それらの結果が出ることが待たれる．

VIII 乳酸菌製剤，オリゴ糖

腸内細菌を変化させることにより，大腸癌の発生を予防する研究が世界中で行われている．腸内細菌を変化させるためには，生菌製剤を投与する方法（プロバイオティクス）と，オリゴ糖などを投与して本来もっている腸内細菌のバランスを変える方法（プレバイオティクス）がある．動物実験で，乳酸菌やビフィズス菌を投与することにより大腸癌の発生を予防できることが多数報告されている．

人の研究でも，乳酸菌やビフィズス菌を含む発酵乳やヨーグルトが大腸癌を予防する可能性が，いくつもの調査で示されている．たとえば，フィンランドでは高脂肪食にもかかわらず，大腸癌が少ないのは，牛乳やヨーグルトなどを多く摂取しているからと考えられている．直接，腸内細菌叢を変えることを試みた臨床試験としては，イタリアでラクツロースを投与する臨床試験が行われ，大腸癌の前癌病変である腺腫の発生を軽度抑制することが報告されている[16]．

IX 食物繊維

以前には，食物繊維は大腸癌を予防する食品成分の一つとして知られていた．しかし，最近になり，食物繊維を摂取しても大腸癌が予防できないとする臨床試験の成績がいくつか続けて報告され，大腸癌の発生予防における食物繊維の位置づけが再検討されている．

1. 食物繊維に対する観察的疫学研究

食物繊維と大腸癌の関係は，1971年にBurkittがアフリカの人に大腸疾患が少ないのは食物繊維を多く含む食品を摂取しているからと考えたのが最初とされている．その後，各国の食物繊維の摂取量と大腸癌の発生率に負の相関があることがいくつも報告された．また，本邦では食物繊維の摂取量の減少とともに大腸癌の増加が認められている．世界13カ所の症例対照研究の成績を集計した検討では，食物繊維の摂取は大腸癌に対し予防的であることを示している[17]．しかし，コホート研究では，1989年の報告で食物繊維の摂取は大腸癌を弱く予防する関係が示されたが，その後に報告された代表的な研究では関連を認めないとするものが多い．

たとえば，1999年に報告された研究[18]では，34～59歳の米国の看護師88,757人に半定量食事頻度調査票を用いた食事内容の把握を1980年に行い，その後，16年間，追跡した研究である．この16年間に，787例の大腸癌，1,012例の大腸腺腫が診断されたが，食事調査で把握した食物繊維の摂取量と大腸癌，大腸腺腫の発生にはまったく関係はみられなかった．さらに，この集団と47,325人の男性医療専門家を対象に，野菜や果物に注目しても解析した報告[19]もあるが，それでも，野菜や果物を多く摂取しても大腸癌の発生を予防する傾向はみられなかった．しかし，ヨーロッパ8カ国での519,978人を10年間追跡する大規模コホート研究[20]では，食物繊維摂取量が最少の群（食物繊維の摂取量が1日平均，男性12.8g，女性12.6g）に対して，最大の群（食物繊維の摂取量が1日平均，男性35.6g，女性31.9g）では，大腸癌の相対危険度は0.75と有意に減少した．より信頼性が高い研究方法である大規模なコホート研究で，食物繊維と大腸癌の関係については異なった結果が相次いで報告されている．

2. 食物繊維に対する臨床試験

食物繊維を投与する臨床試験も複数，実施されている．食物繊維の大腸癌予防の効果を評価するために行われた無作為割付臨床試験の結果はこれまでに五つ報告されている．そのうち，一つの報告では食物繊維の投与により大きな大腸腺腫の発生がわずかに抑制されたが，二つで

は食物繊維の投与は大腸腫瘍の発生率に影響を見出せず，残りの二つでは，食物繊維の投与により，逆に大腸腺腫の発生が増加した．

Albertsらの研究[21]は米国で行われた研究であり，対象者は1,303人である．主エンドポイントである腺腫の発生の有無に差はなかったが，3個以上の腺腫を発生した者は高食物繊維投与群で有意に多かった．

Bonithon-Koppらの研究[22]は欧州で行われた研究であり，対象者は552人である．これらの参加者を，1日2gのカルシウムを投与するグループと，1日3.5gのサイリウムを投与するグループと，それらのプラセボのみを投与するグループの3群に分けた．なお，この論文に書かれているサイリウム（Ispaghula husk）とは，*Plantago ovata* の外皮の英国における薬局方名であり，オオバコの一種の種子から採った天然植物ガムで，インドでは便秘によく用いられている．きわめて保水性が高い．日本でも便秘薬として比較的容易に入手でき，一般にはサイリウムと呼ばれている．3年目の大腸内視鏡検査では，プラセボ群に比べ，カルシウム投与群では有意ではないものの腺腫の発生はオッズ比0.66と減少し，サイリウム投与群ではオッズ比1.67と有意に腺腫の発生が増加した．これら欧米諸国で行われた無作為割付臨床試験の成績からは，食物繊維は腺腫の発生を促進している可能性も考えられるため，大腸癌予防のために食物繊維のサプリメントを投与することは望ましくない．

X その他

βカロテンやビタミンEなどを用いた大腸癌予防のための介入試験が行われてきているが，これまで有効な報告は出ていない．最近になり，心疾患の予防のためにβカロテンやビタミンEを投与した対象者1,000人以上の12件の臨床試験について集計したところ[23]，βカロテンを服用したグループでは，投与していない群に比して死亡率が高いことが報告された．この結果より，βカロテンを用いた臨床試験は中止すべきとこの報告では結論されている．

