臨床利用部門　Department of Clinical Application

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◇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. n-3 系脂肪酸の抗ストレス作用と抗炎症作用，2. n-3 系脂肪酸の欠乏が行動に及ぼす影響，3. 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，4. 漢方方剤が脂質メディエーターに及ぼす影響。

◇研究概要　Research projects

1．n-3 系脂肪酸の一つ DHA の抗ストレス作用を利用して，小学生に DHA を投与することにより，敵意性が制御できるかを調べる二重盲検試験が終了し，現在データを解析中。前値だけでの解析では血中に n-3 系脂肪酸が少ないと攻撃性が増加することが分かった。口内炎のよくできるボランティアに家庭用の油を全てシソ油（n-3 系が多い）か大豆油にしてもらい，口内炎の発生頻度を調べる。第一回の 6 ヶ月間の介入試験では，大きな口内炎はシソ油で有意に減少している。規模を2倍以上にして第 2 回目が進行中。

2．n-3 系脂肪酸欠乏食がネズミの行動異常を起こすかを研究中。LPS 投与による motivated behavior の減弱が，飼料に α-リノレン酸を加えておくことにより抑えられることが判明した。

3．DHA あるいはミード酸をエサに加えることで，マウスの腹腔にマクロファージにおけるロイコトリエンあるいは PAF の産生が低下することが判明した。

4．抗アレルギー作用があるとされる漢方方剤に脂質メディエーターの産生抑制作用があるか否かを現在検討中。

◇著　書　Books

1）Hamazaki, T.:Effect of Docosahexaenoic Acid on Hostility. Eds:Hamazaki,T.,Okuyama,H. Fatty Acids and Lipids-New Findings, pp. 47-52, Karger, 2001.

2）浜崎智仁，中村典雄：改訂版/EPA は心臓を守る潤滑油. ハート出版，東京，2001.

3）渡辺志朗，浜崎智仁：EPAとDHA「プロスタグランジン研究の新展開」. 編集：室田誠逸，山本尚三，176-181，現代化学増刊 38・東京化学同人. 2001.

◇原著論文　Original papers

1）**Inagaki H., Kuroda M., Watanabe S.and Hamazaki T.: Changes in major blood components after adopting the supine position during haemodialysis. *Nephrol Dial Transplant*. 16 : 798-802, 2001.**

Abstract : Background. In Japan haemodialysis (HD) is usually performed with patients in the supine position. However, the effects of changing posture on major blood components have not been investigated in HD patients. It is possible that several fluid components change rapidly when patients change from the upright to the supine position. We therefore investigated the effects of posture on blood component analysis. Methods. A first blood sample was taken from 10 HD patients 5 min after they adopted a supine position; HD was begun immediately after sampling, Additional blood samples were collected 15 and 30 min later while patients remained in the supine position. On an alternate day, blood samples were taken from these same patients in the supine position, but not during HD. The same procedure was performed in 10 healthy volunteers. Results. Haematocrit significantly decreased in patients undergoing HD at 15 and 30 min into the HD session. Similar decreases were observed in HD patients not undergoing HD and in normal control subjects. Haematocrit changes at 15 min were not significantly different between the three groups. Serum albumin concentrations decreased in the same way as haematocrit. Consequently, the reductions in haematocrit and albumin concentrations in HD patients during the HD session were not attributable to the HD procedure or to end-stage renal disease, but rather were due to the supine position and consequent haemodilution caused by redistribution of water from the extra- to the intravascular space. Finally, WBC counts decreased significantly at 15 min in both HD patientgroups and in normal controls. The relative decrease at 15 min was significantly greater in HD patients undergoing HD (61.4% of baseline) than in those not undergoing HD (88.0%) or in normal controls (94.7%). These differences were probably due to previously reported WBC sequestration in the lungs during the early phase of HD. Conclusions. This study suggests that the change from the upright to the supine positions during HD causes changes in blood components that are critical for quality control determinations.

