Effects of dietary linseed, evening primrose or fish oils on fatty acid and prostaglandin E\textsubscript{2} contents in the rat livers and 7,12-dimethylbenz[a]anthracene-induced tumours

Małgorzata Jelińska\textsuperscript{a}, Andrzej Tokarz\textsuperscript{a,*}, Regina Olędzka\textsuperscript{a}, Alicja Czorniuk-Śliwa\textsuperscript{b}

\textsuperscript{a}Department of Bromatology, Faculty of Pharmacy, The Medical University of Warsaw, Banacha 1, 02-097 Warsaw, Poland
\textsuperscript{b}Department of Pathomorphology, Medical Centre for Postgraduate Education, Marymoncka 99, 01-813 Warsaw, Poland

Received 6 June 2002; received in revised form 31 January 2003; accepted 20 February 2003

Abstract

We examined the influence of diets supplemented with fish and vegetable oils on fatty acid and prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) contents in livers of non-7,12-dimethylbenz[a]anthracene (DMBA)- and DMBA-treated rats, and in DMBA-induced tumours. Decreased concentrations of saturated fatty acids and increased unsaturated fatty acid levels were observed in liver phospholipids of rats fed these oils. There was a marked difference in the concentrations of fatty acids found in the tumours and those present in liver lipids. Oleic acid was the main unsaturated fatty acid found in the tumour tissue. Both liver and tumour PGE\textsubscript{2} contents were clearly correlated to the diet. The PGE\textsubscript{2} concentrations were decreased in livers and tumours of rats fed fish (FO) and linseed oils (LO).

© 2003 Elsevier Science B.V. All rights reserved.

Keywords: Fatty acid; Prostaglandin E\textsubscript{2}; Carcinogenesis; Fish and vegetable oils; 7,12-Dimethylbenz[a]anthracene

1. Introduction

Carcinogenesis is a multistage process which frequently depends on the environmental agents. It is considered that 35–40% of all human cancers may be associated with the diet [1,2] and dietary fat is regarded as one of the major risk factors in cancers of the breast, colon and prostate [3–5]. Epidemiological studies [6,7] and experiments on animals [8,9] confirm that both amount and type of fat influence carcinogenesis. It has been reported that fats containing high concentrations of \( n-6 \) polyunsaturated fatty acids (\( n-6 \) PUFA), especially linoleic acid (LA; 18:2), promoted the development of mammary tumours [10–12]. On the other hand, evening primrose oil (EPO) containing about 75\% of linoleic acid and a relatively high level (9\%) of \( \gamma \)-linolenic acid (GLA; 18:3, \( n-6 \)) was reported to inhibit the development of carcinogen-induced rat mammary tumours [13,14]. In contrast to fat containing \( n-6 \) PUFAs, fish oils (FO) rich in \( n-3 \) PUFAs, the most abundant being eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6), were observed to inhibit carcinogen-induced mammary tumorigenesis in the rat and mouse [9,14–16].

A great number of data indicate a correlation between an increased PGE\textsubscript{2} concentration and the risk of cancer development. The PGE\textsubscript{2} level observed in the 1970s in tumour samples gathered from the alimentary tract was higher than in the adjacent intestinal mucosa [17]. It was noted then that the PGE\textsubscript{2} blood concentration in vessels draining a tumour area in the alimentary tract was significantly higher than in arterial blood supplying the tumour, and increased with the development of the tumour [18].

The content of fatty acids, especially unsaturated fatty acids in cellular structures, results from their type supplied with a diet, endogenous synthesis and usage in the body. Accordingly, in our experiments, we tried to explain in which way selected edible oils (EPO, FO, and linseed oil, LO) determine fatty acids composition (wt.\%) in phosphatidylcholine (PC) and phosphatidylethanolamine (PE)
extracted from liver and tumour lipids of 7,12-dimethylben-
z[a]anthracene (DMBA)-treated rats.

The PGE\(_2\) contents in liver and tumour homogenates were also examined to estimate a correlation between the risk of tumour development and the PGE\(_2\) concentration.