これからも，大腸癌予防のための臨床試験が予定されているが，有害事象に関して十分に情報を収集し，慎重に行う必要がある．

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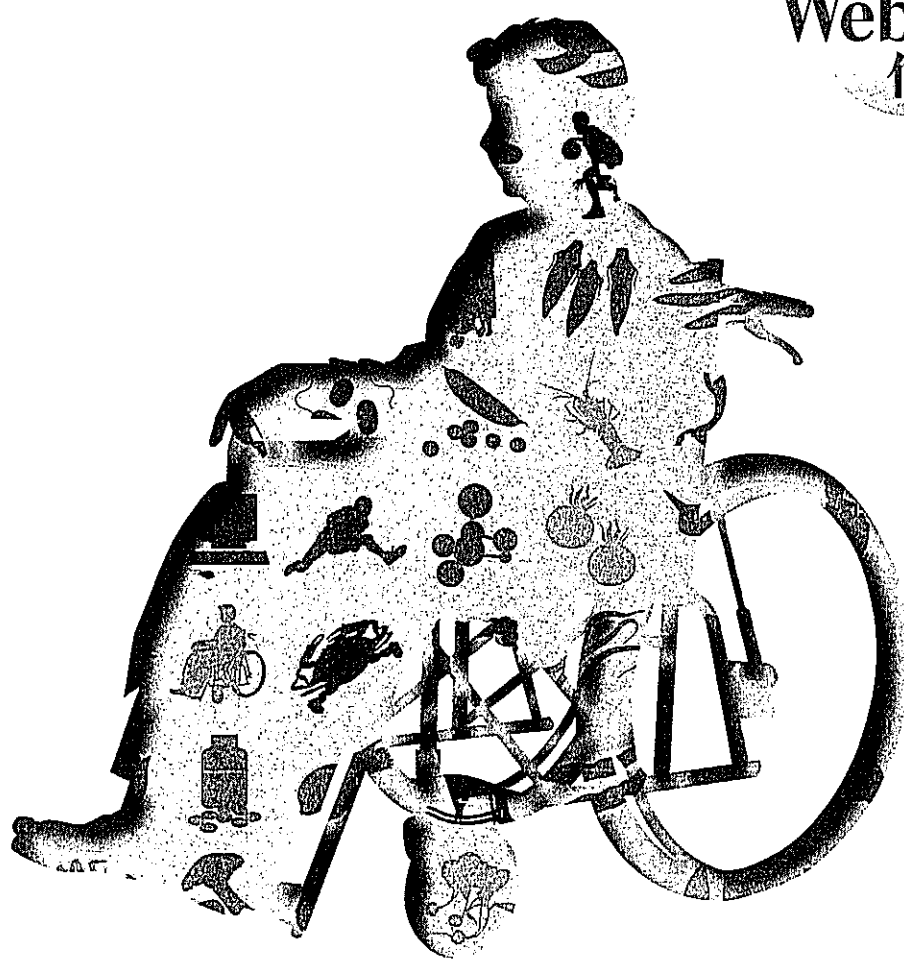

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（五十音順，◎印は編者）

総合判定

C．血液生化学検査および尿検査値

血清アルブミン値 _____ g / dℓ

リンパ球数 _____ / μℓ

総合判定

2．SOAP による指導記録を作成しなさい．

13.4 消化器外科疾患(胃がん術後:早期ダンピング症候群)

実践例

症　例：58 歳，男性，会社員

主　訴：食事直後の冷汗，動悸，下痢，嘔吐

現病歴：3 か月前の胃がん検診にて胃角部に早期胃がんと診断され，某病院にて広範囲胃切除，ビルロートⅡ法の手術を受けた．術後の経過も良好で，2 週間で退院し，通院治療を受けていた．

仕事に復帰し，徐々に食事の量を増やしていたが，術後 3 週間目頃から，食後 10 ～ 15 分程度すると，冷汗，動悸があり，同時に下痢と軽度の腹痛が起こるようになった．

S　昼食や夕食を食べた後，とくに丼物などを急いで食べると，食後 10 分程度で急に冷汗，動悸が起こる．同時に，排便をもよおし，下痢をすることが多い．また，軽い腹痛も起こり，吐いたこともある．

診察時に行った SGA による問診結果(表 13－7①)から，術後の影響による体重の減少が認められるが，栄養状態は良好と診断されている．

O　診察時の食事調査結果(表 13－7②)では，とくに異常は認められなかった．比較的しっかりとした食事をしている．間食が少なく，食べるのが早い傾向がみられた．

診察時の血液検査では，とくに異常所見はみられなかった．

身体計測の結果(表13－7③)では，術後の影響と考えられる軽度の皮下脂肪厚の減少が認められた．

A 術後の体重減少に伴う変化がみられる．食事調査からは各栄養素の過不足は認められず，血液検査からも貧血や栄養状態の不良は認められない．

術後に受けたであろう食生活指導について尋ねたところ，あまりしっかりとは覚えておらず，退院後，調子が良いため，術前と同じような食事をしていたとのことであった．

P 食事方法について，以下のような指導を行う．

・少量の食事を頻回に摂取する．間食を増やし，なるべくゆっくりと食べるようにする．食後，60分間程度は過度な運動などをしないようにする．

表13－7① 診察時栄養スクリーニング

◎主観的包括的評価(SGA)

A．病　歴

1．体重の変化

過去6か月間における体重喪失：＿3＿kg，喪失率＿5＿%

過去2週間における変化：増加＿＿無変化＿○＿　減少＿＿

2．食物摂取における変化(平常時との比較)

無変化　＿○＿

変化：(期間)＿＿＿(週)＿＿＿

タイプ：不十分な固形食＿＿完全液体食＿＿低カロリー液体食＿＿絶食＿＿

3．消化管症状(2週間の持続)

なし＿＿悪心＿＿嘔吐＿○＿下痢＿○＿食欲不振＿＿＿

4．機能性

機能不全なし＿○＿

機能不全：(期間)＿＿＿(週)＿＿＿

タイプ：制限つき労働＿＿＿歩行可能＿＿＿寝たきり＿＿＿

5．疾患，疾患と栄養必要量の関係

初期診断：＿＿＿

代謝亢進に伴う必要量／ストレス：なし＿○＿軽度＿＿＿中等度＿＿＿高度＿＿＿

B．身体(スコアで表示すること：0＝正常，1＋＝軽度，2＋＝中等度，3＋＝高度

皮下脂肪の喪失(三頭筋，胸部)＿1＋＿　筋肉喪失(四頭筋，三角筋)＿0＿

くるぶし部浮腫＿0＿　仙骨浮腫＿0＿　腹水＿0＿

C．主観的包括的評価

栄養状態良好　A＿○＿

中等度の栄養不良　B＿＿＿

高度の栄養不良　C＿＿＿

表13－7② 食事調査結果(1日あたり摂取量)

