臨床利用分野　Division of Clinical Application

教　授	浜崎　智仁	Professor	Tomohito Hamazaki (M.D., Ph.D.)
助教授	渡辺　志朗	Associate Professor	Shiro Watanabe (Ph.D.)
助　手	長澤　哲郎	Assistant Professor	Tetsuro Nagasawa (Ph.D.)

◇研究目的　Aims of the research projects

天然薬物の臨床利用を目指して，以下のテーマについて研究している。

1）天然薬物（特に魚油中のDHA・EPA）の作用機序の解明とその臨床的有効性

2）中枢神経系における自然免疫応答に及ぼす和漢薬および食餌性 n-3 系脂肪酸の影響

3）遺伝的および環境的要因による脂質代謝異常に対する和漢薬の改善効果

◇研究概要　Research projects

1．注意欠陥/多動性障害児における二重盲検試験により DHA が集中力をむしろ低下させる可能性があり（対照群では慣れにより検査値が向上したが，DHA 群では変化がない），DHA は中枢性ノルアドレナリンを抑制している可能性が考えられる。また，二次的症状である攻撃性は，DHA で有意に低下した。これもノルアドレナリンの低下と関連すると思われる。

2．マウスに LPS を投与することにより起こる摂食行動の低下が n-3 系脂肪酸をあらかじめ与えておくことにより抑制されることを明らかにした。この機構を中枢神経系機能の面から解明する。

3．LPS やサイトカインおよびその誘導剤を投与したときに起こる摂食行動の低下を中枢神経系における自然免疫応答の指標として，これに対する和漢薬の効果を評価する。

4．副腎白質ジストロフィーにみられるペルオキシゾーム機能異常による極長鎖脂肪酸の蓄積に対する和漢薬の効果を評価する。

5．高カロリー摂取による肥満に伴う中性脂質蓄積と炎症性応答の相関の解析。またこれに対する和漢薬の効果を評価する。

◇著　書　Books

1）浜崎智仁：コレステロールは高いほうが長生きする．エール出版社，2003.

2）浜崎智仁：臨床試験の組み立て方．ヘルスフード概論 矢澤一良編著，52-67，成山堂書店，東京，2003.

◇原　著　Original papers

1）**Nakamura N., Hamazaki T., Johkaji H., Minami S., Yamazaki K., Satoh A., Sawazaki S., Urakaze M., Kobayashi M., Osawa H., Yamabe H., Okumura K. : Effect of cilostazol on serum lipid concentrations and plasma fatty acid composition in type 2 diabetic patients with peripheral vascular disease.** ***Clin Exp Med.,*** **2: 180-184, 2003.**

Abstract: Cilostazol is an anti-thrombotic and vasodilating agent, reported to have both anti-thrombotic and cerebral vasodilating effects. We investigated the effects of cilostazol on serum lipid concentrations and plasma fatty acid composition in type 2 diabetic patients with peripheral vascular disease. The serum concentrations of total cholesterol, triglycerides, high-density lipoprotein-cholesterol, lipoprotein (a), remnant-like particles-cholesterol, apolipoproteins, and plasma fatty acid composition were measured in 1 7 diabetic patients with peripheral vascular disease before and 1, 3, and 6 months after administration of cilostazol (200 mg/day). Serum triglyceride concentrations were significantly decreased after cilostazol (from 1.31±0.17 mmol/1 to 0.86±0.07 mmol/l at 6 months, P<0.01). Plasma docosahexaenoic acid levels were significantly increased after cilostazol (4.11±0.26% to 4.94±0.26% at 6 months, P<0.01). Our findings show that cilostazol can induce some beneficial changes in serum lipid profile and plasma fatty acid composition.

2）**Hamazaki K., Itomura M., Mingming Huan, Nishizawa H., Watanabe S., Hamazaki T., Sawazaki S., Terasawa K., Nakajima S., Terano T., Hata Y., Fujishiro S. : n-3 Long-Chain FA Decrease Serum Levels of TG and Remnant-Like Particle-Cholesterol in Humans.** ***Lipids.,*** **38:353-358, 2003.**

