臨 床 利 用 部 門

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◇研究目的

天然薬物の臨床利用を目指して，1．n-3系脂肪酸の抗ストレス作用と抗炎症作用，2．必須脂肪酸欠乏症の時に体内で積極的に合成されるアラキドン酸アナログであるミード酸の抗炎症作用，3．イソフラボノイドの一つdaidzinの抗酒効果

◇研究概要

1．n-3系脂肪酸の一つDHAの抗ストレス効果（敵意性制御効果）およびその作用機序を動物モデルを用いて検討中である。n-3の脂肪酸は普通経口投与して用いるが，n-3の静注による各臓器の脂肪酸組成の変化について調べている。さらに，連続静注後の腹腔細胞からのリピッドメディエーターの生産が低下するかを検討中である。

2．ミード酸の抗炎症作用の基礎的研究として，アイコサノイドの合成抑制程度，及びアレルギー性肺疾患への応用のための基礎研究を行っている。

3．daidzinを含む大豆抽出物の抗酒効果を検討中。配糖体の方が効果が強いとの報告があるため，配糖体のまま吸収させる方法（エマルジョンにして口経投与）を検討している。

◇著　書

1）中村典雄，浜崎智仁：アレルギーと行動異常．そして脂質との関連．pp78-92,「油脂（あぶら）とアレルギー」奥山治美・小林哲幸・浜崎智仁編（脂質栄養学シリーズ3　学会出版センター），1999.

2）中村典雄，浜崎智仁：EPAとPTCA．pp174-179，21世紀への医学，平滑筋細胞学動脈硬化研究のフロンティア編集森崎信尋，メディカルセンス，1999.

3）渡辺志朗：アレルギー反応性の軽減をめざした油脂の選択法．pp14-30,「油脂（あぶら）とアレルギー」奥山治美・小林哲幸・浜崎智仁編（脂質栄養学シリーズ3　学会出版センター），1999.

◇原　著

1）**Nakamura N., Hamazaki T., Jokaji H., Minami S.,and Kobayashi M.:Effects of HMG-CoA reductase inhibitors on plasma polyunsaturated fatty acid concentrations in patients with hyperlipidemia. Int J Clin Lab Res, 28 : 192-195, 1998.**

Abstract: We investigated the effect of 12 months' HMG-CoA reductase inhibitor treatment on plasma polyunsaturated fatty acid concentrations in 19 patients with hyperlipidemia. Arachidonic acid concentrations were significantly increased following treatment (from 110.1 ± 20.4 mg/l to 129.2 ± 31.6 mg/1, $P<0.05$). The ratio of eicosapentaenoic acid to arachidonic acid was significantly decreased at the end of 12 months' treatment (from 0.702±0.370 to 0.541±0.204, $P<0.05$). These results suggest that HMG-CoA reductase inhibitors may increase the synthesis of metabolites from arachidonic acid in patients with hyperlipidemia, and that the addition of fish oil is more effective for the prevention of coronary heart disease than HMG-CoA reductase inhibitors alone. [6] reported that lovastatin, an HMG-CoA reductase inhibitor, increased arachidonic acid (AA) levels and thromboxane synthesis in human liver and monocytic cell lines in vitro. Thromboxane induces platelet aggregation and vasoconstriction [7], and increased synthesis may cause thrombosis. In the present study, we examined the effect of 12 months' HMG-CoA reductase inhibitor treatment on plasma concentrations of polyunsaturated fatty acids (PUFA) in 19 patients with hyperlipidemia.

2）**Nakamura N., Hamazaki T., Ohta M., Okubo K., Urakaze M., Sawazaki S., Yamazaki K., Satoh A., Temaru R., Ishikura Y., Takata M., Kishida M.,and Kobayashi M.: Joint effects of HMG-CoA reductase inhibitors and eicosapentaenoic acids on serum lipid profile and plasma fatty acid concentrations in patients with hyperlipidemia. Int J Clin Lab Res, 29 : 22-25, 1999.**