エネルギー	1,654 kcal	ビタミンA	4244 IU
蛋白質	72.0 g	ビタミンC	181 mg
脂　質	53.0 g	飽和脂肪酸	19.8 g
エネルギー比	28.8 %	一価不飽和脂肪酸	17.4 g
糖　質	212.8 g	多価不飽和脂肪酸	8.7 g
食　塩	13.0 g	P／S比	0.50
カルシウム	484 mg	n－6／n－3脂肪酸比	2.69
鉄	8.8 mg		

表13－7③　身体計測値

測定項目	測定値	標準値*
身　長	160 cm	
体　重	58.8 kg	
BMI	21.6	
体脂肪率	14.2 %	
上腕三頭筋部皮脂厚(TSF)	9.5 mm	10.6 ± 4.2
肩甲骨下部皮脂厚(BSF)	15.3 mm	18.3 ± 7.1
上腕囲(AC)	27.2 cm	27.3 ± 2.7
上腕筋囲(AMC)	22.4 cm	23.9 ± 2.6
下腿囲(BKC)	34.3 cm	33.9 ± 3.1
下腿筋囲(BKMC)	32.4 cm	
腹囲(W)	79 cm	
ヒップ囲(H)	95 cm	
W/H	0.83	

*JARD 2001，日本栄養アセスメント研究会

・高蛋白質食，高脂肪食，低炭水化物食で，水分の少ない乾燥した固形食を中心に摂取させる．
・食事方法を指導したところ，食後の冷汗，動悸，嘔吐は起こらなくなり，下痢も徐々に改善した．

演習

症例２(外来例)：57 歳，女性，主婦
診断名：胃がん術後
現病歴：２年４か月前の胃がん検診にて前庭部の進行胃がんと診断され，某病院にて広範囲胃切除，ビルロートⅡ法の手術を受けた．術後の経過も良好で，４週間で退院し，通院治療を受けていた．術後は自宅で家事をしながら養生を続け，状態も安定していたが，２か月前より食後２時間程度で全身倦怠感や脱力感，めまいが起こるようになった．腹痛や下痢などは起こらない．

A．身体計測値

身　長　　148.2 cm
体　重　　48 kg
アルコール歴　　なし
腹　水(－)

B．血液生化学検査値

血清総蛋白質　　7.1 g / dℓ
　アルブミン　　4.7 %
血清コレステロール　　198 mg / dℓ
空腹時血糖値　　108 mg / dℓ

① 考えられる病名を書きなさい.

② この疾患の食事指導方法を書きなさい.

13.5 モニタリングと評価

13.5.1 脳梗塞の患者

症 例：70歳，男性

診断名：脳梗塞

発症時より嚥下障害があるため経腸栄養法(経鼻チューブより 1,000 kcal / 日)で老人保健施設にて療養していた．脳梗塞発症前より痴呆症状がある．3 日前より 38 ℃ 台の熱発がみられ受診，診察の結果，肺炎と診断される．

(1) スクリーニング

(a) 身体計測

身長 165.0 cm，体重 46.0 kg，理想体重 59.9 kg，%IBW 76.8 %

(b) 身体所見チェック項目

◎皮膚の状態

□皮膚の落屑(微量元素／ビタミン類欠乏の疑い)

□乾燥，うろこ状皮膚(必須脂肪酸欠乏の疑い)

□パラフィン様皮膚(蛋白質不足の疑い)

◎全身浮腫著明(蛋白質不足の疑い)

◎口腔内の状態

□舌苔(ぜったい)あり(口腔内感染，誤嚥性肺炎の疑い)

◎毛髪

□ 抜けやすい(微量元素欠乏，蛋白質不足，ビタミン類欠乏の疑い)

◎バイタルサイン

□体温 38 ℃ 以上(侵襲因子増加 BEE × 1.4)

□呼吸回数 35 回(呼吸急迫)

□血圧 105 / 75 mmHg

□腸雑音微弱

◎消化器症状

□水様便(多種栄養素欠乏疑い)

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Randomized trial of dietary fiber and *Lactobacillus casei* administration for prevention of colorectal tumors

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The epidemiologic evidence that dietary fiber protects against colorectal cancer is equivocal. No large-scale clinical study of the administration of *Lactobacillus casei* has been reported. We examined whether dietary fiber and *L. casei* prevented the occurrence of colorectal tumors. Subjects were 398 men and women presently free from tumor who had had at least 2 colorectal tumors removed. Subjects were randomly assigned to 4 groups administered wheat bran, *L. casei*, both or neither. The primary end point was the presence or absence of new colorectal tumor(s) diagnosed by colonoscopy after 2 and 4 years. Among 380 subjects who completed the study, 95, 96, 96 and 93 were assigned to the wheat bran, *L. casei*, both and no treatment groups, respectively. Multivariate adjusted ORs for occurrence of tumors were 1.31 (95% CI 0.87–1.98) in the wheat bran group and 0.76 (0.50–1.15) in the *L. casei* group compared to the control group. There was a significantly higher number of large tumors after 4 years in the wheat bran group. The occurrence rate of tumors with a grade of moderate atypia or higher was significantly lower in the group administered *L. casei*. No significant difference in the development of new colorectal tumors was observed with administration of either wheat bran or *L. casei*. However, our results suggest that *L. casei* prevented atypia of colorectal tumors.
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Key words: colorectal cancer; *Lactobacillus casei*; dietary fiber; probiotic

The incidence of colon cancer is rapidly increasing in Japan.[1] It has been suggested that this trend is caused by the high-fat, low-dietary fiber diet resulting from Westernization of the lifestyle among Japanese. Indeed, intake of dietary fiber by the Japanese has decreased significantly over the past 10 years.[2]

Since Burkitt[3] proposed that a diet high in dietary fiber prevented colorectal cancer, basic studies have suggested the possibility of prevention of colorectal cancer by dietary fiber, through actions including absorption of carcinogens by insoluble dietary fiber[4] and dilution of bile acids and decrease of mutagenicity due to the increase in stool volume.[5,6]

A large number of case-control studies have suggested that dietary fiber may prevent the development of colorectal cancers.[7] However, reports of large-scale cohort studies have failed to show a preventive effect of dietary fiber against colorectal cancer, causing controversy.[8–11]

Randomized clinical trials have been conducted in Western countries[12–16] to evaluate the effectiveness of dietary fiber, using the development of colorectal adenoma as an end point. Many of these studies failed to prove that dietary fiber prevented the development of colorectal adenoma. No intervention study on dietary fiber has been reported in Asians.

