Anticachectic and Antitumor Effect of Eicosapentaenoic Acid and Its Effect on Protein Turnover

Susan A. Beck, Kate L. Smith, and Michael J. Tisdale

Cancer Research Campaign Experimental Chemotherapy Group, Pharmaceutical Sciences Institute, Aston University, Birmingham B4 7ET, United Kingdom

ABSTRACT

The effect of the polyunsaturated fatty acids eicosapentaenoic acid (EPA) and γ-linolenic acid (GLA) on host body weight loss and tumor growth has been investigated in mice bearing a cachexia-inducing colon adenocarcinoma, the MAC16. EPA effectively inhibited both host weight loss and tumor growth rate in a dose-related manner with optimal effects being observed at a dose level of 1.25 to 2.5 g/kg. At these concentrations host body weight was effectively maintained, and there was a delay in the progression of growth of the tumor, such that overall survival was approximately doubled in EPA-treated animals, using the criteria dictated by the United Kingdom Coordinating Committee for the welfare of animals with neoplasms. Even when tumor growth resumed, weight loss did not occur. Animals bearing the MAC16 tumor showed a decreased protein synthesis and an increased degradation in skeletal muscle. Treatment with EPA significantly reduced protein degradation without an effect on protein synthesis.

The effect of GLA on both host body weight loss and tumor growth was much less pronounced than that of EPA, with an effect only being seen at a dose of 5 g/kg, at which some toxicity was observed. In vitro studies showed that while EPA was effective in inhibiting tumor-induced lipolysis, GLA was ineffective in this respect. However, prostaglandin E1, which is formed from GLA in vivo, showed partial reversal of tumor-induced lipolysis and probably accounted for the anticachectic effect of GLA. These results suggest that EPA as the pure fatty acid should be considered for clinical investigation as both an anticachetic and antitumor agent, since prior work has shown that the other major component of fish oil docosahexaenoic acid is without pharmacological activity in this system.

INTRODUCTION

The syndrome of cancer cachexia is a complicating factor in the management of the cancer patient and has a negative impact on overall survival (1). Although various nutritional approaches to the treatment of this condition have been investigated, this has not been proved to be very successful (2). Cachexia is a fairly common condition in cancer, e.g., in a study by De Wys et al. (1), roughly one-half of all patients with disseminated cancer had lost some weight at the time of presentation and about one-third had lost more than 5% of their usual body weight in the preceding 6 months. Despite this the cause of the condition and the relationship, if any, between weight loss and tumor growth are unknown.

During a study into the mechanism of weight loss induced by an experimental colon adenocarcinoma (MAC16) we have noted the presence of a catabolic factor capable of mobilizing triglycerides from adipose tissue (3). This material is present in the circulation only in those tumors capable of inducing weight loss and is also present in patients with clinical cancer cachexia (4). Furthermore, this material is capable of inducing weight loss in previously weight-stable tumor-bearing mice (5), suggesting that it may have a pivotal role in the development of cachexia. The material is composed of three low-molecular-weight acidic peptides and appears distinct from cachectin (tumor necrosis factor-α) (5). We have recently shown that the (ω-3) PUFA eicosapentaenoic acid is capable both of inhibiting the activity of this factor in vitro and of inhibiting cachexia in the MAC16 tumor model in vivo (6). In addition, the inhibition of cachexia is followed by an inhibition of tumor growth, a feature also observed with other inhibitors of cachexia (7), suggesting a relationship between growth of the tumor and utilization of host products liberated during cachexia.

This study further investigates the antitumor and anticachectic effect of EPA and its effect on protein synthesis and degradation in skeletal muscle of animals with cachexia, using the MAC16 tumor model. In addition a comparison has been made with another PUFA, GLA, not included in the initial study (6), in order to determine the specificity of the EPA effect.

MATERIALS AND METHODS

Animals. Pure strain NMRI mice were obtained from our own inbred colony and were fed a rat and mouse breeding diet (Pilsbury Ltd., Birmingham, United Kingdom) and water ad libitum. Female mice (average body weight, 20 g) were transplanted with fragments of the MAC16 tumor into the flank by means of a trocar as described (8), and were fed on the rat and mouse breeding diet for 10 to 12 days after transplantation, when the tumors became palpable and weight loss had started to occur. This point was chosen to ensure complete tumor take and weight loss prior to initiation of therapy. The average tumor volume on initiation of therapy was 114 ± 12 mm³, the body weight was 19.04 ± 0.26 g, and the average weight loss was 1.18 ± 0.14 g (5.9 ± 0.7%). At this point animals were randomized and received either solvent (liquid paraffin:water, 2:1) or EPA daily by gavage as a single dose up to a maximum of 9 days. Host body weight was measured daily and recorded as a percentage of the body weight prior to p.o. dosing. Tumor volumes were also measured daily by means of calipers and were recorded as a percentage of the starting tumor volume. Animals were housed in groups and the average food and water intake was measured daily. Food and water was provided ad libitum during the study.

