Archives of Medical Research 45 (2014) 195−202

Archives of Medical Research

ORIGINAL ARTICLE

Effects of Fish Oil with a High Content of n-3 Polyunsaturated Fatty Acids on Mouse Gut Microbiota

Hai-Ning Yu,[a] Jing Zhu,[a] Wen-sheng Pan,[b] Sheng-Rong Shen,[c] Wei-Guang Shan,[a] and Undurti N. Das[d]

[a]*College of Pharmaceutical Science, Zhejiang University of Technology, Hangzhou, China*
[b]*The Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou, China*
[c]*School of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou, China*
[d]*UND Life Sciences, Federal Way, Washington*

Received for publication October 19, 2013; accepted January 29, 2014 (ARCMED-D-13-00577).

Background and Aims. Many studies show that fish oil with high content of n-3 polyunsaturated fatty acids (PUFAs) plays an important role in human health and disease. But the effects of fish oil with high content of PUFAs on gut microbiota, which are also known play a significant role in several human diseases, is not clear. In the present study we evaluated the effects of fish oil with high content of n-3 PUFAs on gut microbiota.

Methods. Changes in gut microbiota in ICR mice after supplementation of fish oil (containing eicosapentaenoic acid and docosahexaenoic acid: ~40 and 27% respectively) for 15 days was characterized using the hypervariable V3 region of the 16 rRNA gene-based polymerase chain reaction (PCR)-denaturing gradient gel electrophoresis (DGGE) profiling, DNA sequencing, and phylogenetic analysis techniques.

Results. Fish oil treatment resulted in a decrease in *Helicobacter, Uncultured bacterium clone WD2_aaf07d12 (GenBank: EU511712.1), Clostridiales bacterium, Sphingomonadales bacterium and Pseudomonas* species *Firmicutes*, and several uncultured bacteria.

Conclusions. Fish oil with a high content of n-3 PUFAs are capable of producing significant changes in the gut microbiota that may, at least in part, explain the health benefits or injury induced by fish oil use.

Key Words: Gut microbiota, Fish oil, PCR-DGGE, Phylogenetic analysis.

Gut microbiota plays an important role in human health and disease (1). Intestinal microbes influence physiological functions including nutrition, production of vitamins, gut maturation and angiogenesis, development and maintenance of the innate immune system, regulation of host fat storage, development of obesity, type 1 and type 2 diabetes mellitus and metabolic syndrome and autoimmune diseases (2). N-3 polyunsaturated fatty acids (PUFAs), which are rich in fish oil, have a positive influence on human health. Many studies show that fish oil rich in n-3 polyunsaturated fatty acids prevent DNA damage induced by smoking, suppress the growth of tumor cells, and are of benefit in the management of diabetes mellitus (3−5). Recent studies suggested that fish oil may influence the content of gut microbiota (6,7). In the present study we evaluated the effect of fish oil with high content of n-3 PUFAs on gut microbiota.

Gut microbiota can be identified and characterized by many molecular techniques such as polymerase chain reaction (PCR), temperature gradient gel electrophoresis (TGGE), denaturing gradient gel electrophoresis (DGGE), restriction fragment length polymorphism (RFLP), quantitative-PCR (qPCR), fluorescence in situ hybridization (FISH) and deep-sequencing techniques (454 16S rRNA gene sequencing and shot-gun sequencing methods) (8−13).

DGGE has frequently been applied to study microbial composition of the gut of animals (14). By employing the DGGE method most dominant microorganisms can be detected but the obtained sequence information is limited (15). The dominant bacterial population representing over

Address reprint requests to: Dr. Undurti N. Das, MD, FAMS, FRSC, UND Life Sciences, 2020 S 360th St, #K-202, Federal Way, WA 98003; Phone: 216-231-5548; FAX: 928-833-0316; E-mail: Undurti@hotmail.com

http://dx.doi.org/10.1016/j.arcmed.2014.03.008

1% of the total population is reflected in the gels (14), and amplification of the V3 region of 16S rRNA is sufficient to identify the organism(s). In studies concerning the gut microbiota such as ours described here, identification of only the dominant bacteria is sufficient. Because identification of the hypervariable V3 region of the 16 rRNA genes is characteristic of a bacterium, we chose this method to identify the gut microbial community in the present study. For this study, we took advantage of the PCR technique combined with DGGE. This method allows for the simultaneous analysis of multiple samples in one gel, and it has proven to be effective in elucidating the diversity of the bacterial community living in human gut (16–21). Our results emphasize a significant association between fish oil supplementation and gut microbiota, which may explain some of the reported health benefits of fish oil.