It has been shown that *Lactobacillus casei* strain Shirota reduces DNA damage induced by chemical carcinogens in laboratory studies[17] and prevents carcinogenesis in animal experiments.[18,19] In addition, it has been reported, in humans, that lactobacilli reduce the level of mutagens in stool.[20] Furthermore, oral administration of *L. casei* strain Shirota preparation decreased the recurrence of superficial bladder cancer after transurethral resection,[21,22] and habitual intake of a fermented product with *L. casei* strain Shirota reduced the risk of bladder cancer in an epidemiologic study.[23] Thus, we decided to use a *L. casei* strain Shirota preparation in the present study. It has been suggested that high intake of yogurt and fermented milk is responsible for the low incidence of colon cancer in Finland, where consumption of fat is higher than in other countries.[24] Two case-control studies have shown that yogurt[25] and fermented milk[26] prevent colon cancer. In the Netherlands Cohort Study, it was reported that fermented milk intake showed an inverse relationship with the development of colon cancer, although there was no statistical significance.[27]

In 1993, we initiated a randomized clinical trial to determine whether dietary fiber from wheat bran and *L. casei* prevented the occurrence of colorectal tumors.

Material and methods

Study design and subjects

Part of the study design and methods have been previously described in detail.[28] Subjects were recruited at the Osaka Medical Center for Cancer and Cardiovascular Diseases between June 1993 and September 1997. The study protocol was approved by the Ethics Committee of the Osaka Medical Center for Cancer and Cardiovascular Diseases. Written informed consent was obtained from all subjects.

Inclusion criteria were men and women aged 40–65 years who had had at least 2 colorectal tumors (adenomas and/or early cancers) removed endoscopically within 3 months before recruitment. Endoscopic examination had been conducted twice, to detect and resect polyps, respectively. It must have been performed on the entire large intestine, and the subjects must have had an adequate nutritional status. Excluded were subjects with other malignant tumors, a history of intestinal or gastric resection (except appendectomy), familial adenomatous polyposis and severe illness.

Four regimens were incorporated for prevention of colorectal cancer: A, dietary instruction and regular intake of wheat bran biscuits; B, dietary instruction and regular intake of *L. casei* preparation; C, dietary instruction and regular intake of wheat bran biscuits and *L. casei* preparation; and D, dietary instruction alone.

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One of the 4 regimens was assigned randomly in advance each week. Physicians recruited subjects according to the regimen decided at the beginning of the week. Patients received consultation, including histologic diagnosis of the resected polyp, from group physicians as soon as possible from 1 week following endoscopic treatment. Since the regimen assignment could not be changed by the physicians or participants or arbitrarily manipulated by the authors, it was regarded as random. Trial physicians recruited all outpatients who met the inclusion criteria.

The amount of wheat bran biscuits and *L. casei* preparation to be consumed in 1 month was given to the participant at the start of the trial, and thereafter the amount for 3 months was given. The trial was started after confirming that the subject understood the procedure of the regimen. During the trial, consultation was performed every 3 months to check the participant's physical condition and to confirm the intake of wheat bran biscuits and the *L. casei* preparation. The regimen was continued for 4 years. Participants' compliance with taking wheat bran biscuits and/or the *L. casei* preparation was assessed at the consultations performed every 3 months. At each consultation, the numbers of unconsumed biscuit packages and silver packages of *L. casei* preparation were estimated by verbal inquiry of the patients, and the amounts of wheat bran biscuits and *L. casei* preparation taken in the past 3 months were calculated as the percentage of the target intake. Consultations were performed 16 times, every 3 months for 4 years, and the average at those 16 consultations was taken as the compliance over 4 years.

The target number of subjects was 100 in each group, for a total of 400 subjects. As the incidence of tumors in the control group can be estimated to be about 60%, a significant difference would be obtained if the occurrence rate of tumors could be reduced to 50% (suppression rate 17%) by administration of wheat bran or *L. casei* preparation. No midpoint analysis was performed.

Tumors discovered by colonoscopy performed at the end of the 2nd and 4th years were included in the analysis. The analysis included subjects with poor compliance with the regimen for intake of wheat bran biscuits and *L. casei* preparation on an intention-to-treat basis. For patients with early colorectal cancers resected during colonoscopy before entry in the trial, colonoscopy was performed 6 months after entry (35, 29, 25 and 24 subjects of groups A, B, C and D, respectively). Tumors discovered by colonoscopy performed at 6 months were excluded from analysis. Subjects who refused participation and dropouts were excluded from analysis.

Dietary instruction

The core purpose of the dietary instruction was to restrict fat intake so that the energy from fat constituted 18–22% of total energy intake. Subjects were asked to record, on a diet record form, the contents of their meals for the 3 days before consultation; and nutritionists calculated, from these records, the total energy intake and intake of fat and oil. Compliance with the dietary instructions on the restriction of fat intake was evaluated at dietary checkups 3 months and 4 years after beginning the regimen, and, when necessary, instruction was given again.

Wheat bran biscuits

Biscuits containing wheat bran at 30% of dry weight were prepared.[29] Patients were instructed to eat 25 g/day wheat bran biscuits (7.5 g as wheat bran) before each meal. Biscuits were developed by Ezaki Glico (Osaka, Japan) and Horii Pharmaceutical Industry (Osaka, Japan). The components and contents of the wheat bran biscuits were as follows: energy, 454 kcal/day; protein, 2.9 g/day; lipid, 3.3 g/day; and nonfibrous carbohydrate, 17.5 g/day.