Abstract: HMG-COA reductase inhibitors reduce serum total cholesterol concentrations and the risk of coronary heart disease in patients with hypercholesterolemia. Recently, it has been reported that patients with combined hyperlipidemia are also at risk of coronary heart disease. However, HMG-CoA reductase inhibitor therapy alone does not sufficiently reduce serum triglyceride concentrations. Epidemiological and clinical evidence has shown that fish

oil can lower plasma lipid levels, especially triglycerides. Consequently, we investigated the effects ofthe combination of HMG-COA reductase inhibitors and eicosapentaenoic acid, a major component of fish oil, on hyper lipidemia. We administered 900-1,800 mg/day of the ethyl ester of eicosapentaenoic acid to patients with hyperlipidemia who had been treated with HMG-CoA reductase inhibitors for 30±6 months (means±SE). Serum total cholesterol and triglyceride concentrations were significantly decreased 3 months after the administration of eicosapentaenoic acid (from 5.63±0.23 mmol/1 to 5.20±0.20 mmol/1, P<0.05; from 2.07 ± 0.41 mmol/1 to 1.08 ± 0.17 mmol/1, P<0.01,respectively).Serum high-density lipoproteincholesterol concentrations were significantly increased after the treatment (from 1.23±0.12 mmol/1 to 1.34±0.13 mmol/1, P<0.05). Plasma eicosapentaenoic acid concentrations and the ratio to arachidonic acid in plasma were also significantly increased 3 months after the treatment (from 101.9±8.1mg/1 to 181.8±23.9mg/1, P<0.001; from 0.640±0.075 to 1.211±0.170, P<0.001, respectively). These results suggested that the combination therapy of HMG-CoA reductase inhibitors and eicosapentaenoic acid was effective for patients with hyperlipidemia.

3) **Taki H., Sakai E., Sugiyama T., Mino T., Kuroda A., Taki K., Hamazaki T., Koizumi H.,and Kobayashi M.: Monokine stimulation of interleukin-11 production by human vascular smooth muscle cells in vitro. Atherosclerosis, 144 : 375–380, 1999.**

Abstract : Human vascular smooth muscle cells (VSMC) are a component of blood vessels, and secrete a variety of cytokines in atherosclerotic loci. Interleukin- 11 (IL-11), a member of IL-6-1ike cytokines, is reported to be involved in inflammation and tissue remodeling, both of which are observed in atherosclerosis. However, no information is available as to the production of IL-11 by VSMC. Therefore, the expression of IL-11 in VSMC is investigated. The amounts of IL-11 protein and mRNA were determined by enzyme-linked immunosorbent assay (ELISA) and Northern blot analysis, respectively. The expression of IL-11 in VSMC was also immunohistochemically determined. IL-1 α, transforming growth factor-P (TGF β) and, to a lesser extent, tumor necrosis factor- α (TNF α) stimulated the IL-11 production by VSMC, and the stimulatory effects of IL- 1 α and TGF β on IL-11 production were dose-dependent. IL-1 α and TNF α synergistically augmented TGF β-stimulated IL-11 production by VSMC. Immunohistochemical staining also revealed the expression of IL-11 protein in VSMC. Furthermore, IL-1 α, TGF β, and TNF α induced IL-11 gene expression in VSMC. Because IL-6-like cytokines are reported to be cytoprotective, monokine-stimulated IL-11may have a potent protective role in atherosclerotic lesions.

4) **Hamazaki T., Sawazaki S., Nagasawa T. Nagao Y., Kanagawa Y., and Yazawa K.: Administration of Docosahexaenoic Acid Influences Behavior and Plasma Catecholamine Levels at Times of Psychological Stress. Lipids, 34s : 33–37, 1999.**

Abstract : The purpose of the present research was to clarify the effect of docosahexaenoic acid (DHA) intake on behavior and plasma catecholamines (CA). In Study 1, 42 students

took either DHA-rich oil capsules containing 1.5-1.8 g DHA/d or control oil capsules containing 97% soybean oil plus 3% of another fish oil for 3 mon in a double-blind fashion. They took a psychological test (PF Study) at the start and end of the study. This study started at the end of summer vacation and ended just before the final exams. In the control group, external aggression (aggression against others) in PF Study was significantly increased at the end of the study as compared with that measured at the start (+8.9%), whereas it was not significantly changed in the DHA group (-1.0%). In a similar double-blind study (Study 2), we measured external aggression under nonstressful conditions. External aggression slightly decreased in the control group, whereas there were no significant changes in the DHA group. In Study 3 with 1 4 students, plasma CA were measured at the start and end of capsule administration period of 2 mon. Subjects were under continuous stress of the final exams that lasted throughout the whole study period. The ratio of plasma epinephrine to norepinephrine concentrations was significantly increased in the DHA group (78%), whereas it stayed at the same level in the control group. In Study 4, mice were fed either DHA-deficient diet or -sufficient diet for 4 wk, and their rearing frequency (an anxiety index) was measured. In the DHA-sufficient group, the rearing frequency was significantly less than in the other group. These effects of DHA intake may be applied to people in an attempt to ameliorate stress-related diseases.

