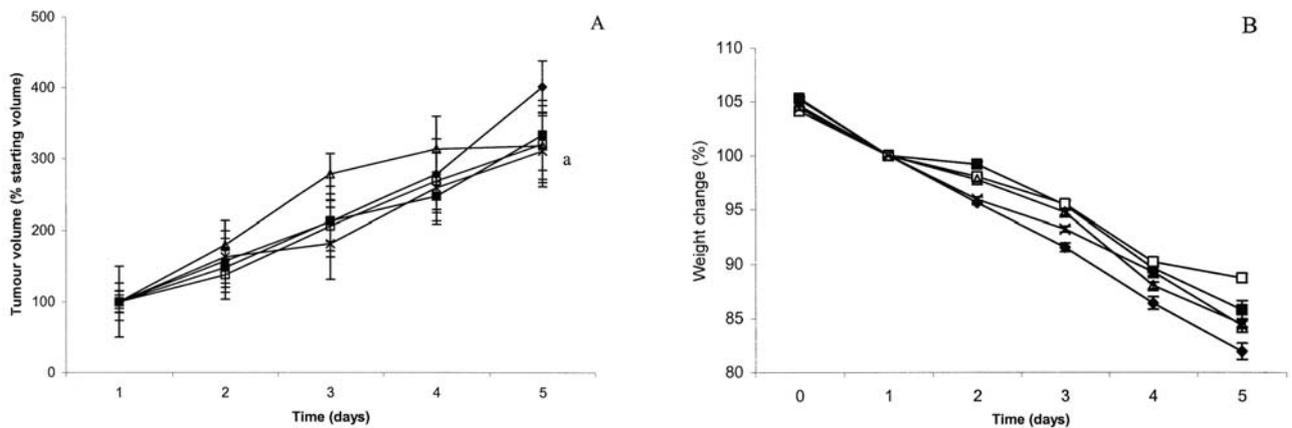


Figure 2. Effect of epoehilone (2.5mg/kg in 10% DMSO + PBS) administered *i.p.* as a single dose on day 2, alone (■), or combined with EPA (2g/kg; □), DHA (2g/kg; X) or EPA and DHA (△) on tumour growth rate (A) and body weight loss (B) in mice bearing the MAC16 tumour. The fatty acids were administered *p.o.* daily in olive oil and control animals (◆) received olive oil alone. Differences from control are shown as a, $p < 0.05$. Treatment with EPA and DHA alone is shown in Figure 1.

