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Effects of a fish oil–based emulsion on rat hepatoma cell invasion in culture

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Abstract

Objective: Total parenteral nutrition containing a lipid emulsion is often employed after surgical tumor resection. This study investigated the effects of a fish oil–based infusion on rat hepatoma cell invasion.

Methods: Rat ascites hepatoma cell line AH109A was precultured with a fish oil–based or safflower oil–based emulsion for 48 h. Changes in membranous fatty acid composition were evaluated by gas chromatography. The invasiveness of hepatoma cells was assessed by coculturing with mesentery-derived mesothelial cells. To examine ex vivo effects of the fish oil–based infusion on hepatoma invasion, sera were prepared from rats infused with fish oil– or safflower oil–based emulsion and the effects of these sera were assessed. To clarify the mechanism of inhibition of invasion by the fish oil–based emulsion, the effects of prostaglandin (PG) E_2 and PGE_3 on invasion were examined.

Results: Pretreatment with the fish oil–based emulsion reduced invasiveness without affecting growth compared with the safflower oil–based emulsion. Pretreatment with the sera from rats infused with the fish oil–based emulsion also reduced invasiveness compared with the sera from rats infused with the safflower oil–based emulsion. The addition of PGE_2 eliminated the inhibitory effect of the fish oil–based emulsion, and the addition of PGE_3 reduced the invasiveness of hepatoma cells pretreated with the safflower oil–based emulsion.

Conclusion: These results suggest that the fish oil–based emulsion may have anti-invasive effects. Changes in the membranous fatty acid composition and consequent changes in the prostaglandins produced may be involved in this inhibitory effect.

Keywords: Lipid emulsion; Hepatoma; Invasion; Prostaglandin; Fish oil

Introduction

Some evidence from epidemiologic and experimental studies have suggested that there is a relation between dietary fat and the risk of tumor development [1–5]. In particular, it has been shown that a high intake of saturated and ω-6 polyunsaturated fatty acids (PUFAs), such as linoleic acid, is associated with a high risk of developing cancer, whereas a high intake of ω-3 PUFAs, found in oily fish and fish oils, such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), reduces the risk of breast, colon, and prostate cancer [1–5]. Experimental studies have shown that ω-6 PUFAs stimulate tumor growth and metastasis, whereas ω-3 PUFAs suppress them [6–9]. Many studies have suggested that the ratio of ω-6 to ω-3 fatty acids may be an important factor in controlling tumor development. However, most of these previous studies were concerned with carcinogenesis or the proliferation and/or apoptosis of tumor cells, and less information is available concerning the effects of the type of fatty acid on tumor cell invasion and metastasis. Some reports have suggested that the ratio of ω-6 to ω-3 PUFAs may also be an important factor in the effects of fatty acids on tumor cell invasion [10–13].

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Total parenteral nutrition is often employed after the surgical resection of tumors. Infusions containing ω-3 PUFAs may have beneficial effects on the prognosis of patients with tumors by inhibiting tumor cell invasion.

Tumor metastasis is a complicated and sequential process that can lead to the death of the patient [14]. The development of metastasis severely affects the prognosis of patients with tumors, especially after resection of the tumor tissue. Among the many steps in the process of metastasis, invasion is thought to be the most characteristic and important [14], and the inhibition of invasion may lead to the inhibition of tumor metastasis. The possibility that some infusions may have an inhibitory effect on tumor invasion may potentially influence the postoperative use of total parenteral nutrition.

In the present study, the effects of a fish oil (FO)–based emulsion on rat hepatoma cell invasion were examined in culture, and the effects of the sera of rats infused with a solution containing an FO-based emulsion on hepatoma invasion were also investigated by comparison with a safflower oil (SO)–based emulsion. Pretreatment with an FO-based emulsion or an SO-based emulsion resulted in the replacement of fatty acids in membranous phospholipids. To clarify the possible modes of action of the FO-based emulsion on hepatoma invasion, the effects of prostaglandin (PG) E_2 and PGE_3 on the invasiveness of hepatoma cells pretreated with each emulsion were also assessed, because some prostaglandins have been reported to affect tumor cell invasiveness.

Materials and methods

Materials

The SO-based and FO-based emulsions were prepared according to the formulas presented in Table 1. The fatty acid compositions of the SO and FO used are listed in Table 2. The ω-6/ω-3 fatty acid ratios of the SO and FO were 401 and 0.12, respectively. PGE_2 and PGE_3 were purchased from Cayman Chemical Co., Ltd. (Ann Arbor, MI, USA). The WST-8 cell proliferation assay kit was purchased from Dojindo Laboratories (Kumamoto, Japan). All other reagents were of the highest commercially available grade.

Table 1
Compositions of SO-based and FO-based emulsions*

Ingredients (g/100 mL)	SO	FO
SO	10	—
FO	—	10
Egg yolk lecithin	1.2	1.2
Glycerol	2.2	2.5
dl-α-Tocopherol	0.022	0.022

FO, fish oil; SO, safflower oil

* The FO-based emulsion was obtained in the form of Omegaven-Fresenius, Fresenius Kabi Deutschland GmbH (Bad Homburg v.d.H, Germany); the SO-based emulsion was prepared by Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan).

Table 2
Fatty acid compositions of SO-based and FO-based emulsions*

wt%	SO	FO
C18:3 ω-3	0.1	1.2
C20:5 ω-3	0.0	24.1
C22:5 ω-3	0.0	2.5
C22:6 ω-3	0.1	19.8
C18:2 ω-6	73.5	2.8
C18:3 ω-6	0.0	0.3
C20:2 ω-6	0.0	0.2
C20:3 ω-6	0.0	0.3
C20:4 ω-6	0.4	1.6
C22:4 ω-6	0.2	0.5
C16:1 ω-7	0.1	8.9
C18:1 ω-9	13.1	12.5
C18:1 ω-7	0.6	2.6
C20:1 ω-9	0.1	0.6
C20:3 ω-9	0.0	0.0
C12:0	0.0	0.2
C14:0	0.0	6.3
C16:0	7.7	12.4
C18:0	3.0	2.3
C20:0	0.3	0.0
C22:0	0.2	0.0
C24:0	0.0	0.1
Others	0.6	0.6
ω-6/ω-3 fatty acid ratio	401	0.12

FO, fish oil; SO, safflower oil

* The FO-based emulsion was obtained in the form of Omegaven-Fresenius, Fresenius Kabi Deutschland GmbH (Bad Homburg v.d.H, Germany); the SO-based emulsion was prepared by Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan).

Animals and cell culture

Male Donryu rats were purchased from Charles River Japan (Yokohama, Kanagawa, Japan). The animals were treated in accordance with the guidelines established by the animal care and use committee at Tokyo Noko University or Otsuka Pharmaceutical Factory, Inc. AH109A cells were obtained from the Institute of Development, Aging and Cancer of Tohoku University (Sendai, Japan) and were maintained in the peritoneal cavity of Donryu rats as described previously [15]. AH109A cells cultured in minimum essential medium (MEM; Nissui Pharmaceutical Co., Tokyo, Japan) containing 10% calf serum (CS; JRH Biosciences, Lenexa, KS, USA; hereafter 10% CS/MEM) for at least 2 wk after preparation from accumulated ascites were used in the following experiments.

Pretreatment with each emulsion and in vitro proliferation and invasion assays

AH109A cells were pretreated with 1% CS/MEM containing a 1% SO- or FO-based emulsion for 48 h. After 48 h of preculture, the cells were collected, washed three times with 10% CS/MEM, and used for the following proliferation or invasion assays. To confirm the effective replace-