5) **Sawazaki S., Hamazaki T., Yazawa K., and Kobayashi M.: The Effect of Docosahexaenoic Acid on Plasma Catecholamine Concentrations and Glucose Tolerance during Long-Lasting Psychological Stress: A Double-Blind Placebo-Controlled Study.J Nutr Sci Vitaminol, 45 : 655–665, 1999.**

Summary : We previously found that docosahexaenoic acid (DHA) intake prevented aggression from increasing at times of mental stress. In the present study, we investigated whether DHA intake modified the plasma catecholamines and cortisol of medical students during a 9-wk period of final exams. We also investigated the effects of DHA intake on a 75g oral glucose tolerance test (oGTT). Fourteen medical students participated in the present study. They were randomly allocated to either control or DHA group in a double-blind manner. Subjects in the control group (4 males and 3 females) took 10 control capsules/d, each capsule containing 280 mg of mixed plant oil, and those in the DHA group (4 males and 3 females) took 10 DHA capsules/d containing 1.5 g DHA for 9 wk, during which subjects underwent more than 20 stressful final exams. At the start and end of the study, plasma catecholamines (epinephrine, norepinephrine (NE) and dopamine) and cortisol were measured ; a 75 g oGTT was also performed. There were no intra- or intergroup differences in plasma glucose concentrations. However, NE concentrations were significantly reduced after DHA administration (- 31%, $p < 0.03$). The other catecholamines and cortisol did not change significantly. The plasma ratio of epinephrine to NE increased in every DHA subject (+ 78%, $p < 0.02$), and intergroup differences were significant ($p < 0.03$). We conclude that these effects of DHA may be applied to people under long-lasting psychological stress to prevent

stress-related diseases.

6) **Yasuda S., Watanabe S., Kobayashi T., Hata N., Misawa Y., Utsumi H., and Okuyama H.: Dietary Docosahexaenoic Acid Enhances Ferric Nitrilotriacetate-Induced Oxidative Damage in Mice but not when Additional α-Tocopherol is Supplemented . Free Rad.Res., 30 : 199–205, 1999.**

Abstract : Weaning mice were fed a diet supplemented with beef tallow (BT) or BT plus docosahexaenoic acid (DHA) containing 100 mg α-tocopherol/kg (α-Toc100) or 500 mg α-tocopherol/kg (α-Toc500) for 4 wk to modify membrane fatty acid unsaturation, and then were administered ferric nitrilotriacetate (Fe-NTA). The mortality caused by Fe-NTA was higher in the group fed the DHA (α-Toc100) diet than in the BT diet groups but the DHA (α-Toc500) diet suppressed this increase. Serum and kidney α-tocopherol contents were slightly influenced by the dietary fatty acids but not significantly. These results indicate that the increased unsaturation of tissue lipids enhances oxidative damage induced by Fe-NTA in mice fed DHA (α-Toc100) but not when additional α-tocopherol is supplemented, The apparent discrepancy between the observed enhancement by dietary DHA of oxidative damage and the beneficial effects of dietary DHA on the so-called free radical diseases is discussed in terms of strong bolus oxidative stress and moderate chronic oxidative stress.

7) **Oh-hashi K., Takahashi T., Tanabe A., Watanabe S.,and Okuyama H.: Dietary α-Linolenate Suppresses Endotoxin-Induced Platelet-Activating Factor Production in Rat Kidney.Lipids 34, 31–37, 1999.**

Abstract: In comparison with dietary high-linoleate safflower oil, high α-linolenate perilla oil decreased alkylacyl- and alkenylacyl-glycerophosphocholine (CPC) content in rat kidney by roughly 30 and 25%, respectively. The fatty acid composition was also modified by high α-linolenate oil; arachidonic acid (AA) level in alkylacyl-GPC, a platelet-activating factor (PAF) precursor, decreased by 30% along with concomitant increases in the n-3 fatty acid levels. PAF contents under resting conditions were similarly low in the two dietary groups. Fifteen minutes after endotoxin administration, PAF and lyso-PAF contents increased significantly, and the PAF content in the high α-linolenate group was 60% lower than in the high linoleate group; the lyso-PAF contents also tended to be lower. Lyso-PAF acetyltransferase and CoA-independent transacylase activities in kidney microsomes increased significantly after endotoxin administration, while PAF acetylhydrolase activity in the cytosol was relatively unchanged. The lyso-PAF acetyltransferase and PAF acetylhydrolase activities did not differ between the two dietary groups, but the CoA-independent transacylase activity was roughly 30% lower in the high α-linolenate group. In agreement with in vitro study, our present study demonstrates that dietary high α-linole nate suppresses PAF production in rat kidney during systemic endotoxemia, and which is mainly due to the decrease in alkylacyl-GPC content, altered fatty acid compositions of the

precursor lipids and lower CoA-independent transacylase activity.