L. casei *preparation*

The *L. casei* strain Shirota preparation was a powder containing approximately 10^{10} viable cells/g. It was stored in a refrigerator, and 1 g was taken after every meal. The *L. casei* preparation was provided by Yakult Honsha (Tokyo, Japan). The viable cell count of *L. casei* and absence of bacterial contamination were confirmed for all lots every 6 months during the 2-year storage period. To confirm the viable cell count of *L. casei*, MRS agar medium for detection of *L. casei* was used. It has been confirmed in previous studies that the number of bacteria per 1 g of *L. casei* preparation remained in the range of 1.5×10^9 to 2.1×10^{10} during 24 months when stored in a cool place (15°C). In addition, the average number of bacteria is 8.0×10^9 after 24 months.

Colonoscopy

The main end point of the trial was the presence or absence of new colorectal tumor(s). Colonoscopy was performed 2 and 4 years after the start of the regimen. The entire large intestine, from the anus to the cecum, was examined. Examinations for detection of new lesions were performed by 2 physicians. All lesions, except hyperplastic polyps clearly evaluated by colonoscopy, were examined histologically on the basis of the guidelines of the Japanese Society for Cancer of the Colon and Rectum.[30] All histologic diagnoses (inflammatory polyp; hyperplastic polyp; adenoma with mild atypia, with moderate atypia, with severe atypia; early cancer) were performed blindly without identification of the participant's dietary regimen.

In patients with early colorectal cancer, which was diagnosed from tumor tissue resected by colonoscopy before entry in the trial, colonoscopy was performed to detect local recurrence after 6 months of participation. All colorectal tumors discovered with this procedure were resected.

Statistical analysis

All colorectal tumors discovered at the end of the 2nd and 4th years were defined as "new". Analyses at years 2 and 4 were performed separately, and 2×2 contingency table analysis was performed. Comparison of baseline characteristics of subjects with or without wheat bran biscuits or *L. casei* intake was performed by appropriate tests such as *t*-test and the χ^2 test. Logistic regression models were used to estimate the odds ratio (OR) adjusted for covariates such as age and sex. Confidence intervals (CIs) based on Wald statistics were used to assess significance.

Results

Enrollment and randomization

The number of patients who met the inclusion criteria during the screening period was 470 (Fig. 1). All were invited to participate in the trial, but 60 patients (13%) declined. Of 410 patients who agreed to participate, 12 were excluded because of incompatibility with the protocol, including detection of cholangiocarcinoma and gastric cancer in 4, history of gastrectomy in 3, colectomy in one, familial adenomatous polyposis in one, advanced age in one, young age in one and more than 3 months after endoscopic treatment in one. Thus, 398 patients were assigned to the 4 groups.

Baseline characteristics of subjects

Table I shows the baseline characteristics of the 398 patients randomly assigned and the number of dropouts. There was no difference in baseline characteristics of subjects such as dietary content among the 4 groups. A total of 18 patients (4.5%) did not complete endoscopic examinations. The reasons for not receiving endoscopic examinations were death in 2 patients (from lung cancer and cerebral hemorrhage), serious illness in 5 patients and trial discontinuation in 11 patients. There was no difference in the rate of dropouts among all groups. Excluding 18 dropouts, 380 patients were included in the analysis.

Colonoscopy

Colonoscopic examination was possible throughout the length of the large intestine, up to the cecum, in all cases. There was no difference in the intervention period among groups (Table II).