Abstract: A large number of papers have reported that administration of n-3 FA reduced serum TG concentrations in hypertriglyceridemic patients. However, few studies have examined the effect of n-3 FA on serum concentrations of remnant-like particle (RLP) cholesterol. Volunteers (n =41) whose serum TG concentrations were 100-300 mg/dL were recruited and randomly assigned to either an n-3 FA group or a control group with stratification by sex, age, and serum TG level in a double-blind manner. The subjects in the n-3 FA group were administered 125 mL of fermented soybean milk with fish oil containing 600 mg of EPA and 260 mg of DHA/d for 12 wk. The controls consumed control soybean milk with olive oil. Fasting blood samples were obtained before the start of administration and at 4, 8, and 12 wk. EPA concentrations in red blood cells increased significantly in all but one subject in the n-3 FA group, with no significant changes in the control group. TC levels decreased more in the n-3 FA group than in the control group at weeks 4 (P< 0.05), 8 (P< 0.01), and 12 (P< 0.05) with their baseline as covariate. RLP cholesterol levels decreased more in the n-3 FA group than in the control at weeks 8 (P< 0.01) and 12 (P< 0.05) with their baseline as covariate. The groups did not differ in the other lipid levels. It is likely that n-3 long-chain FA may exert anti-atherosclerotic effects by lowering serum TG and RLP-cholesterol levels even at the dose of 860 mg/d.

3）**Harada S., Sugiyama E., Takebe S., Taki H., Shinoda K., S.G.K. Mohamed., Maruyama M., Hamazaki T., and Kobayashi M. : Cooperative induction of 15-lipoxygenase in rheumatoid synovial cellsby IL-4 and proinflammatory cytokines.** ***Clin. Exp. Rheumatol.,*** **21:753-758, 2003.**

Abstract Objective: To clarify the role of interleukin-4 (IL-4) in the expression of 15-lipoxygenase (15-LOX), whose metabolites are known to suppress the inflammatory reaction, in freshly prepared rheumatoid synovial cells.

Methods: Adherent synovial cells were prepared by enzymatic digestion of synovia obtained from patients with rheumatoid arthritis (RA). Protein expression of 15-LOX was determined by Western blot analysis. The messenger RNAs of 15-LOXwere determined by reverse transcription and the polymerase chain reaction (RT-PCR).

Results: Freshly prepared rheumatoid synovial cells did not express 15-LOX at either the mRNA or protein levels. IL-4 induced the protein expression of 15-LOX after 24 hours of culture. Although interleukin- 1α(IL-1α) and tumor necrosis factor α(TNFα), major inflammatory cytokines in rheumatoid synovia, did not induce the expression of 15-LOX, IL-4 and these inflammatory cytokines synergistically enhanced the protein expression of 15-LOX. The synergistic effect was also observed at the level of mRNA.

Conclusions: We demonstrate that IL-4 cooperated with the inflammatory cytokines IL-1α and TNFα to enhance the expression of 15-LOX in rheumatoid synovial cells. Since 15-LOX metabolites have potent anti-inflammatory actions, our data suggest that IL-4 might downregulate rheumatoid inflammation via the induction of 15-LOX and its metabolites.

4) **Watanabe S., Doshi M., and Hamazaki T.: n-3 Polyunsaturated fatty acid (PUFA) deficiency elevates and n-3 PUFA enrichment reduces brain 2-arachidonoylglycerol level in mice.** ***Prostaglandins, Leukotrienes and Essential Fatty Acids,*** **69: 51-59, 2003.**

Abstract: 2-Arachidonoylglycerol (2-AG) is a putative endogenous ligand for cannabinoid receptors and was suggested to play an important role in both physiological and pathological events in the central nervous system (CNS) as well as in peripheral organs. The sequential hydrolysis of arachidonic acid (20:4n-6, AA)-containing phospholipids has been proposed as a major biosynthetic route of 2-AG. On the other hand, the manipulation of the dietary n-3 polyunsaturated fatty acid (PUFA) status changes the AA level in tissue phospholipids. We, therefore, conducted two separate experiments to confirm whether the dietary n-3 PUFA status influences the 2-AG level in the mouse brain. In the first experiment, we fed mice with n-3 PUFA-deficient diet, which resulted in a marked decrease in the docosahexaenoic acid (22:6n-3, DHA) levels without a change in the AA level in brain phospholipids as compared with the mice fed with an n-3 PUFA-sufficient diet. The brain 2-AG level in the n-3 PUFA-deficient group was significantly higher than in the n-3 PUFA sufficient group. In the second experiment, we found that short-term supplementation of DHA-rich fish oil reduced brain 2-AG level as compared with the supplementation with low n-3 PUFA. The decrease in the AA level and the increase in the DHA level in the major phospholipids occurred in the brains of the mice fed the fish oil diet compared with those fed the low n-3 PUFA diet. Our results indicate that the n-3 PUFA deficiency elevates and n-3 PUFA enrichment reduces the brain 2-AG level in mice, suggesting that physiological and pathological events mediated by 2-AG through cannabinoid receptor in the CNS could be modified by the manipulation of the dietary n-3 PUFA status.

