

## 恒常性機能解析分野 Division of Analysis of Homeostasis

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### ◇研究目的 Aims of the research projects

植物が作り、動物の機能維持に必須な脂肪酸には二種類ある。リノール酸 (n-6) は成長や生殖生理の維持に必須であるが、体内でアラキドン酸に変換されエイコサノイドと呼ばれる種々の生理活性物質 (炎症メディエーター) の前駆体となっている(リノール酸カスケード)。 $\alpha$ -リノレン酸 (n-3) はエイコサペンタエン酸 (EPA) やドコサヘキサエン酸 (DHA) に変換され、EPA はエイコサノイド合成の前駆体として働き、DHA は脳・神経機能の維持に必須の役割をしている。n-6 系と n-3 系は多くの酵素、受容体の段階で競合的であり、そのバランスが身体の恒常性維持 (健康と病気) に重要な因子となっている。

本研究は、摂取食品によって変わるリノール酸 (n-6) 系と  $\alpha$ -リノレン酸 (n-3) 系のバランスが、過去半世紀に急増しているアレルギー過敏症、米国型癌、心疾患などに及ぼす影響を、長期投与の結果の解析によって評価することを目的としている。

### ◇研究概要 Research projects

#### 1. アレルギー過敏症の体質改善—基礎と臨床

リノール酸カスケードを介して作られる n-6 系メディエーター (プロスタグランジン, トロンボキサン, ロイコトリエンなど) は、異物(アレルゲン)の体内への侵入を防ぐ役割を果たしている。しかし、リノール酸の摂取過剰により細胞膜にアラキドン酸が増えるとメディエーターが過剰に作られ、その防御反応が過剰に、長期にわたっておこり、病的なアレルギー症状となる。一方、 $\alpha$ -リノレン酸系の EPA から類似のメディエーターが作られるが、一般に作られる量は少なく活性もよわい。そして、摂取食品を選び、n-6/n-3 の比を低くすると、アレルゲン刺激で作られる炎症メディエーターの産生量と活性を低くすることができる (体質の改善)。動物実験の結果に基づき、「リノール酸を減らし、 $\alpha$ -リノレン酸系を増やす食事療法」が、アトピー性皮膚炎の治療に有効であることが証明されつつある (共同研究)。薬により対症療法としてリノール酸カスケードを抑えると同時に、食物の選択による体質改善が勧められる。

#### 2. n-6/n-3 比を下げることによる米国型癌の予防—基礎と臨床

米国で先行しわが国で増えている癌 (肺腺癌, 大腸癌, 乳癌, 前立腺癌, 膵臓癌, 皮膚癌など) に対し、リノール酸は促進的に、 $\alpha$ -リノレン酸系は抑制的にはたらく (動物実験)。リノール酸カスケードを介して作られる過剰のプロスタグランジン, ロイコトリエンなどが炎症を持続させ、炎症細胞からの過剰の活性酸素 (ROS) が遺伝子傷害をひきおこし、細胞増殖的にはたらく。動物実験の結果、薬の作用機構、疫学データの解析に基づき、n-6/n-3 比を下げることによる大腸腫瘍再発予防の介入試験を継続中である (共同研究)。

#### 3. シソ油(エゴマ油)の開発と食用油の安全性評価

工業的に使われていたシソ油の安全性と有効性を、長期投与の結果を評価することにより示して、食用に再開発した。シソ油 (エゴマ油) は、癌の化学予防に最も有効性が高いと評価され、脳卒中発症予防効果もある。この過程で、数種の食用油が脳卒中ラットの寿命を異常に短縮することが明らかとなり、その内分泌攪乱作用の本体を探索している。

## ◇著書 Books

- 1) Okuyama H., Tatematsu K., Hirose, N., Kato T., Chiba S. and Nagata Y. : Suppression of allergic hyper-activity by lowering the n-6/n-3 ratio of dietary fatty acids: basic and clinical studies. 1<sup>st</sup> International Congress on the Columbus Concept, Belovo S.A., Belgium, pp119~126, 2003

## ◇原著 Original papers

- 1) Miyazawa D., Ikemoto A., Fujii Y. and Okuyama H. : **Partial purification and characterization of phosphatidic acid-specific phospholipase A1 in porcine platelet membranes**, *Biochim. Biophys. Acta.*, 1631 : 17~25, 2003