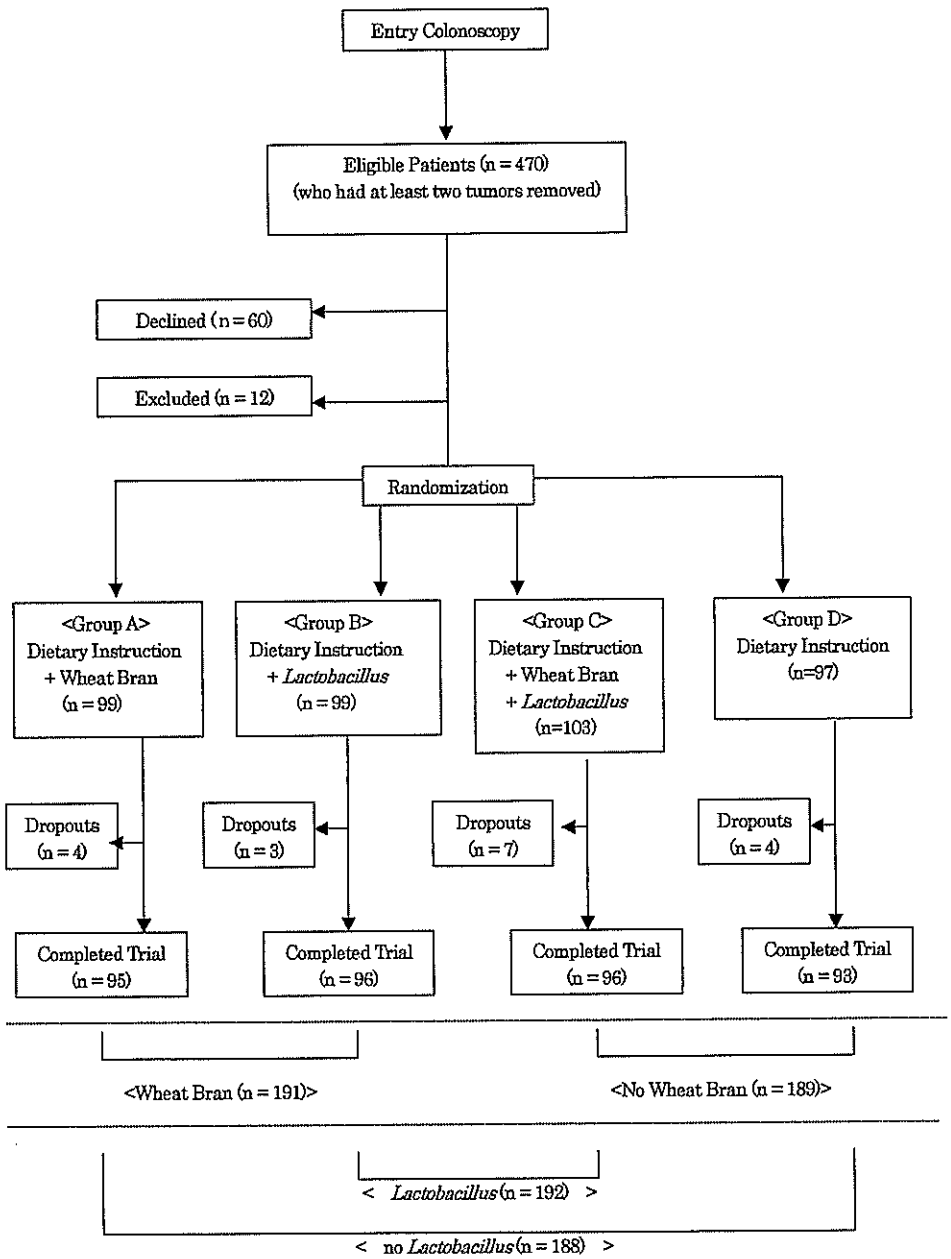

FIGURE 1 – Study participant data.

There was no difference in the time required for insertion into and removal from the cecum in all groups. No difference was found in the proportion of nonneoplastic lesions (inflammatory polyps and hyperplastic polyps).

Compliance

Compliance with intake of wheat bran biscuits was over 90% in 77 persons (40%) and over 70% in 135 persons (71%) for the entire 4 years. Compliance with intake of the *L. casei* preparation was over 90% in 130 persons (68%) and over 70% in 168 persons (88%).

Occurrence of tumors

The results after intake of wheat bran biscuits are shown in Table III. The wheat bran biscuit administration group included 191 persons, while the nonadministration group included 189 persons. The adjusted OR of developing at least one tumor was 1.31 (95% CI 0.87–1.98) after 2 years in the administration group compared to the nonadministration group. It was 1.31 (95% CI 0.87–1.97) after 4 years, showing some increase in adjusted OR, although not statistically significant. The adjusted OR for developing tumors larger than 3 mm was 1.14 (95% CI 0.76–1.72) in the administration group compared to the nonadministration group after 2 years and 1.57 (95% CI 1.04–2.37) after 4 years, showing a significant increase. In particular, the occurrence of tumors larger than 10 mm after 2 years showed no difference between the wheat bran administration and nonadministration groups. However, after 4 years, these tumors did not occur in the nonadministration group while they occurred in 7 patients (3.7%) in the administration group, showing a significant increase. There was no difference in the occurrence of more than one or more than 3 tumors with moderate or severe atypia.

Table IV shows the results after *L. casei* administration. The *L. casei* administration group included 192 persons and the nonad-

TABLE I – BASELINE CHARACTERISTICS OF SUBJECTS[1]

	Group A: wheat bran (n = 99)	Group B: *Lactobacillus* (n = 99)	Group C: wheat bran + *Lactobacillus* (n = 103)	Group D: no treatment (n = 97)
Age (years)	54.7±6.1	54.8±6.5	54.9±6.2	55.5±6.2
Male sex, number (%)	83 (83.8)	79 (79.8)	80 (77.7)	83 (85.6)
Height (cm)	164.6±8.4	164.6±7.3	163.0±7.1	164.1±7.6
Weight (kg)	66.1±10.5	64.6±10.5	62.7±8.7	63.2±9.4
Dietary intake				
Energy (kcal/day)	2,075±368	2,066±411	2,058±402	2,163±404
Total fat (g/day)	54.6±15.2	53.0±13.5	52.8±16.7	56.6±14.9
Dietary fiber (g/day)	15.1±3.9	14.5±3.9	15.4±4.7	15.5±4.0
Calcium (mg/day)	635.4±237.1	638.7±218.4	636.6±246.7	661.4±247.7
Alcohol drinking every day, number (%)	50 (50.5)	37 (37.4)	49 (47.6)	48 (49.5)
Current smoker, number (%)	47 (47.5)	41 (41.4)	43 (41.7)	44 (45.4)
Tumors before recruitment				
Total tumors	5.9±4.3	5.8±5.6	5.2±3.6	5.0±3.4
Adenomas with mild atypia	2.8±2.9	2.7±3.9	2.5±2.6	2.0±2.7
Adenomas with moderate atypia	2.1±2.2	2.4±2.1	1.9±1.8	2.0±1.9
Adenomas with severe atypia	0.6±0.8	0.4±0.7	0.5±1.1	2.0±1.9
Early cancers, number (%)	37 (37.4)	31 (31.3)	29 (28.2)	28 (28.9)
History of colorectal cancer in one parent or sibling, number (%)	15 (15.2)	8 (8.1)	15 (14.6)	11 (11.3)
Dropped out, number (%)	4 (4.0)	3 (3.0)	7 (6.8)	4 (4.1)

[1]Values are means ± SD.

TABLE II – INTERVENTION PERIOD OF COLONOSCOPY

Intervention period	Group A: wheat bran (n = 95)	Group B: *Lactobacillus* (n = 96)	Group C: wheat bran + *Lactobacillus* (n = 96)	Group D: no treatment (n = 93)
Period of 2nd year from entry (days)				
Mean ± SD	679.4±60.8	674.2±31.0	672.1±27.6	680.3±56.9
Maximum	1,009	827	778	925
Minimum	568	617	600	617
Period of 4th year from entry (days)				
Mean ± SD	1,339.6±46.9	1,339.7±51.1	1,338.1±40.5	1,367.4±120.4
Maximum	1,611	1,660	1,617	2,129
Minimum	1,275	1,275	1,233	1,201

ministration group, 188 persons. The adjusted OR of developing at least one tumor was 0.76 (95% CI 0.50–1.15) in the administration group compared to the nonadministration group after 2 years. After 4 years, it was 0.85 (95% CI 0.56–1.27), showing a decrease after both 2 and 4 years, although not statistically significant. For the occurrence of tumors with moderate or severe atypia, the adjusted OR was 0.80 (95% CI 0.52–1.22) in the administration group compared to the nonadministration group after 2 years and 0.65 (95% CI 0.43–0.98) after 4 years, showing a significant decrease after 4 years. There was no difference in the size and number of new tumors that developed.

When the results were examined separately for the different levels of compliance, they were similar to those described above.