2）**Nakaya A., Wakabayashi H., Imamura L., Fukuta K., Makimoto S., Orihira T., Minemura M., Shimizu Y., Nagasawa T., Hamazaki T. and Watanabe A.: Helicobacter pylori alters n-6 fatty acid metabolism and prostaglandin E_2 synthesis in rat gastric mucosal cells. *Journal of Gastroenterology and Hepatology*. 16 : 1197-1205, 2001.**

Abstract : Background and Aims: Little is known about whether *Helicobacter pylori* infection alters fatty acid metabolism in gastric mucosal cells. By using cultured rat gastric mucosal cells (RGM-1), we investigated the effect of *H. pylori* broth culture filtrates on this point. Furthermore, our study aimed to find out whether n-6 long chain polyunsaturated fatty acids from linoleic acid are formed in RGM-1 cells. Methods: Rat gastric mucosal cells were incubated with 10, 20 and 40 μg/mL of linoleic acid or medium alone. Phosphatidylcholine content extracted from whole RGM-1 cells was quantitated by using a densitometer, and its fatty acid composition was analyzed by using gas chromatography. Prostaglandin E_2 concentration in the culture medium was measured by using radioimmunoassay. The expression of cyclooxygenase (COX)-1 and COX-2 was examined by using reverse transcription-polymerase chain reaction. In addition, after incubation with 〔1-^{14}C〕 linoleic acid, radioactivities of both linoleic acid and arachidonic acid components of the PC fraction were counted. The effects of *H. pylori* broth culture filtrates on PC

content, its fatty acid composition and prostaglandin (PG) E_2 synthesis were also assessed. Results: Linoleic acid addition caused an increase in the composition of arachidonic acid, as well as linoleic acid, and also in PGE_2 concentration. Cyclo-oxygenase-2 expression was induced in RGM-1 cell by the addition of linoleic acid. In addition, 〔1-^{14}C〕 linoleic acid added to the culture medium was converted to 〔1-^{14}C〕 arachidonic acid in RGM-1 cells. *Helicobacter pylori* broth culture filtrates decreased linoleic acid composition and increased arachidonic acid composition. Moreover, after incubation with *H. pylori* broth culture filtrates, PGE_2 concentrations were higher than that of the controls. Conclusions: These findings suggest the presence of fatty acid elongase and $\triangle^5$-and $\triangle^6$ desaturases synthesize arachidonic acid from linoleic acid in RGM-1 cells. Thus, *H. pylori* infection may enhance PGE_2 synthesis and accelerate n-6 fatty acid metabolism in gastric mucosal cells, which could make the gastric mucosal barrier more fragile.

3) **Watanabe S., Doshi M., Akimoto K., Kiso Y. and Hamazaki T.: Suppression of platelet activating factor generation and modulation of arachidonate metabolism by dietary enrichment with (n-9) eicosatrienoic acid or docosahexaenoic acid in mouse peritoneal cells. *Prostaglandins and other Lipid Mediators*: 66 : 109-120, 2001.**

Abstract : Several studies have shown that dietary n-3 polyunsaturated fatty acids (PUFAs) suppress platelet-activating factor (PAF) generation in leukocytes of humans and rodents, which is associated with the antagonism of arachidonic acid metabolism. Dietary eicosatrienoic acid (20 : 3n-9, ETrA) is also suggested to antagonize arachidonic acid (AA) metabolism, but its effect on PAF generation in leukocytes has not been defined. In the present study, we investigated the effects of an ETrA-rich diet on PAF generation and AA metabolism in mouse peritoneal cells, which were compared with those of a docosahexaenoic acid (DHA) - rich diet. Mice were fed a diet supplemented with a lipid preparation rich in ETrA, a DHA-rich fish oil (FO) or palm oil (PO) for 3 weeks, and peritoneal cells containing more than 80% of monocytes/macrophages were obtained. The peritoneal cells in the DHA and ETrA diet groups generated upon zymosan stimulation a smaller amount of PAF than cells in the PO diet group. In the peritoneal cells of the DHA diet group, AA contents in phosphatidylcholine (PC) and phosphatidylethanolamine (PE) were significantly lower than those in cells of the PO diet group, but those in phosphatidylinositol (PI) were not significantly different between the two dietary groups. A considerable amount of ETrA was incorporated into the peritoneal cells of the ETrA diet group, and AA was reduced as compared with the PO diet group. These changes occurred preferentially in PI but to a less extent in PC and PE. The amount of free AA released by the peritoneal cells upon zymosan stimulation was significantly reduced in the DHA diet group as compared with that in the PO diet group, whereas AA release was similar between the PO and ETrA diet groups. In conclusion, the effects of dietary ETrA on AA content in the phospholipid subclass and AA release were quite different from those of dietary DHA, although both diets suppressed PAF generation in mouse peritoneal cells to a similar extent.