5) **Chiang N., Takano T., Arita M., Watanabe S., and Serhan, C.N.: A novel rat lipoxin A4 receptor that is conserved in structure and function** ***Br. J. Pharmacol.,*** **139: 89-98, 2003.**

Abstract: 1 Lipoxin (LX) A4 and aspirin-triggered-LX (ATL) are endogenous lipid-derived mediators that regulate leukocyte trafficking via specific LXA4 receptors (ALX), and are involved in endogenous anti-inflammation and resolution. Both LXA4 and ATL are produced by rat tissues in vitro as well as in vivo. In rats, LXA4 and ATL exhibit potent physiological and pathophysiological roles. Thus, we set out to determine whether ALX is expressed in rat tissues and its potential role in modulating leukocyte trafficking with LXA4 and ATL.

2 In rats, a stable analog of ATL, when given intravenously with two consecutive doses at approximately 60 μg kg^{-1} each injection, significantly inhibited neutrophil infiltration (-43%) and protein extravasation (-42%) in a casein-induced peritonitis.

3 The rat orthologue of ALX was cloned from peripheral blood leukocytes encoding a putative G protein-coupled receptor (GPCR). It gave - 74 and - 84% homology, respectively to the deduced amino-acid sequences of the human

and mouse ALX.

4 Tissue distribution analysis by RNase protection revealed that this rat receptor is expressed in tissues/cells, where LXA4 displays physiological and pathophysiological roles, namely, lung, kidney and leukocytes.

5 The rat orthologue of ALX gave specific radioligand binding with [^{3}H]LXA4 and [^{125}I-Tyr]-annexin 1-derived peptide with apparent Kd values of 5 and 820 nM, respectively, that are at levels comparable to those of the human ALX.

6 Activation of rat ALX inhibited tumor necrosis factor alpha-mediated nuclear factor kappaB activity in a ligand-dependent manner utilizing a luciferase reporter gene system.

7 Together, these results are the first demonstration of a rat ALX that is conserved in both structure and function suggesting that ALX plays key roles in regulating effector immune responses from murine to human species.

6）**Du, C., Sato A., 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: 766-770, 2003.**

Abstract: Hypocholesterolemic activity of dietary polyunsaturated fatty acids is observed after relatively short-term but not long-term feeding 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-α 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-methylglutary-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）Hamazaki T., 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.

2）浜崎智仁，澤崎茂樹，糸村美保，浜崎景，渡辺志朗，平山論：n-3 系多価不飽和脂肪酸と行動，特集「機能性食品のトップバッター－n-3 系多価不飽和脂肪酸－」，機能性食品と薬理栄養，1 : 153-158，2003.

3）渡辺志朗：n-3 多価不飽和脂肪酸の化学・代謝・生理，特集「機能性食品のトップバッター－ n-3 系多価不飽和脂肪酸－」，機能性食品と薬理栄養，1 : 129-134，2003.

4）渡辺志朗，浜崎智仁：n-3 系不飽和脂肪酸によるエイコサノイド産生制御，特集「プライマリケア医のための最新栄養学」，治療，85 : 107-111，2003.

◇学会報告　Scientific presentation

1）平山論，浜崎智仁，寺澤捷年 : DHA 含有食品が注意欠陥/多動性障害児に及ぼす影響，日本脂質栄養学会 2003，9，東京.