Synergistic effects

Tumor occurrence in the group administered both wheat bran and *L. casei* was higher than that in the groups administered wheat bran or *L. casei* and lower than that in the nonadministered group (data not shown). No notable synergistic effects between the treatments were observed.

Adverse events

During the study period, colorectal cancers were discovered in 4 persons by endoscopy, including one person each in groups B, C and D after 2 years and one person in group B after 4 years. There was no bias in their occurrence among the groups. All were cancer invasion of mucosa and were completely resected endoscopically, not requiring colectomy. During the study period, 2 patients died, one of lung cancer in group A and one of cerebral hemorrhage in group C. One person each in groups A and C underwent surgery for peritonitis resulting from acute appendicitis. There was no other serious adverse event.

Discussion

It was found that *L. casei* intake appeared to suppress the development of colorectal tumors; in particular, it prevented, with statistical significance, the development of tumors with moderate and severe atypia. This large-scale randomized clinical study shows that an *L. casei* preparation prevented the development of colorectal tumors.

Since our study was performed at one hospital, the evaluations of endoscopic findings were thought to be consistent. All patients who satisfied the conditions for participation were asked to participate, and the rate of consent to participation was extremely high at 88%, supporting the high validity of the results. The reasons for the high rate of consent could be that a special organization was instituted in this hospital for this trial and that all participants were offered dietary instruction. In addition, the dropout rate was low at 4.5% and compliance was high, indicating that the results were highly reliable. Endoscopic examination was conducted twice before entry so that we could minimize oversights.

In our previous prevention studies, subjects were patients with at least one tumor, whereas the present study included patients with at least 2 tumors. It is known that patients with at least 2 tumors in the large intestine have a higher risk of colon cancer than those with only one tumor. It is difficult to apply the results of our clinical study to the general population. Many of the patients participating in this trial, different from other reports in the past, had a larger number of colorectal tumors together with a history of cancer. This difference appears to have resulted from the background of the population, who had a higher risk of colorectal cancer than those participating in previous clinical trials. Accordingly, our results should be discussed not on the basis of the general population but on the basis of a population with a high risk of colorectal cancer. Nonetheless, our study included patients

TABLE III – RISK OF TUMOR OCCURRENCE WITH WHEAT BRAN BISCUITS

	Year	Wheat bran (groups A + C) (n = 191)	No treatment (groups B + D) (n = 189)	Crude		Adjusted	
				relative risk	(95% CI)	OR	(95% CI)[1]
Number of tumors							
At least one	2	119 (62.3%)	106 (56.1%)	1.11	(0.94–1.31)	1.31	(0.87–1.98)
	4	106 (55.5%)	93 (49.2%)	1.13	(0.93–1.37)	1.31	(0.87–1.97)
≥ 2	2	57 (29.8%)	60 (31.7%)	0.94	(0.70–1.27)	0.92	(0.60–1.43)
	4	51 (26.7%)	53 (28.0%)	0.95	(0.69–1.32)	0.95	(0.60–1.50)
≥ 4	2	11 (5.8%)	14 (7.4%)	0.78	(0.36–1.67)	0.78	(0.34–1.76)
	4	11 (5.8%)	12 (6.3%)	0.91	(0.41–2.00)	0.91	(0.39–2.13)
Size of largest tumor (mm)							
≥ 3	2	95 (49.7%)	88 (46.6%)	1.07	(0.87–1.32)	1.14	(0.76–1.72)
	4	97 (50.8%)	76 (40.2%)	1.26	(1.01–1.58)	1.57	(1.04–2.37)
≥ 4	2	51 (26.7%)	52 (27.5%)	0.97	(0.70–1.35)	0.97	(0.61–1.54)
	4	52 (27.2%)	51 (27.0%)	1.01	(0.73–1.40)	1.02	(0.65–1.60)
≥ 10	2	4 (2.1%)	4 (2.1%)	0.99	(0.25–3.90)	1.00	(0.25–4.06)
	4	7 (3.7%)	0 (0.0%)	—	$p < 0.01$[2]		
Atypia of tumors							
≥ With moderate	2	64 (33.5%)	66 (34.9%)	0.96	(0.73–1.27)	0.94	(0.61–1.44)
	4	77 (40.3%)	74 (39.2%)	1.03	(0.80–1.32)	1.06	(0.70–1.60)

[1]OR of recurrent tumors in the wheat bran biscuits group compared to the no treatment group, adjusted for age, sex and *Lactobacillus* group.–[2]χ^2 test.

TABLE IV – RISK OF TUMOR OCCURRENCE WITH *LACTOBACILLUS* PREPARATION

	Year	*Lactobacillus* (groups B + C) (n = 192)	No treatment (groups A + D) (n = 188)	Crude		Adjusted[1]	
				relative risk	(95% CI)	OR	(95% CI)
Number of tumors							
At least one	2	107 (55.7%)	118 (62.8%)	0.89	(0.75–1.05)	0.76	(0.50–1.15)
	4	96 (50.0%)	103 (54.8%)	0.91	(0.75–1.11)	0.85	(0.56–1.27)
≥ 2	2	56 (29.2%)	61 (32.4%)	0.90	(0.66–1.22)	0.88	(0.57–1.36)
	4	53 (27.6%)	51 (27.1%)	1.02	(0.73–1.41)	1.08	(0.68–1.71)
≥ 4	2	10 (5.2%)	15 (8.0%)	0.65	(0.30–1.42)	0.67	(0.29–1.53)
	4	15 (7.8%)	8 (4.3%)	1.84	(0.79–4.23)	1.98	(0.81–4.83)
Size of largest tumor (mm)							
≥ 3	2	86 (44.8%)	97 (51.6%)	0.87	(0.70–1.07)	0.77	(0.51–1.15)
	4	83 (43.2%)	90 (47.9%)	0.90	(0.72–1.13)	0.85	(0.56–1.28)
≥ 4	2	41 (21.4%)	62 (33.0%)	0.65	(0.46–0.91)	0.56	(0.35–0.89)
	4	58 (30.2%)	45 (23.9%)	1.26	(0.90–1.76)	1.38	(0.87–2.19)
≥ 10	2	4 (2.1%)	4 (2.1%)	0.98	(0.45–3.86)	1.01	(0.25–4.12)
	4	4 (2.1%)	3 (1.6%)	1.31	(0.30–5.75)	1.29	(0.28–6.00)
Atypia of tumors							
≥ With moderate	2	61 (31.8%)	69 (36.7%)	0.87	(0.65–1.14)	0.80	(0.52–1.22)
	4	66 (34.4%)	85 (45.2%)	0.76	(0.59–0.98)	0.65	(0.43–0.98)