Materials and Methods

Fish oil was obtained from General Nutrition Centers (Pittsburgh, PA). As per the specification given by the supplier its contents contain ~60% DHA + EPA. To verify the claim of the supplier, we performed fatty acid analysis of the sample that showed that it contained ~40% EPA (eicosapentaneoic acid, 20:5 n-3) and ~27% DHA (docosahexaenoic acid, 22:6 n-3) (Table 1).

ICR mice were purchased from the Laboratory Animal Center of Shanghai Jiao Tong University (Shanghai, China). Thirty ICR mice (15 males and 15 females, 4 weeks, 17–21 g) were housed under a controlled environment , the humidity is between 40% and 60%, the temperature is from 20–24°C with a 12 h light–dark cycle according to China GB 14925–2010 (Laboratory animal requirements of environment and housing facilities). All efforts were made to minimize discomfort. The animals were accustomed to the laboratory for 1 week and then randomly divided into three groups, i.e., natural saline group (CK), high-dose fish oil group (10 mg/kg), and low-dose fish oil group (5 mg/kg). Each group consisted of five male and five female mice and all animals had free access to water and food. Fish oil was administrated orally every day at the same time of the day by gavage along with the feed (composition of the normal chow shown in Table 2) for 15 days. Mice were weighed every day.

Sample Collection and DNA Extraction

Prior to the institution of fish oil treatment and after the adaptive phase, fresh fecal samples were collected for analysis. On the 15th day of the treatment of fish oil, fresh stool samples were collected to study the effect of fish oil treatment. In order to note the significant differences in the content of microbiota between saline treatment and fish oil groups and to avoid minor differences that could occur in the intestinal bacteria of each animal within the groups, we pooled the stools of 10 animals of each group. Thus, stools from one group (five male and five female mice) were pooled as one sample for further analysis. All samples were stored at −80°C until extraction of total DNA.

The total genomic DNA was isolated from the fecal samples as per the instructions of the QIAamp DNA Stool Mini Kit (QIAGEN, Hilden, Germany). In order to gain DNA with higher concentration, the extracted DNA was eluted in 100 μL AE buffer (according to the protocol) and packed into four tubes to avoid multigelation. All DNA was stored at −20°C before use.

PCR-DGGE

All primers used in this study were synthesized by the Sangon Biotech Co., Ltd. (Shanghai, China). To investigate bacterial community composition in extracted DNA, the hypervariable V3 region of the 16 rRNA genes was the target region for amplification. Universal primers for

Table 1. Fatty acid content of the fish oil used in the study

	Fatty acids	Percentage (%)
C16:0	Palmitic acid	0.689 ± 0.038
C16:1	Palmitoleic acid	0.413 ± 0.019
C18:0	Stearic acid	1.453 ± 0.046
C18:1	Oleic acid	4.958 ± 0.031
C18:2	Linoleic acid	0.902 ± 0.064
C18:3 n6	γ-Linolenic acid	0.361 ± 0.014
C18:3 n3	α-Linolenic acid	0.190 ± 0.012
C20:0	Arachidic acid	0.490 ± 0.057
C20:2	Eicosadienoic acid	1.357 ± 0.028
C20:1	Eicosenic acid	0.638 ± 0.017
C21:0	Heneicosanoic acid	0.449 ± 0.070
C20:3 n6	Eicosatrienoic acid	0.366 ± 0.053
C20:4 n6	Arachidonic acid	0.555 ± 0.089
C20:3 n3	Eicosatrienoic acid	2.007 ± 0.025
C22:0	Behenic acid	0.385 ± 0.078
C20:5 n3	EPA	40.060 ± 2.898
C22:1 n9	Erucic acid	3.049 ± 0.244
C22:2	Docosadienoic acid	0.397 ± 0.155
C23:0	Tricosanoic acid	1.971 ± 0.068
C24:0	Lignoceric acid	3.054 ± 0.028
C22:6 n3	DHA	27.36 ± 0.340

Table 2. Composition of the normal chow

	Content
Water	8–10%
Protein	20–25%
Fat	4–5%
Fiber	4–5%
Ash	6–8%
Diaminocaproic acid	1.20–1.42%
Methionine + micysteine	0.78–1.1%
Tryptophan	0.27–0.34%
Calcium	1.2–1.8%
Phosphorus	0.8–1.2%
Vitamin A	12500–15000 (IU/kg)
Vitamin D	12500–15000 (IU/kg)