The impact of omega-3 fatty acids on lung histopathology in mice model of chronic asthma

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ABSTRACT

We aimed to determine the impact of the diet enriched with omega-3 fatty acids, a potential anti-inflammatory agent, on chronic changes of asthma in a mice model. Therapeutic modalities have not yet been proved to be successful in reversing already-established chronic changes of airways in asthma. For this reason, interventions including dietary changes before the sensitization and remodeling period would prevent those changes. Six-week old female Balb/c mice (n=18) were divided into two groups. During the development of chronic asthma model, mice in omega-3 fatty acid group received a diet enriched with omega-3 fatty acids, whereas mice in the other group received a diet with saturated fatty acids. Balb/c mice were sensitized intraperitoneally in both groups using 10 μg/100 μl of ovalbumin (OVA) and 1.5 mg Al (OH)3 on days 1, 14 and 21. Primed mice were challenged by repeated intranasal instillation of 20 μg/10 μl OVA three times a week. Mice were sacrificed and bronchoalveolar lavage fluid was obtained 24 h after the last challenge (day 77). The lungs were removed for histological examination. The omega-3 fatty acid group showed significantly reduced neutrophil percentage in bronchoalveolar lavage fluid. There was no statistically significant difference between the two groups in lymphocyte, macrophage and eosinophil percentages. The mean basement membrane thickness was less severe in the omega-3 group than the saturated fatty acid group. There was no significant difference between the two groups for goblet cell numbers, subepithelial smooth muscle of airways, and bronchial-associated lymphoid tissue. The findings of this study suggest that consumption of omega-3 fatty acids would prevent some of the chronic changes of airways due to asthma. Future studies are needed to evaluate the potential preventive and therapeutic effects of omega-3 fatty acids in asthma.


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that often have different biological actions or potencies than those formed from AA (James et al., 2000; Calder, 2001). A couple of animal studies indicate that fish oil can have potent effects on immune function and inflammatory cell responses (Jolly et al., 1997; Peterson et al., 1998a, 1998b).

Clinical trials about supplementing omega-3 fatty acids to adults with established allergies and bronchial asthma have been generally disappointing. However, it is known that the immature immune system is highly susceptible to immune-modulation in the very early life (Calder et al., 2002). Until now, only a limited number of clinical studies were published about the beneficial effects of fish oil supplementation on asthma in postnatal period (Blümer and Renz, 2007).

In this study, we aimed to determine the impact of the diet enriched with omega-3 fatty acids, a potential anti-inflammatory and immune-modulatory agent, on chronic changes of asthma in mice model.

2. Materials and methods
The Animal Ethics Committee of Adnan Menderes University approved the experimental procedures, and maintenance of animals in accordance with institutional guidelines.

Mice
Eighteen 6-week old female BALB/c mice (obtained from Cerrahpaşa Medical Biology Laboratories, Istanbul, Turkey) were maintained in the Laboratory Animals Breeding and Experimental Research Unit of Adnan Menderes University during the study between March-June 2008. Cages were placed into a ventilated cabinet in a quiet room on a 12:12 hour light/dark cycle and were kept at a constant room temperature (24°C).

Experimental diets
After a 30-days acclimation period, mice were randomly assigned to diets containing lard or fish oil. Tap water and food were available ad libitum throughout the study. Nutritionally complete experimental diets (MBD Provender, Gebze, Kocaeli, Turkey) were based on the semi-purified AIN93G diet (Reeves, 1997). The original basal diet with lower fat was modified to contain 200 g fat/kg diet by addition of fish and corn oils or lard, while maintaining the same nutrient to energy ratio. The test diet contained 18% mackerel fish oil (a gift from Orzax Medicine, Quincy, USA) and 2% corn oil to prevent essential fatty acid (18:2n-6) deficiency. The higher than usual fat content of this experimental diet allows a greater level of n-3 PUFA enrichment with a widely used fish oil source of these fatty acids, i.e., mackerel fish oil. The control diet contained 20% lard. Both lard and fish oils were stabilized with a synthetic antioxidant (0.2 g/kg tert-butyl-hydroquinone). Other diet ingredients included (per kg of diet): 354.6 g corn starch, 230 g casein, 100 g sucrose, 57.4 g fiber, 40.2 g AIN-93 mineral mix, 11.2 g AIN-93 vitamin mix, 3.4 g L-cysteine, 2.9 g choline bitartate, and 200 g fat. The fatty acid composition of these diets has been previously reported by Irons et al (2003).