4) **Yoshida S., Miyazaki M., Zhang QZ., Sakai K., Fujimoto I., Ikenaka K., Ikemoto A., Watanabe S. and Okuyama H.: Change of oligosaccharides of rat brain microsomes depending on dietary fatty acids and learning task. *J. Neurosci. Res.* 63, 185-195, 2001.**

Abstract : We have analyzed oligosaccharide chains in brain microsomes of rats fed an n-3 polyunsaturated fatty acid-deficient (safflower oil group; S group) or -rich (perilla oil group; P group) diet before and after brightness-discrimination learning tasks. The amount of concanavalin A-binding sites (mainly mannoside) of the brain microsomes was found to be significantly less in the S group than the P group before the learning task. Detailed analysis of glycoprotein glycans demonstrated that high mannose type oligosaccharides were dominant in brain microsomes before the learning task in both dietary groups, whereas multiantennary complex-type oligosaccharides became dominant after the learning task and especially a tetra-antennary glycan, that had a core structure of the

glycan of neural cell adhesion molecule, was more increased in the S-group than the P group. When polysialylated glycans were analyzed on serotonin-conjugated HPLC column, the glycans in the S-group microsomes before the learning task contained larger amount of higher affinity-polysialylated glycans to serotonin column than those in the P-group, and also contained larger amount of phosphoglycans that showed also high affinity to serotonin column than the P-group. Removal of mannoside from microsomes by -mannosidase-treatment changed the membrane surface physical property, especially permittivity, as revealed by analysis of the interaction with 1-anilinonaphthalene-8-sulfonate. These results suggest that high mannose content and several multiantennary glycans including polysialylated and phospho-glycans were changed by dietary n-3 fatty acid deficiency and learning task in rat brain microsomal glycoproteins and that these changes may affect membrane functions through changes of membrane surface physical properties and reactivity against serotonin.

5）**Du C., Sato A., Watanabe S., Ikemoto A., Fujii Y. and Okuyama H .: Effect of dietary oils enriched with n-3 fatty acids on survival of mice. *J. Nutr. Biochem.* 12 : 474-480, 2001.**

Abstract : Female mice were fed a conventional diet, shifted at 119 days of age to a diet supplemented with 10 wt % lard (Lar), high-linoleic (n-6) safflower oil (Saf), rapeseed oil (low-erucic, Rap), high--linolenic (n-3) perilla oil (Per) or a mixture (1:9) of ethyl docosahexaenoate (n-3) and soybean oil (DHA/Soy). Weight gain was less in the Per group than in the other groups at 497 days of age. In the Rap group, proteinuria was more severe than in the Saf, Per and DHA/Soy group, and hepatic triacylglycerol accumulation was greater than in the other groups. The mean survival time of the DHA/Soy group (753 days) was significantly longer than in the Lar group (672 days) and Saf group (689 days); the differences among other groups (e.g., 701 days in the Per group and 712 days in the Rap group) were not statistically significant. Although DHA is more susceptible to auto-oxidation than other major fatty acids in the air, an oil containing DHA was found to increase the survival of mice. Rapeseed oil that decreases the survival time of SHRSP rats was found to be safe in the mouse strain used in this study when survival was an end point.