2. Materials and methods

2.1. Animals and experiment

Female Sprague–Dawley rats (Maria Skłodowska-
Curie Memorial Cancer Centre and Institute of Oncology) were used in the study, which was approved by the Ethics Committee of the Medical University of Warsaw. At the beginning of the study, the rats (30 days old) were divided into three groups, which were fed ad libitum the rat standard diet composed of 19.5% protein, 6.8% fat, 53.1% carbohydrates, 4.5% fibre and 1.7% minerals. After 1-week adaptation to the experimental regimen, the rats were administered by intragastric intubation 0.4 ml of the following oils daily: EPO (Oeparol, Agropharm, Poland), FO (Trienyl, Lek Pharmaceutical and Chemistry Company, Ljubljana, Slovenia), or LO (Gal, Poland). The test oils were stored in dark bottles in a refrigerator. The content of fat in each diet was then about 10%, i.e. equivalent to 24.3% of the caloric value. Fatty acid compositions of the study oils are presented in Table 1. However, because the standard diet was a laboratory chow and oils were administered intragastrically, the type and amount of fatty acids were sums of their contents in the chow and in the oil. The daily food intake by adult animals was about 10 g per rat. The fatty acid daily intake is shown in Table 2.

When 50 days old, each of dietary groups (EPO, FO, LO) was subdivided into two groups. One subgroup selected from each dietary group was administered DMBA (Sigma Chemical Company, USA) in the amount of 65 mg/kg body weight [19]. As a result, six groups were formed; three non-
DMBA-treated groups (EPO, FO, LO) and three DMBA-
treated groups (EPO + DMBA, FO + DMBA, LO + DMBA). Twenty weeks after DMBA administration, the rats were decapitated. Livers and tumours were collected and stored at −70 °C until further analyses.

2.2. Histopathological examination

Tumours, which could be identified with the naked eye, were collected and their sections were fixed in 10% formalin. They were diagnosed by a pathologist as adenocarcinomas of the mammary gland. Tumour incidence (percentage of animals with tumours) was recorded. No spontaneous tumours were observed in non-DMBA-treated rats.

2.3. Analysis

Lipids from liver and tumour homogenates were extracted with chloroform/methanol mixture (2:1; v/v) according to the method of Folch et al. [20]. The separation of lipids and phospholipids into PC and PE was accomplished by thin-layer chromatography [21]. Fatty acids contained in PC and PE were esterified with 14% BF\(_3\) in methanol (Sigma) as described by Raclot and Groscolas [22]. Fatty acids methyl esters were analysed on a gas chromatograph.
Table 4
Percentage composition of fatty acids in liver phospholipids of non-DMBA- and DMBA-treated female rats fed the experimental diets (wt.%)  

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>EPO</th>
<th>FO</th>
<th>LO</th>
<th>EPO + DMBA</th>
<th>FO + DMBA</th>
<th>LO + DMBA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PC</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18:0</td>
<td>34.89 ± 3.83&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.36 ± 2.12&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.77 ± 2.48</td>
<td>32.64 ± 2.21</td>
<td>29.19 ± 1.06</td>
<td>30.25 ± 2.48</td>
</tr>
<tr>
<td>18:1</td>
<td>5.06 ± 0.72</td>
<td>6.72 ± 1.02</td>
<td>5.61 ± 1.87</td>
<td>4.69 ± 1.46</td>
<td>5.38 ± 0.72</td>
<td>5.96 ± 1.65</td>
</tr>
<tr>
<td>18:2 n-6</td>
<td>7.76 ± 1.17</td>
<td>9.11 ± 0.65</td>
<td>8.25 ± 1.26</td>
<td>7.41 ± 0.78</td>
<td>8.18 ± 0.76</td>
<td>9.10 ± 0.43</td>
</tr>
<tr>
<td>20:4 n-6</td>
<td>15.80 ± 3.15</td>
<td>13.26 ± 2.25</td>
<td>16.93 ± 1.78</td>
<td>20.95 ± 2.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.53 ± 1.88&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.32 ± 3.62&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20:5 n-3</td>
<td>0.074 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.14 ± 0.36&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.015 ± 0.28&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.12 ± 0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.39 ± 0.40&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.64 ± 0.31&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>22:6 n-3</td>
<td>5.11 ± 2.08&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.54 ± 2.16&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.93 ± 1.59&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.31 ± 1.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10.54 ± 1.46&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.68 ± 2.07&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