In a separate experiment GLA was also administered once daily by means of gavage for a period of 4 days and the effect was compared with that obtained with EPA. Animals were killed when the tumor ulcerated, weight loss reached 25 to 30%, the tumor weight reached 10% of the host body weight, or the animals became moribund, as agreed by the Coordinating Committee on Cancer Research of the United Kingdom for the welfare of animals with neoplasms.

Chemicals. L-[4-3H]Phenylalanine (specific activity, 30 Ci/mmol) was purchased from Amersham International, Amersham, United Kingdom. GLA (99.4%) and EPA (80%, expressed as a percentage of fatty acid methyl esters prepared) were kindly donated by Dr. D. Horrobin, Scotia Pharmaceuticals Ltd., Guildford, Surrey, United Kingdom.

Preparation of Lipid-mobilizing Activity from the MAC16 Tumor. Tumors were obtained from animals which had lost up to 30% of their original body weight and were homogenized at 4°C in Krebs-Ringer buffer at a concentration of 0.2 g/ml. The homogenate was centrifuged for 10 min at 600 x g to remove debris, and the supernatant was heated to 60°C for 10 min to inactivate proteases.

Received 5/17/91; accepted 9/9/91.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work has been supported by a grant from the Cancer Research Campaign.

2 Recipient of a research studentship from the Cancer Research Campaign.

3 To whom requests for reprints should be addressed.

The abbreviations used are: PUFA, polyunsaturated fatty acid; EPA, eicosapentaenoic acid; GLA, γ-linolenic acid; PGE1, prostaglandin E1.
RESULTS

The effect of daily p.o. dosage with EPA on tumor growth rate and host body weight loss in weight-losing animals bearing the MAC16 tumor is shown in Fig. 1. At a dose of 2.5 g/kg of EPA host body weight was maintained at the same level as at the initiation of the experiment (day 1) throughout the 9 days of treatment (values not significantly different from day 1), while solvent-treated control animals had lost an additional 20% of their original body weight after 5 days and had to be terminated, as agreed by the Coordination Committee on Cancer Research of the United Kingdom for the welfare of animals with neoplasms (Fig. 1A). Animals treated with 1.25 g/kg of EPA initially lost weight at the same rate as solvent-treated controls but started to regain weight by day 5, and maintained levels not significantly different from the starting value throughout the remaining 5 days. Animals treated with EPA at a dose of 0.625 g/kg lost weight at the same rate as controls.

The effect of EPA on tumor volume was similar to the effect on host body weight with doses of 2.5 and 1.25 g/kg, causing an initial growth delay followed by a regrowth, which paralleled the control growth pattern (Fig. 1B). The overall survival of animals treated with EPA based on tumor growth and weight loss was about twice that of controls, using the criteria defined by the United Kingdom Coordinating Committee for the welfare of animals with neoplasms. The pattern of tumor growth inhibition produced by EPA differed from the effect on host body weight loss. Thus, while host body weight loss decreased by day 5 at a dose of EPA of 1.25 g/kg, tumor volume increased at this time (Fig. 1B). Also by day 10 with EPA at a dose of both 2.5 and 1.25 g/kg host body weight was normalized, but tumor volume was similar to that of controls at day 6, where the animals have lost 25% of their original body weight.

Despite the fact that animals treated with EPA at 2.5 g/kg were not losing weight, the average calorie intake (including the EPA) was 11.7 ± 0.5 (SE) kcal/day and was not significantly different from weight-losing controls (11.6 ± 0.7 kcal/day) or non-tumor-bearing animals (10.9 ± 1.1 kcal/day). In addition, the water intake of EPA-treated animals (3.8 ± 0.4 ml/day) was not significantly different from saline-treated control animals (4.5 ± 0.6 ml/day).

Animals bearing the MAC16 tumor showed a significantly depressed (P < 0.001) protein synthesis in gastrocnemius muscle and an increased degradation (P < 0.01) when compared with non-tumor-bearing control animals (Fig. 2). Muscle from tumor-bearing animals that had received EPA (2.5 g/kg) also showed a depressed protein synthesis when compared with non-tumor-bearing control animals, but a significantly reduced protein degradation (P < 0.05), when compared with tumor-bearing saline-treated controls. However, the rate of protein degradation in EPA-treated animals was still significantly (P < 0.05) greater than in non-tumor-bearing controls, although the degree of protein turnover was much less than in tumor-bearing animals administered solvent alone.

In vitro studies showed EPA to inhibit tumor-induced proteolysis in mouse diaphragm in the presence of an extract of the MAC16 tumor (Fig. 3). The apparent K_i value for the inhibition was 50 μM and the effect was not shown by other polyunsaturated fatty acids.