8) **Sato A., Osakabe T., Ikemoto A., Watanabe S., Kobayashi T., and Okuyama H.: Long-term n-3 Fatty Acid Deficiency Induces No Substantial Change in the Rate of Protein Synthesis in Rat. Brain and Liver. Biol. Pharm. Bull, 22 : 775–779, 1999.**

Abstract : The influence of long-term n-3 fatty acid deficiency on the rate of protein synthesis in rat brain and liver was investigated in relation to learning behavior or a presumed survival time-shortening factor (SSF) in rapseed oil, using a large-dose [^{3}Hlphenylalanine (Phe) injection method. When Wistar rats were made n-3 fatty acid-deficient by feeding a safflower oil (α-linolenate-deficient) diet for 2 generations, conditions under which the safflower oil group had been shown to exhibit altered learning behaviors, compared with the perilla oil group, no significant changes in the rate of protein synthesis were observed compared with the perilla oil (α-linolenate sufficient) or rapeseed oil (α-linolenate-sufficient but SSF-containing) groups. However, the rapeseed oil group had a reduced specific radioactivity of free Phe in the cerebral cortex, compared with the safflower oil group. In contrast to the reported observation of very long-term n-3 fatty acid deficiency inducing an almost 2-fold increase the rate of protein synthesis in the brain, our results indicate that altered learning behavior resulting from n-3 fatty acid deficiency in rats is not associated with any substantial changes in the rate of protein synthesis in the brain.

9) **Ikemoto A., Kobayashi T., Emoto K., Umeda M., Watanabe S., and Okuyama H.: Effects of Docosahexaenoic and Arachidonic Acids on the Synthesis and Distribution of Aminophospholipids during Neuronal Differentiation of PC12 Cells. Archives of Biochemistry and Biophysics. 364: 67-74, 1999.**

Abstract : We have shown previously that docosahexaenoic acid (DHA) promotes and arachidonic acid (AA) suppresses neurite outgrowth of PC12 cells induced by nerve growth factor (NGF) and that incorporation of [^{3}H]ethanolamine into phosphatidylethanolamine (PE) is suppressed in PC12 cells by AA while DHA has no effect. In the present study, the effects of these fatty acids on PE synthesis via decarboxylation of phosphatidylserine (PS), another pathway of PE synthesis, and distribution of aminophospholipids were examined. Incorporation of [3H]serine into PS and PE was elevated in the course of NGF-induced differentiation and was further stimulated significantly by DHA, but not by AA. [^{3}H]Ethanolamine uptake by PC12 cells was significantly suppressed by AA but not by DHA while these fatty acids did not affect [^{3}H]serine uptake, indicating that the suppression by AA of [^{3}H]ethanolamine incorporation into phosphatidylethanolamine is attributable, at least in part, to a reduction in [^{3}H]ethanolamine uptake. The distribution of PE in the outer leaflet of plasma membrane decreased during differentiation, which is known to be accompanied by an increase in the surface area of plasma membrane. Supplementation of PC12 cells with DHA or AA did not affect the distribution of aminophospholipids. Thus, DHA and AA affected aminophospholipid synthesis and neurite outgrowth differently, but not the

transport and distribution of aminophospholipids, while the PE concentration in the outer leaflet of the plasma membrane decreased in association with morphological changes in PC12 cells induced by NGF.

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◇講　演

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◇共同研究

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◇非常勤講師

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◇研究費取得状況

1）科学技術庁科学技術振興調整費（代表：浜崎智仁）：「老齢者の敵意性抑制効果を有する食品成分の探索」（第 3 年度）18,781千円
2）富山県受託研究費「和漢薬・バイオテクノロジー研究」（分担：浜崎智仁),「生体防御に有効な和漢薬の開発研究」, 434千円

3）科学研究費補助金（奨励研究(A)）（代表：渡辺志朗),「食餌 n-3 系不飽和脂肪酸が・型糖尿病に及ぼす影響とその機構の解析」, 1,600千円

4）平成11年度富山第一銀行奨学財団（代表：渡辺志朗）：「エイコサペンタエン酸及びドコサヘキサエン酸乳剤の新規臨床利用法の開発に関する基礎的研究」, 400千円

5）国際研究集会派遣研究費旅費（浜崎智仁）：「ω6 及びω3 系脂肪酸の必須性に関するワークショップ（ベセスダ, 米国)」

◇研究室在籍者

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