**Abstract:** We have shown previously that the phospholipase A (PLA) activity specific for phosphatidic acid (PA) in porcine platelet membranes is of the A(1) type (PA-PLA(1)) [J. Biol. Chem. 259 (1984) 5083]. In the present study, the PA-PLA(1) was solubilized in Triton X-100 from membranes pre-treated with 1 M NaCl, and purified 280-fold from platelet homogenates by sequential chromatography on blue-Toyopearl, red-Toyopearl, DEAE-Toyopearl, green-agarose, brown-agarose, polylysine-agarose, palmitoyl-CoA-agarose and blue-5PW columns. In the presence of 0.1% Triton X-100 in the assay mixture, the partially purified enzyme hydrolyzed the acyl group from the sn-1 position of PA independently of Ca(2+) and was highly specific for PA; phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI) were poor substrates. The enzyme exhibited lysophospholipase activity for l-acyl-lysoPA at 7% of the activity for PA hydrolysis but no lipase activity was observed for triacylglycerol (TG) and diacylglycerol (DG). At 0.025% Triton X-100, the enzyme exhibited the highest activity, and PA was the best substrate, but PE was also hydrolyzed substantially. The partially purified PA-PLA(1) in porcine platelet membranes was shown to be different from previously purified and cloned phospholipases and lipases by comparing the sensitivities to a reducing agent, a serine-esterase inhibitor, a PLA(2) inhibitor, a Ca(2+)-independent phospholipase A(2) inhibitor, and a DG lipase inhibitor.

- 2) Umezawa M., Tatematsu K., Korenaga T., Fu. X., Matsushita T., Okuyama H., Hosokawa M., Takeda T. and Higuchi K. : **Dietary fat modulation of apoA-II metabolism and prevention of senile amyloidosis in the senescence-accelerated mouse**, *J. Lipid. Res.*, 44 : 762~769, 2003

**Abstract:** Senescence-accelerated mouse-prone (SAMP1; SAMP1@Umz) is an animal model of senile amyloidosis with apolipoprotein A-II (apoA-II) amyloid fibril (AApoAII) deposits. This study was undertaken to investigate the effects of dietary fats on AApoAII deposits in SAMP1 mice when purified diets containing 4% fat as butter, safflower oil, or fish oil were fed to male mice for 26 weeks. The serum HDL cholesterol was significantly lower ( $P < 0.01$ ) in mice on the diet containing fish oil (7.4 +/- 3.0 mg/dl) than in mice on the butter diet (38.7 +/- 12.5 mg/dl), which in turn had significantly lower ( $P < 0.01$ ) HDL levels than mice on the safflower oil diet (51.9 +/- 5.6 mg/dl). ApoA-II was also significantly lower ( $P < 0.01$ ) in mice on the fish oil diet (7.6 +/- 2.7 mg/dl) than on the butter (26.9 +/- 7.3 mg/dl) or safflower oil (21.6 +/- 3.7 mg/dl) diets. The mice fed fish oil had a significantly greater ratio ( $P < 0.01$ ) of apoA-I to apoA-II, and a smaller HDL particle size than those fed butter and safflower oil. Severe AApoAII deposits in the spleen, heart, skin, liver, and stomach were shown in the fish oil group compared with those in the butter and safflower oil groups (fish oil > butter > safflower oil group,  $P < 0.05$ ). These findings suggest that dietary fats differ in their effects on serum lipoprotein metabolism, and that dietary lipids may modulate amyloid deposition in SAMP1 mice.

- 3) Miyazawa D., Ikemoto A., Fujii Y. and Okuyama H. : **Dietary alpha-linoleic acid suppresses the formation of lysophosphatidic acid, a lipid mediator, in rat platelets compared with linoleic acid**, *Life Sci.*, 73 : 2083~2090, 2003

**Abstract:** Rats fed a high linoleic acid (LA, 18:2n-6) diet or a high alpha-linolenic acid (ALA, 18:3n-3) diet for 4

months after weaning. Platelets from the high-LA group contained more arachidonic acid (AA, 20:4n-6) and less eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) compared with those from the high-ALA group. Incorporation of [<sup>32</sup>P]orthophosphate into platelet phospholipids was increased by thrombin-treatment, and was greater by ca. 30% in the high-LA group than in the high-ALA group both in the presence and absence of thrombin. The formation of [<sup>32</sup>P] lysophosphatidic acid (LPA), a lipid messenger, in [<sup>32</sup>P] orthophosphate-labeled platelets was increased 6.6-fold in the high-LA group and 4.1-fold in the high-ALA-group by thrombin-treatment. The formation of [<sup>32</sup>P] LPA in activated platelets was reduced by 35% in the high-ALA group.