Administration p.o. of GLA at a dose of 5 g/kg produced some growth retardation of the MAC16 tumor (Fig. 4A) and also had a protective effect on the loss of host body weight (Fig. 4B), which was more pronounced than the effect on tumor growth.
Fig. 2. Protein synthesis (G) and degradation (B) in gastrocnemius muscle of non-tumor-bearing female NMRI mice (A) or of tumor-bearing animals with cachexia and treated with solvent (B), or EPA (2.5 g/kg) (C). Measurements were made on tumor-bearing animals on the day following termination of the experiment detailed in Fig. 1. Results are expressed as means ± SEM and differences were determined by Student's t test as a, \( P < 0.05 \); b, \( P < 0.01 \); c, \( P < 0.001 \) from non-tumor-bearing controls, and d, \( P < 0.05 \) from tumor-bearing controls.

DISCUSSION

While PUFAs of the (\(\omega-6\)) series such as linoleic acid have often been shown to enhance the growth rate of tumors both in vitro (14) and in vivo (15), PUFAs of the (\(\omega-3\)) series have been shown to have an inhibitory effect on the growth of mammary (16), colon (17), and prostatic (18) tumors in mice. In none of these studies, however, were pure fatty acids administered, so it is difficult to decide on the active ingredients of the preparations. This study addresses this problem by the administration of pure fatty acids by gavage to tumor-bearing animals bearing established tumors and with a weight loss of about 6%. The MAC16 is a particularly chemoresistant tumor, being nonresponsive to a range of clinically useful antitumor agents (19), and thus the antiproliferative effect of EPA is particularly impressive. Although cures were not obtained, the life span of the animals was approximately doubled at a dose of 1.25 to 2.5 g/kg/day. Such a dose when translated on a surface area basis to a human would be between 6.4 and 12.8 g/day, assuming an average human surface area of 1.7 m\(^2\). This dose is similar to...
the average daily intake (7 g) among Greenland Eskimos (20), and is about twice that previously administered to breast cancer patients as max EPA capsules (21). In this previous study a measurable clinical response was observed in 2 of 12 heavily pretreated patients, without toxicity, although further dose escalation would have been prohibitive, considering the large number (20) of capsules per day. With the advent of pure fatty acids on a large scale, further studies should now be possible.

In addition to its antiproliferative effect, EPA also demonstrated a marked suppression of the cachexia accompanying tumor growth. This inhibition of weight loss occurred without an alteration in nutrient intake. Weight loss in animals bearing the MAC16 tumor is accompanied by a decrease in skeletal muscle mass, which is proportional to the tumor burden in the animals (3). This loss of lean body tissue appears to be due to both a depression in protein synthesis and an increase in protein degradation in skeletal muscle. The effect of cachexia on protein synthesis may be complicated by reductions in food intake. However, in animals bearing the MAC16 tumor, food intake is not different from non-tumor-bearing controls (8), and a recent study (22) indicated that newly diagnosed lung cancer patients showed increased protein turnover rates despite being noncachexic and suffering no reduction in food intake. Previous studies on animals bearing the Ehrlich ascites tumor (23), Novikoff hepatoma, and Yoshida sarcoma (24) have also demonstrated elevated protein turnover in skeletal muscle in tumor-bearing animals, although in these models the depression in protein synthesis appeared to be more important than the increase in degradation.

Maintenance of host body weight in animals bearing the MAC16 tumor and treated with EPA is associated with a decreased protein degradation in skeletal muscle without an effect on protein synthesis. Previous studies (25) have shown EPA to have no effect on protein synthesis or degradation in rabbit forelimb digit extensor muscles in vitro. While confirming the lack of effect of EPA on protein synthesis this study shows a significant reduction in protein degradation both in vivo and in vitro, using isolated diaphragm muscle, under the influence of an extract of the MAC16 tumor. The reason for this difference between the two studies is not known.

We have previously shown (6) that the ant cachectic effect of EPA correlates with the in vitro inhibition of a lipid mobilizing
Table 1  Inhibition of MAC16-induced lipolysis in fresh epididymal adipocytes by PGE,

<table>
<thead>
<tr>
<th>PGE (µM)</th>
<th>Inhibition of lipolysis (% of control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14</td>
<td>15 ± 5*</td>
</tr>
<tr>
<td>28</td>
<td>31 ± 3*</td>
</tr>
<tr>
<td>57</td>
<td>33 ± 3*</td>
</tr>
<tr>
<td>283</td>
<td>38 ± 4*</td>
</tr>
</tbody>
</table>

* Results are shown as mean ± SEM for 3 separate determinations.

ACKNOWLEDGMENTS

We thank M. Wynter for the tumor transplantation.

REFERENCES

Anticachectic and Antitumor Effect of Eicosapentaenoic Acid and Its Effect on Protein Turnover

Susan A. Beck, Kate L. Smith and Michael J. Tisdale