Study design
Mice were divided into two equal size groups by their feeding modality (Table 1). During the development of chronic asthma model, mice in omega-3 fatty acid group received diet enriched with omega-3 fatty acids, whereas mice in control group received a diet enriched with saturated fatty acids.

Establishment of chronic asthma model
Balb/c mice were sensitized intraperitoneally in both groups using 10 μg/100 μl of ovalbumin (OVA) and 1.5 mg Al(OH)3 on days 1, 14 and 21. Primed mice were challenged by repeated intranasal instillation of 20 μg/10 μl OVA three times a week during 8 weeks.

Histopathological examination
Mice were sacrificed and bronchoalveolar lavage fluid (BALF) was obtained by flushing the lung 3 times with 1 ml PBS 24 h after the last challenge (day 77). Slides were prepared by cytocentrifugation (Cytospin 3, Shandon Scientific, Runcorn, Cheshire, UK) and stained with May-Grunwald-Giemsa. Differential cell counts were made on two different slides for each animal, counting at least 200 inflammatory cells per slide, and the means of two slides were recorded.

Lung tissues were fixed in 10% phosphate-buffered formalin for 24 hours and embedded in paraffin wax. Sections (2 to 3mm) were cut and stained with hematoxylin-eosin (H&E) and periodic acid-Schiff (PAS). The stained sections were visualized by light microscopy and examined for thickness of epithelia, basement membrane, bronchial-associated lymphoid tissue, subepithelial smooth muscle and goblet cell hyperplasia. The density of changes was scored in relative units (RU), with 0=no, 1=weak, 2=moderate, and 3= strong.

Statistical analysis
SPSS 15 for Windows® was used for statistical analysis of the data. Mann-Whitney U test was used to compare data between two groups. A p value of less than 0.05 was considered to be significant.

3. Results
Three mice from the omega-3 fatty acid group and six mice from the control group died during the study. Compared with the controls, the omega-3 fatty acid group showed significantly

Table 1. Repartition of each fatty acid in the two diet groups (gram per 100 grams of lipids)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>ω 3 diet</th>
<th>SF Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0 (PA)</td>
<td>9.0</td>
<td>26.0</td>
</tr>
<tr>
<td>C18:0 (SA)</td>
<td>3.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Total of saturated fatty acids</td>
<td>12.0</td>
<td>34.0</td>
</tr>
<tr>
<td>MUFA oleic acid</td>
<td>45.0</td>
<td>30.0</td>
</tr>
<tr>
<td>C18:2 ω6 (LA)</td>
<td>23.0</td>
<td>25.0</td>
</tr>
<tr>
<td>C18:3 ω3 (ALA)</td>
<td>10.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C18:3 ω6 (GLA)</td>
<td>3.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C18:4 ω3 (STA)</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C20:4 ω6 (AA)</td>
<td>0.5</td>
<td>11.0</td>
</tr>
<tr>
<td>C20:5 ω3 (EPA)</td>
<td>5.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C22:6 ω3 (DHA)</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Total of PUFA</td>
<td>43.0</td>
<td>27.0</td>
</tr>
</tbody>
</table>

MUFA: Mono-unsaturated fatty acid; PA: Palmitic acid; SA: Stearic acid; LA: Linoleic acid; AA: Arachidonic acid; ALA: α-linolenic acid; GLA: γ-linolenic acid; STA: Stearidonic acid; EPA: Eicosapentaenoic acid; DHA: Docosahexaenoic acid; PUFA: Polyunsaturated fatty acid; SF: Control diet with saturated fatty acids.
reduced neutrophil percentage in BALF (p=0.024). Although the animals fed on fish oil-containing diet had higher mean number of lymphocytes than that of the controls, the difference between the two groups was not found to be statistically significant (p=0.085). The subepithelial smooth muscle thickness of the airways was higher in the group fed on lard-containing diet compared with that of the fish-oil group, but this was also statistically insignificant (p=0.083). The mean percentages of macrophages and eosinophil leukocytes as well as the goblet cell numbers and bronchial-associated lymphoid tissues were similar in animals of both groups (p>0.05). The comparison of the two groups showed statistically significant difference in basement membrane thickness. The mean basement membrane thickness of animals fed on a lard-enriched diet was significantly greater than that of animals fed on a fish oil-enriched diet (p=0.038).

4. Discussion

The objective of this study was to determine the effects of a diet enriched either with n-3 PUFAs or saturated fatty acids on structural changes of airways in a chronic mice model of asthma.