| **PE**     |     |    |    |            |           |           |
| 18:0       | 37.46 ± 3.46<sup>ab</sup> | 31.11 ± 1.68<sup>a</sup> | 32.91 ± 2.74<sup>b</sup> | 33.88 ± 3.30 | 30.05 ± 1.32 | 32.32 ± 2.58 |
| 18:1       | 3.40 ± 0.48<sup>ab</sup> | 2.41 ± 0.24<sup>a</sup> | 2.48 ± 0.40<sup>b</sup> | 3.08 ± 0.88 | 3.34 ± 0.62 | 3.30 ± 1.28 |
| 18:2 n-6   | 3.61 ± 0.38     | 3.17 ± 0.49     | 3.16 ± 0.63     | 3.11 ± 0.32     | 3.68 ± 0.74     | 4.01 ± 1.40     |
| 20:4 n-6   | 14.64 ± 3.12<sup>a</sup> | 10.75 ± 1.34<sup>b</sup> | 14.45 ± 0.91<sup>b</sup> | 16.97 ± 2.47 | 14.22 ± 1.55 | 14.56 ± 2.32 |
| 20:5 n-3   | 0.12 ± 0.05<sup>ab</sup> | 1.13 ± 0.42<sup>a</sup> | 1.27 ± 0.25<sup>b</sup> | 0.18 ± 0.03<sup>ab</sup> | 1.54 ± 0.54<sup>a</sup> | 1.71 ± 0.55<sup>b</sup> |
| 22:6 n-3   | 11.90 ± 2.86<sup>ab</sup> | 22.47 ± 4.12<sup>a</sup> | 20.07 ± 2.50<sup>b</sup> | 13.75 ± 1.68<sup>a</sup> | 19.27 ± 2.47<sup>ab</sup> | 14.61 ± 1.15<sup>b</sup> |

Data are expressed as mean ± S.D. Values sharing a letter (a, b) are statistically different (P < 0.05, analysis of variance combined with Tukey–Kramer’s Multiple Comparison Test). Abbreviations: EPO, evening primrose oil; FO, fish oil; LO, linseed oil; PC, phosphatidylcholine; PE, phosphatidylethanolamine.

(Shimadzu GC-17A, Japan) equipped with a 30-m capillary column, 0.32-mm internal diameter and 0.25-μm stationary phase HP-225 (50% CNPrPh Me Siloxane). The column temperature was programmed from 140 to 220 °C. Helium was used as a carrier gas. Individual fatty acid methyl esters were identified by comparison with known standards (Sigma).

The PGE2 was isolated from liver and tumour tissues using the solid phase extraction (SPE) method and determined by HPLC [23]. The PGE2 was separated on Lichrospher 100RP-18/5 μm column. The mobile phase was a mixture of acetonitrile and 0.0174 M H3PO4 (328:672, v/v) and the detection wavelength was 196 nm.

2.4. Statistical analysis

The results were subjected to an analysis of variance and a Tukey–Kramer’s Multiple Comparison Test. Values of P < 0.05 were considered as significant. Data are presented as means ± S.D.

3. Results

A number of tumour-bearing rats in each DMBA-treated group is shown in Table 3. The highest tumour incidence (84%) was observed in the LO group (21 of 25 animals...
developed tumours, and 4 animals had 2 tumours each). However, although more numerous, the tumours in that group were smaller in size than in the other groups (Table 3). The tumour incidence in the other groups was as follows: EPO—28%; FO—40%, which is similar to the soybean oil group described in our previous study [24].