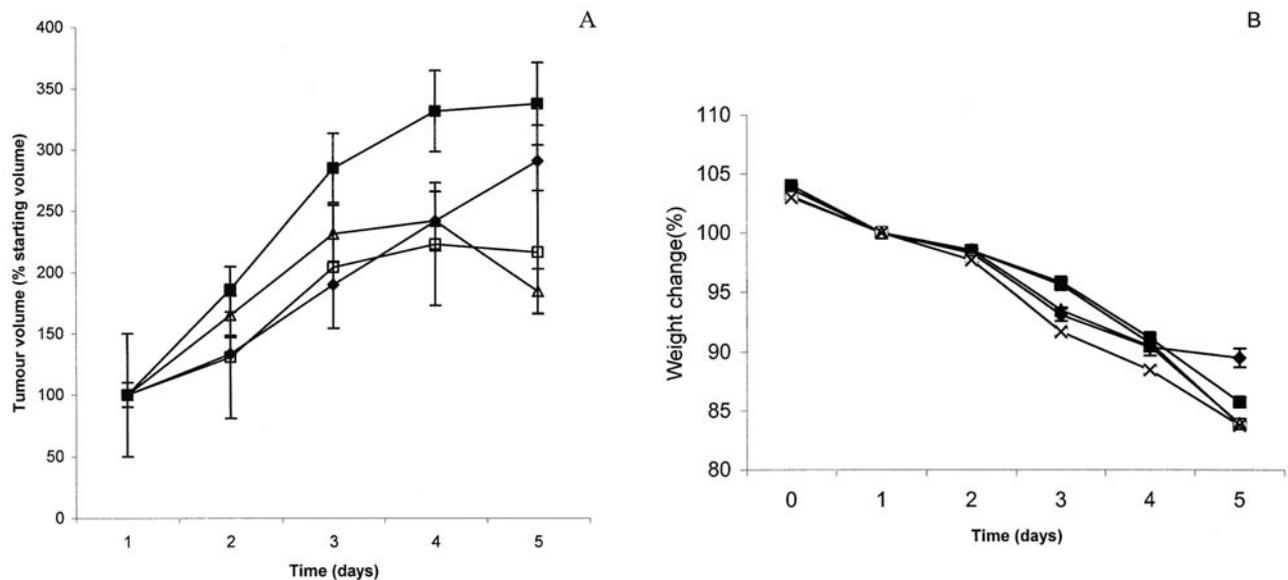


Figure 3. Effect of gemcitabine (50mg/kg in PBS) administered *i.p.* on alternate days, alone (■) or combined with EPA (2g/kg; □), DHA (2g/kg; X) or EPA and DHA (△) on tumour growth rate (A) and body weight loss (B) in mice bearing the MAC16 tumour. The fatty acids were administered *p.o.* daily in olive oil and control animals received olive oil alone (◆). There was no difference in any parameter from control animals administered PBS. Differences from control are indicated as a, $p < 0.05$. Treatment with EPA and DHA alone is shown in Figure 1.

effective in preventing the development of cachexia compared with 5-FU alone.

Effect of combination with cyclophosphamide. Cyclophosphamide is an alkylating agent, which requires metabolic activation in the liver to produce the active species, and is widely used in the treatment of chronic leukaemia, the lymphomas and solid tumours. Cyclophosphamide (300 mg/kg) alone produced a small non-significant inhibition of

tumour growth, and this was significantly potentiated by EPA, but not DHA (Figure 5A). EPA had a minimal effect on the weight loss profile in mice receiving cyclophosphamide (Figure 5B).

Discussion

Combinations of antitumour agents have proved more efficacious in the treatment of human cancer than single agent

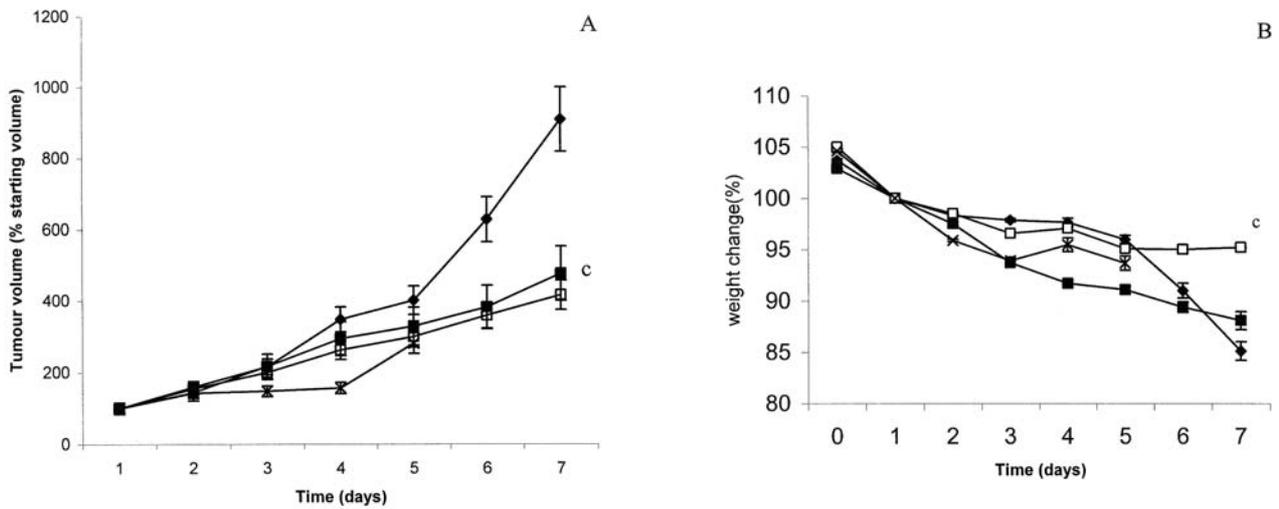


Figure 4. Effect of 5-FU (80mg/kg in PBS) administered i.p. on alternate days alone (■), or combined with EPA (2g/kg; □), DHA (2g/kg; X) or EPA and DHA (△) on tumour growth rate (A) and body weight loss (B) in mice bearing the MAC16 tumour. The fatty acids were administered p.o. daily in olive oil and control animals (◆) received either olive oil or PBS. Differences from control are indicated as a, $p < 0.05$, b, $p < 0.01$ or c, $p < 0.001$. Treatment with EPA and DHA alone is shown in Figure 1.