2）仁井本剛，道志勝，浜崎智仁，渡辺志朗：In vivo での脂質ケミカルメディエーター産生に及ぼすオウゴンの効果，日本薬学会123年会 2003，3，長崎.
3）道志勝，渡辺志朗，仁井本剛，秋元健吾，木曽良信，浜崎智仁：食餌 DHA および n-9 エイコサトリエン酸がマウスにおける脂質性ケミカルメディエーター産生と急性及び慢性炎症に及ぼす影響，日本薬学会123年会 2003，3，長崎.
4）竹中瑞貴，直井一久，金田智子，浜崎智仁，渡辺志朗：n-3 系脂肪酸給餌が LPS 投与によるマウスの強制水泳における行動変化に及ぼす影響，日本薬学会123年会 2003，3，長崎.
5）渡辺志朗，金田智子，竹中瑞貴，浜崎智仁：n-3 系脂肪酸給餌による LPS 誘発 sickness behavior の抑制，日本薬学会123年会 2003，3，長崎.
6）渡辺志朗，金田智子，竹中瑞貴，浜崎智仁：n-3 系脂肪酸給餌によるリポポリサッカライド誘発 sickness behavior の抑制，日本脂質生化学会 2003，6，仙台.
7）渡辺志朗，金田智子，竹中瑞貴，浜崎智仁：n-3 系脂肪酸給餌による LPS 誘発 sickness behavior の抑制，日本脂質栄養学会 2003，9，東京.

◇その他　Others

1）浜崎智仁：スタチン治療に偏っていないか？，リノール酸摂取制限の提言，循環 Plus，3：6〜8，2003.
2）浜崎智仁：連載「魚油と人の健康」，アクネット，Vol.6，8-12号，2003.
3）浜崎智仁：招待講演「脂肪酸と行動」，学術フロンティア推進研究組織第三回ワークショップ」杏林大学，2003，2. 3，東京.
4）浜崎智仁：コレステロールは悪者か？,産経新聞，2003，2. 16
5）浜崎智仁：講演「ドコサヘキサエン酸が精神面に与える影響」，JOCS-ILSI Japan Joint Symposium 2003，2003，6.1，東京.
6）浜崎智仁：魚油でマラリア予防，北日本新聞，2003，7，28
7）浜崎智仁：講演「脂肪酸と精神疾患」. 国立がんセンター研究所支部，2003，10. 17，千葉.
8）浜崎智仁：講演「最近の油事情と健康」. 富山県厚生部，2003，10. 25，富山.
9）浜崎智仁：講演「脂肪酸：医学の新しい切り口」東京農業大学，2003，11. 7，東京.
10）浜崎智仁：講演「魚油の薬効に関する基礎から臨床まで」第 2 回とやま産学官交流会，2003. 11. 11，富山.

◇共同研究　Co-operative researchs

1）孫　月吉：中国・大連医科大学神経精神医学教授「自殺未遂患者の血中脂肪酸構成」2000.11〜
2）平山　論：倉敷市立短期大学「ADHA の治療研究」2001.6〜2003.
3）東原英二：杏林大学医学部泌尿器科学教授「前立腺癌の再発予防研究」2001.9〜
4）Insan Tunru, Syafruddin：インドネシア・ハサヌディン大学，アイクマン研究所「魚油によるマラリア予防の大規模介入試験」2002.6〜

◇研究室在籍者　Research Members

学部 3 年生：藤岡俊太郎，山田泰広
学部 4 年生：熊谷知子，長野見知子
大学院前期 1 年生：岡藤文人，斎藤正隆，直井一久
大学院後期 1 年生：桐原祐子
大学院後期 3 年生：道志　勝
大学院医学研究科 1 年生：西澤弘人
大学院医学研究科 3 年生：ホワンミンミン

大学院医学研究科4年生：浜崎　景
研究支援推進員：武部鎮子
事務補佐員：浜谷裕子
外国人客員研究員：Dr. Syafruddin（アイクマン研究所研究員，ハサヌディン大学医学部講師
2003,11/27-12/26）

◇学位および論文名（2002.3）　Academic degrees and theses

卒業研究：
直井一久「n-3系脂肪酸給餌がLPS投与によるマウスの強制水泳における行動変化に及ぼす影響」
修士論文：
竹中瑞貴「末梢での免疫応答に起因する行動抑制の食餌n-3系脂肪酸による制御の行動学的解析」
仁井本剛「ザイモサン誘発空気嚢炎モデルにおける脂質性ケミカルメディエーター産生に及ぼす天然薬物の効果」
西澤弘人「NC-FAN装置による血液流動性測定と白血球数の影響について」
博士論文：
糸村美保「The Effect of Docosahexaenoic Acid on Physical Aggression in Schoolchildren- a randomized, double-blind, placebo-controlled trial」医学博士（富山医科薬科大学）