In our experiment, we analysed 11 fatty acids (myristic acid, C14:0; palmitic acid, C16:0; palmitoleic acid, C16:1; stearic acid, C18:0; oleic acid, C18:1 n-9; vaccenic acid, C18:1 n-7; linoleic acid, LA, C 18:2 n-6; α-linolenic acid, ALA, C 18:3 n-3; arachidonic acid, AA, C 20:4 n-6; eicosapentaenoic acid, EPA, C20:5 n-3; and docosahexaenoic acid, DHA, 22:6 n-3) in livers and tumours. In the present paper, however, we focus on the essential PUFAs such as LA, AA, EPA, and DHA as well as on stearic acid and oleic acid.

There were significant differences in fatty acid concentrations among study groups. The lowest AA level was observed in the PC and PE extracted from livers of the FO non-DMBA-treated rats and in the PE of DMBA-treated rats (Table 4). However, statistically significant differences in AA concentrations appeared in the PC extracted from livers of DMBA-treated groups. In the EPO group, the AA level was increased when compared with the FO and LO groups. In contrast, the FO and the LO groups showed higher concentrations of liver EPA and DHA than the EPO group. The EPA level was significantly lower in the FO and LO groups. Similar statistically significant differences where
found in the DHA level, which was lower in the EPO than in the FO group (PC and PE of both non-DMBA- and DMBA-treated rats) and in the LO group (Table 4). The highest DHA contents were observed in the FO group, and the highest EPA contents in the LO group. There were not any statistically significant changes in LA contents. The FO group showed the lowest, and the EPO group the highest, level of stearic acid. However, except data concerning the PC of non-DMBA-treated group, the differences were not statistically different.

In the phospholipids extracted from tumour tissue, decreased levels of stearic acid, AA and DHA were observed regardless the diet, compared to the liver (Figs. 1 and 2; Table 4). EPA was not detected at all. On the contrary, the oleic acid contents were elevated and that acid became the main unsaturated fatty acid in tumour tissue. The highest concentrations of LA, AA, and DHA were noted in the LO, when compared to the EPO and FO groups.

The prostaglandin E2 (PGE2) concentrations in both liver and tumour tissues were significantly higher in the EPO than in the FO and LO groups (Fig. 3) where the levels were similar. Increased PGE2 levels were also noted in livers of all DMBA-treated groups compared to the non-DMBA-treated animals.

4. Discussion

In many studies concerning the relationship between dietary fat and the incidence of breast cancer, fish n-3 PUFA-exert an inhibitory effect against tumorigenesis, slow primary mammary tumour growth, and metastases in animal models of mammary tumorigenesis [25–27]. On the contrary, n-6 fatty acids enhance the incidence, growth, and metastases of the tumours [28,29]. In our study, the lowest tumour incidence was observed in the EPO group where LA (C 18:2 n-6) was about 77%. However, EPO also contains about 9% of GLA, which is LA delta-6-desaturated metabolite. GLA appears to be the most effective PUFAs killing cancer cells followed by AA, EPA, cis LA, and ALA, whereas DHA was less effective [30–33]. In the in vitro studies, GLA stimulated the expression of nm-23, a metastasis suppressor gene, and as a result inhibited the invasion of tumour cells [34]. It is also possible that oenothein B, a strong polyphenolic substance present in the EPO, is responsible for beneficial activities of this oil [35]. The current study supports the results by Munoz et al. [36] who reported the absence of mammary tumour promotion in mice fed diets enriched with n-6 PUFA—LA or GLA (EPO).