Preliminary studies demonstrated almost always an increase in the numbers of neutrophils, eosinophils, macrophages and lymphocytes in the respiratory system of animals sensitized via OVA (Nabe et al., 2005; Nabe et al., 2011; Yang et al., 2011; Brandenberger et al., 2013; Kim et al., 2013). In this study, there was no difference between the two groups for eosinophilia and macrophage, with a slight difference in the number of lymphocytes (p=0.085). However, there was a significant difference in neutrophils due to their decrease in BALF of omega-3 PUFA group (p=0.024). This finding demonstrates that omega-3 fatty acids have phase-dependent anti-inflammatory effects, affecting the early phase of asthma by reducing the number of neutrophils.

Airway mucosa remodeling is the consequence of chronic inflammatory processes and represents the final stage of asthma (Fahy et al., 2000). Histological examination of the lung tissue did not reveal any difference for goblet cells and bronchia-associated lymphoid tissues. However, the comparison of the two groups showed a slightly increased subepithelial smooth muscle thickness of airways, and a statistically significant increase in basement membrane thickness in the omega-3 group (p=0.083 and p=0.038, respectively). This data indicate that dietary omega-3 fatty acids could reduce some of the structural chronic changes seen in asthma.

It is now well known that consumption of fish oil containing omega-3 fatty acids reduces the risk of many human diseases (Sidhu, 2003). Dietary lipid manipulation may affect different immune parameters and the immune-modulation induced by dietary fatty acids may be beneficial in inflammatory diseases. A dietary immune modulation with omega-3 polyunsaturated fatty acid should represent an interesting alternative to immune suppressive molecules, like corticosteroids, which concomitantly has many undesired side-effects (Dahl, 2006). In this study, two different diet groups were used (Table 1). One diet was dominated by EPA and DHA which have less potential inflammatory products such as PGE3 and LTB5. Nevertheless, EPA and DHA act as a competitive inhibitor of AA conversion to the pro-inflammatory key mediators PGE2 and LTB4. And, the other diet was dominated by saturated fatty acids and arachidonic acid, which give rise to PGE2 and LTB4 (James et al., 2000).

Some studies have focused on the anti-inflammatory effects of omega-3 fatty acids on asthma (Blümer et al., 2001). Wallace et al. (2001) reported that omega-3 fatty acids are effective on T-helper 1 cells and eicosanoid metabolism. Korotkova et al. (2004a; 2004b) showed that omega-3 fatty acid-enriched diet in pregnant rats suppressed the allergic reactions of their offsprings. Yokoyama and co-workers.

Table 2. Structural changes in the airways

<table>
<thead>
<tr>
<th></th>
<th>Goblet cell hyperplasia</th>
<th>Muscle thickening</th>
<th>Basal membrane thickening</th>
<th>BALT</th>
</tr>
</thead>
<tbody>
<tr>
<td>ω 3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ω 3</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>ω 3</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>ω 3</td>
<td>2</td>
<td>1</td>
<td>0</td>
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</tr>
<tr>
<td>ω 3</td>
<td>0</td>
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<tr>
<td>ω 3</td>
<td>1</td>
<td>0</td>
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<td>1</td>
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<tr>
<td>SF</td>
<td>1</td>
<td>1</td>
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<td>SF</td>
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</tr>
</tbody>
</table>

Sections stained with hematoxylin-eosin (H&E) or periodic acid-Schiff (PAS). The stained sections were examined for inflammatory changes and goblet cell metaplasia. The density of changes was scored in relative units (RU) with 0=no, 1=weak, 2=moderate, and 3=strong goblet cell hyperplasia

ω 3: Omega-3 fatty acid group; SF: Saturated fatty acid group
showed that nebulized DHA has anti-inflammatory effects on an acute asthma model in mice (Yokoyama et al., 2000).

In the future, it will be necessary to develop preventive strategies as well as strategies which specifically and sufficiently interfere in structural changes of the airways, since none of the currently available therapies are able to prevent or stop the beginning of airway remodeling (Wegmann and Renz, 2005).

In addition to previous studies evaluating the inhibitory effects of omega-3 fatty acids on acute asthma, the current study is the first to determine the immune-modulatory effects of dietary fatty acids on the structural changes of chronic asthma and provides an evidence of diminished neutrophil production in inflammation process.

Therefore, the notion of preventing or at least modifying the development of airway remodeling by supplementing with omega-3 PUFAs in asthma seems to be a promising approach. However, there is an increasing need for future studies to evaluate the potential benefits of omega-3 fatty acids in asthma.

REFERENCES