◇総　説　Review papers

1）浜崎智仁：「二重盲検法を用いたヒトでの機能評価」. 食品機能研究法. 3-3-13：310-317, 2000.
2）浜崎智仁：「N-3 系多価不飽和脂肪酸と血液粘度」. FOOD STYLE 21. 2：58-61, 2001.

◇学会報告　Scientific presentation

1）浜崎智仁：N-3 系脂肪酸はキレを予防するか？ 第 4 回日本補完代替医療学会学術集会. 2001, 11, 11, 大阪.
2）道志勝, 渡辺志朗, 浜崎智仁, 秋元健吾, 木曽良信：ミード酸含有脂質の給餌がマウス腹腔細胞の血小板活性化因子（PAF）産生に及ぼす影響：日本薬学会 121年会 2001, 3, 28-30, 札幌.
3）道志勝, 渡辺志朗, 浜崎智仁：食餌 DHA によるマウス脳内 2-アラキドノイルグリセロールレベルの変動：第10回日本脂質栄養学会, 2001, 9, 7-8, 富山.

◇その他　Others

1）浜崎智仁, 澤崎茂樹：高齢者食品機能研究成果発表会「敵意性制御高価を示す食品成分の探索」. ここまでわかった！高齢者のための健康的で豊かな食生活.農水省食総研/科技庁. 84-90, 2000.
2）浜崎智仁：学術講演会「EPA と DHA の最近の研究」. やくせき. 19：50-55, 2001.
3）浜崎智仁：「魚の油 EPA と DHA の最近の研究」. 京都実地医報. 6-13, 2001.
4）浜崎智仁：「健康食品ノート/DHA」他 1 件, 2001, 4, 毎日新聞.
5）浜崎智仁：「DHAを摂るとキレない？」2001, 6, 15, MINATO.
6）浜崎智仁：「DHA関連記事」2001, 7, 5, 水産週報.

7）浜崎智仁：「第10回日本脂質栄養学会関連記事」2001, 8月号, 食生活.
8）浜崎智仁：「DHA関連記事」2001, 11, 25, 教育医事新聞.
9）浜崎智仁：講演「高脂血症治療について」国際シンポジウム 2001. 2001, 1, 27, 東京.
10）浜崎智仁：「21世紀における東洋医学教育, 研究, 診療のあり方と将来について」Kampo Medical Symposium 2001. 2001, 2, 3, 東京.
11）浜崎智仁：講演「魚油と健康」杏林大学学術フロンティア研究. 2001, 7, 21-22, 東京.
12）浜崎智仁：講演「魚の油と健康-心疾患から精神疾患までー」第13年度全国大学保健管理協議会東海・北陸地方部会/第28回保健婦・看護班研究集会. 2001, 7, 24, 富山.
13）浜崎智仁：「二重盲検法による口内炎の予防」和漢研夏期セミナー. 2001, 8, 7-9, 富山.
14）浜崎智仁：講演「生活習慣病について」富山市北保健福祉センター. 2001, 9, 13, 富山.
15）浜崎智仁：産業医認定研修会講演「職場における高脂血症対策」福山市医師会. 2001, 11, 19, 広島.
16）浜崎智仁：講演「さかなの油が健康に及ぼす影響」第22回鎌野会講演会. 2001, 11, 24, 東京.
17）浜崎智仁：講演「熟年のイライラ解消」富山市南保健センター. 2001, 12, 3, 富山.

◇共同研究　Co-operative researches

1）孫　月吉：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）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～
3）平山　論：倉敷市立短期大学「ADHD の治療研究」2001.6～

◇研究費取得状況　Acquisition of research funds

1）学術研究費助成（立仁会）(代表：浜崎智仁)：100千円
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（基盤研究(C)(2)）(代表：浜崎智仁),「α-リノレン酸によるアフタ性口内炎の予防研究（二重盲検法)」, 800千円
4）(社) 大日本水産会,水産物消費改善推進事業補助金（代表：浜崎智仁),「ドコサヘキサエン酸（DHA）含有食品が児童の及ぼす影響の研究」, 3,600千円

◇研究室在籍者　Research Members

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