In the FO group, tumour incidence was higher than in the EPO (40%), despite of high EPA and DHA concentrations in this diet as well as in the liver lipids. However, the highest susceptibility to tumorigenesis was observed in the LO group (84%), although the fatty acid contents in liver lipids of that group are similar to the FO. LO is the richest source of ALA. Higher tumour incidence in the FO and LO groups might be caused by optimal n-3/6 ratio disturbances after FO and LO supplementation. The n-3/ n-6 ratios for the diets supplemented with the FO and LO are 1.5 and 1.75, respectively. These results agree with the results of Sasaki et al. [37]. They fed the rats 10% fat diets in which n-3/n-6 ratio was as follows: 0.01, 1.03, 3.96, and 7.84. The incidence of mammary tumours tended to be lower in the first (0.01) group than in the other three groups. What is more, the total number and weight of the mammary gland tumours in each group increased in a stepwise manner with the increasing n-3/n-6 ratio and were the highest in the fourth group (n-3/n-6 = 7.84).

The fatty acid profile and levels of mammary tumour phospholipids differed from those of healthy liver tissue but were similar to those observed by other authors in the prostate [38], spleen, and liver tumour cells [39]. The highest oleic acid concentrations probably resulted from its synthesis from stearic acid in the body [40]. One of the stearic acid sources in the body is dietary glucose. As a result, a phospholipid structure in tumours make cell membranes less fluid and permeable [41], and more resistant to the cytotoxic impact of various compounds such as PUFA peroxides. Cancer cells are characteristically resistant to lipid peroxidation, in comparison to the normal, healthy cells [42–44]. This phenomenon is caused by relatively low PUFA contents in tumours and decreased activities of cytochrome P-450 and NADPH, whose enzymes participate in the initiation of lipid peroxidation. Simultaneously, the antioxidant activity of tumour tissue increases. Cancer cell membranes are supposed to change cell responses to hormone effects and their susceptibility to the immune system [43,44]. In our experiment, data regarding the fatty acid composition of tumours induced in the rats fed the LO appear to be of interest. PC and PE isolated from tumour tissue of that group contained the highest levels of LA, AA, and DHA and identical oleic acid levels (Figs. 1 and 2) in comparison to the FO and EPO.

Many experiments [13,17,18] show the relationship between PGE2 content in animal tissues and susceptibility to tumorigenesis. However, the results are inconsistent. The analysis of PGE2 concentrations in the current study shows the absence of correlation between the PGE2 concentrations and mammary tumour incidence. The PGE2 contents in livers and tumours of animals fed the diets supplemented with the EPO, FO, and LO indicate that high PGE2 (EPO) concentrations may occur together with a low cancer incidence and conversely the low PGE2 concentrations appear with the high cancer risk (LO). The dietary fatty acid composition determines the PGE2 synthesis in the body. The high contribution of n-3 fatty acids (LO, FO) inhibits the PGE2 synthesis. However, despite the high n-3 fatty acid concentrations in the LO (Table 1) and similar PGE2 concentrations in the FO and LO groups, the tumour incidence is higher by 50% in the LO than in the FO group. The present study agrees with the results of Sasaki et al.
This finding suggests that the PGE₂ may be one of the agents affecting carcinogenesis, but it is not of the crucial importance.

Our findings confirm the importance of the appropriate type and amount of fatty acids in the diet. They also show how complex and difficult to prevent tumorigenesis is. It may be concluded that fatty acids participating in various pathological processes act at the molecular level and, having various chemical structures, cause numerous pharmacological effects.

5. Conclusions

1. Tumour incidence was strictly correlated to the type of the fat fed.
2. The process of tumorigenesis is positively correlated to the higher n – 3/n – 6 ratios.
3. The presence of GLA and polyphenolic antioxidant was probably the reason of the lowest tumour incidence in the EPO group, despite of a high LA level in that oil.
4. Tumour incidence was negatively correlated to PGE₂ synthesis in livers and tumours. That indicates PGE₂ is probably not a decisive factor in tumorigenesis, although it participates in the process.
5. Oleic acid is the main unsaturated fatty acid in rat tumour tissue, which may determine physicochemical properties of tumour cell membranes.

Acknowledgements

This study was supported by research grant No. 4PO5 D 08712 from the State Committee for Scientific Research in Poland. The authors are grateful to Elżbieta Karpinska and Małgorzata Kozikowska for excellent technical assistance.

References