4) Du. C, Sato S., Watanabe S., Wu. C-Z, Ikemoto A., Ando K., Kikugawa K., Fujii Y. and Okuyama H. : **Cholesterol synthesis in mice is suppressed but lipofuscin formation is not affected by long-term feeding of n-3 fatty acid-enriched oils compared with lard and n-6 fatty acid-enriched oils**, *Biol. Pharm. Bull.*, 26(6) : 766~770, 2003

**Abstract:** Hypocholesterolemic activity of dietary polyunsaturated fatty acids is observed after relatively short-term but not long-term feedings, and their long-term feedings are suspected to accelerate aging through tissue accumulation of lipid peroxides and age pigments (lipofuscin). To define the long-term effects of fats and oils in more detail, female mice were fed a conventional basal diet supplemented with lard (Lar), high-linoleic (n-6) safflower oil (Saf), rapeseed oil (Rap), high-alpha-linolenic (n-3) perilla oil (Per), or a mixture of ethyl docosahexaenoate and soybean oil (DHA/Soy) from 17 weeks to 71 weeks of age. The DHA/Soy and Per groups had decreased serum cholesterol levels compared with the Lar and Saf groups, but the difference between the Lar and Saf groups was not significant. The 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase activity in the liver was also significantly lower in the Per and DHA/Soy groups. However, no significant difference in lipofuscin contents in the brain and liver was observed among the 5 dietary groups, despite significant differences in peroxidizability indices of the dietary and/or tissue lipids. These results indicate that n-3 fatty acid-rich oils are hypocholesterolemic by suppressing hepatic HMG-CoA reductase activity compared with animal fats and high-linoleic (n-6) oil, but tissue lipofuscin contents are not affected by a long-term feeding of fats and oils with different degree of unsaturation in mice.

◇総説 Review papers

- 1) Brisibe EA., Okada N., Mizukami H., Okuyama H. and Fujii Y. : RNA interference: potentials for the prevention of HIV infections and the challenges ahead, *TRENDS in Biotech.*, 21(7) : 306~311, 2003
- 2) Hamazaki T. and Okuyama H. : The Japan Society for Lipid Nutrition Recommends to Reduce the Intake of Linoleic Acid. A Review and Critique of the Scientific Evidence, Simopoulos AP, Cleland LG (eds) : Omega-6/Omega-3 Essential Fatty Acid Ratio: The Scientific Evidence. *World Rev Nutr Diet.* 92 : 109~132, Basel, Karger, 2003

◇学会報告 Scientific presentation

- 1) Okuyama H. : Cardiovascular disease -from cholesterol hypothesis to  $\omega 6/\omega 3$  balance, IX ASIAN CONGRESS OF NUTRITION, 2003. 2, New Delhi
- 2) 奥山治美 : 必須脂肪酸バランスと行動, 日本精神科病院協会, 千葉県支部定期総会, 2003. 5. 30, 千葉.
- 3) 奥山治美 : 油脂栄養から見たアレルギー・炎症性疾患の予防, 第54回日本皮膚科学会, 中部支部学術大会, 2003. 11. 9, 大阪.

◇その他 Others

- 1) 奥山治美 : 特別講演「脂質栄養の新方向-コレステロールからの脱却-」, 岐阜地域保健所研修会, 2003. 1. 10, 岐阜.

- 2) 奥山治美：特別講演「油脂の選び方の新方向-アレルギー・癌，心臓病予防のために-，天白区医師会臨床懇談会，2003. 1. 16, 名古屋.
- 3) 奥山治美：特別講演「油脂栄養の新方向-コレステロール仮説から n-6/n-3バランスへ-」，病院栄養士協議会研修会，2003. 2. 8, 大分.
- 4) 奥山治美：特別講演「油脂（あぶら）の正しい選び方」～血液サラサラで究極の健康づくり～，和歌山県健康センター健康講座，2003. 3. 15, 和歌山.
- 5) 奥山治美：特別講演「脂質栄養の新方向-心疾患・アレルギー・癌予防のために-」，第3回44病診連携談話会，2003. 7. 5, 名古屋.
- 6) 奥山治美：特別講演「脂質栄養の新方向-心疾患・アレルギー・癌予防のために-」，和歌山市医師会学校医部会学術講演会，2003. 10. 25, 和歌山.