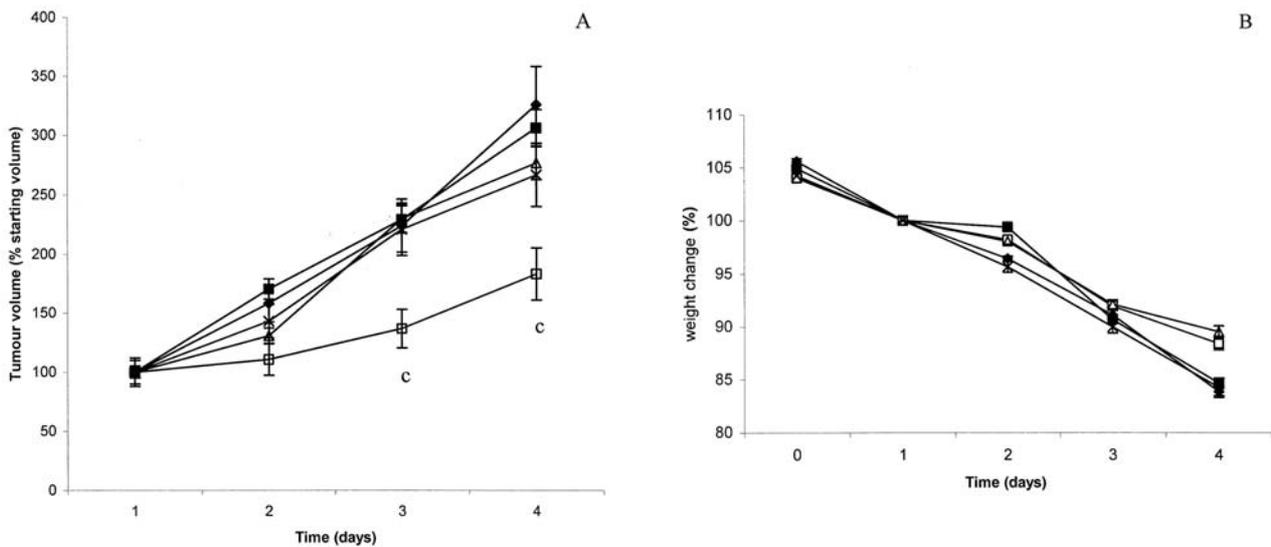


Figure 5. Effect of cyclophosphamide (300mg/kg in PBS) administered i.p. as a single dose on day 1 (■), or combined with EPA (2g/kg; □), DHA (2g/kg; X) or EPA and DHA (△) on tumour growth rate (A) and body weight loss (B) in mice bearing the MAC16 tumour. The fatty acids were administered p.o. daily in olive oil and control animals (◆) received either olive oil or PBS. Differences from control are indicated as a, $p < 0.05$. Treatment with EPA and DHA alone is shown in Figure 1.

treatment alone. Treatments tend to be additive if the locus for tumour inhibition is different for each agent in the combination. Since fish oil preparations rich in EPA and DHA are increasingly being used in the treatment of cancer cachexia (3), it is important to look for any interactions with commonly used antineoplastic drugs, that may be either synergistic or antagonistic. Tumour growth inhibition by EPA

has been shown to be effectively reversed by treatment with the n-6 PUFA linoleic acid (15), suggesting the involvement of prostaglandin or lipoxygenase metabolites in its antitumour action. However, the anticachectic effect of EPA was not reversed by linoleic acid, suggesting that the two effects were separate (15), and the results with the chemotherapeutic agents show that inhibition of tumour growth does not

substantially effect the rate of weight loss. DHA appears to be devoid of antitumour activity in the MAC16 model, suggesting that the two PUFAs act by different mechanisms, as also observed in their ability to prevent weight loss, where EPA exerts a profound anticachectic effect, while DHA is devoid of anticachectic activity (16).

The MAC16 tumour model is highly chemoresistant (10) and most of the agents used had minimal antitumour activity, thus allowing analysis of any synergistic combinations. For gemcitabine, neither PUFA had any additional effect on tumour growth inhibition. However, for epothilone tumour growth inhibition was increased when in combination with DHA and for 5-FU and cyclophosphamide tumour growth inhibition was enhanced in combination with EPA, while the antitumour effect of gemcitabine was not altered by either PUFA. Of the four agents studied, the anticachectic effect of EPA was only seen in combination with 5-FU, suggesting that anticachectic treatment with EPA combinations may be more effective if administered between the cycles of chemotherapy. This might be expected, since EPA would not be expected to counter any weight loss due to drug toxicity. The results suggest that innate drug resistance is not overcome by an alteration of the lipid domain of the cell membrane, but nevertheless the antitumour efficacy of certain agents may be enhanced by concomitant administration of a fish oil preparation rich in EPA and DHA, while these PUFAs do not interfere with the antitumour effectiveness of other agents, suggesting that a fish oil-containing supplement can be taken during chemotherapy.

Acknowledgements

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