[1]OR of recurrent tumors in the *Lactobacillus* group compared to the no treatment group, adjusted for age, sex and wheat bran biscuit group.

with at least 2 tumors for the following reasons: *(i)* it is more efficient for the analysis of preventive methods against colon cancer to use subjects in higher-risk groups and *(ii)* since the occurrence rates of colon tumors after 2 and 4 years were higher in patients in the high-risk group, a preventive effect would be more prominent in this group.

The weak point of this trial is that it was not a double-blind study. Therefore, there could be bias from the fact that the participants and medical professionals did know the group to which each participant belonged. However, since it is widely believed in Japan that dietary fiber prevents colorectal cancer and nobody would think that dietary fiber would cause tumors to enlarge, it is highly unlikely that the unexpected results obtained in this study were biased. Histologic evaluations were performed blindly, without group identification, by pathologists. Therefore, there is unlikely to be a bias resulting from this not being a double-blind study in the result that administration of *L. casei* prevented the development of tumors with moderate or severe atypia.

The occurrence of tumors larger than 4 mm was significantly suppressed by *L. casei* administration after 2 years but not after 4 years. This might have resulted from a suppressive effect of *L. casei* administration against enlargement of colon tumors lasting for only a limited period. At the present time, it is not clear how *L. casei* influences the early stages of tumor development. We are planning to examine the effect of *L. casei* administration on cellular proliferation histopathologically, to find the best administration method that will clearly show a suppressive effect on tumor development.

Although clinical studies on the administration of *L. casei* for the prevention of colorectal tumors have not been reported, there are a few reports of clinical studies aimed at changing the intestinal flora. Roncucci *et al.*[31] reported that lactulose appeared to slightly suppress the development of colorectal tumors, although without statistical significance.

The *L. casei* preparation used in our study was a quality-controlled homogeneous live preparation. *L. casei* survives well in gastric acid[32] and is used as an intestinal conditioning agent in Japan. It is known to augment immunity[33] and inhibit enzyme activity involved in carcinogenesis.[17] It has been reported to suppress the development of colorectal tumors in rats.[34]

The mechanism of the suppression by *L. casei* of the development of colorectal tumors with moderate or severe atypia is not clear. Further analyses are in progress examining stools, colonic mucous membrane and serum collected from patients who participated in this study.

Several similar studies from Western countries have reported that dietary fiber supplementation did not prevent or promote the

development of colorectal tumors. In the clinical study by Bonithon-Kopp *et al.*,[16] dietary fiber-rich psyllium significantly increased the development of adenomas after 3 years as analyzed by endoscopy (OR = 1.67), consistent with our results. Alberts *et al.*[15] reported, from a clinical study with large and small quantities of wheat bran cereal, that there was no difference in the development of adenomas but that the number of patients who developed at least 3 adenomas was significantly higher in the high-dietary fiber group. Since it was found in a previous study[29] that the diets of participants were changed by administration of a large quantity of dietary fiber, the quantity of dietary fiber was lower in the present study than that used in other studies. To target the high-risk group for colorectal cancer, patients with multiple colorectal tumors were included as subjects. In spite of these differences from previous studies, the development of colorectal tumors was not prevented by dietary fiber also in this study.

Thus, there has been no consensus on the efficacy of dietary fiber against colorectal cancer. From the results of our study as well as the previous results of supplementation studies, it is not recommended to take supplements containing a high concentration of dietary fiber for the prevention of colorectal cancer.

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J Toxicol Pathol 2005; 18: 53–59

Original

Lack of Chemoprevention or Promotion Effects of Docosahexaenoic Acid on Small Intestine, Colon, Liver, Lung, Thyroid, Esophagus, Kidney, and Forestomach Carcinogenesis in a Rat Medium-Term Multi-Organ Carcinogenesis Model

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Abstract: Modifying effects of docosahexaenoic acid (DHA) were examined using a medium-term multi-organ carcinogenesis model (DMBDD model). Groups of twenty F344 male rats were treated sequentially with *N*-diethylnitrosamine (DEN, i.p.), *N*-methyl-*N*-nitrosourea (MNU, i.p.), 1,2-dimethylhydrazine (DMH, s.c.), *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN, in drinking water) and dihydroxy-di-*N*-propylnitrosamine (DHPN, in drinking water) during the first 4 weeks (DMBDD treatment), and then DHA-ethyl ester (DHA-E), DHA-triglyceride (DHA-TG) and/or tocopherol were administered intragastrically 3 times a week for 31 weeks. Significant inhibition of the development of glutathione *S*-transferase placental form (GST-P) positive foci was observed in DMBDD treated 30% DHA-TG 404 mg and 128 mg + tocopherols groups and with tocopherol alone; however, this appeared to be due to the tocopherol. DHA treatment did not influence the development of aberrant crypt foci in the large intestine. Histopathologically, the incidences of preneoplastic and neoplastic lesions in other organs were also not increased or decreased by DHA treatment. Thus, the results indicate a lack of chemopreventive and tumor promotion effects of any type of DHA in male rats under the present experimental conditions. (J Toxicol Pathol 2005; **18**: 53–59)

Key words: docosahexaenoic acid, medium-term multi-organ carcinogenesis model, F344 rat, promotion

Introduction

The n-3 polyunsaturated fatty acid, docosahexaenoic acid (DHA) is a major component of fish oil, which has been frequently reported to have chemopreventive potential for colon, mammary gland and pancreas carcinogenesis in rats[1–6]. For example, DHA was found to suppress aberrant crypt foci (ACF) in the colon induced by azoxymethane (AOM) or 1,2-dimethylhydrazine (DMH)[1,3]. Furthermore, induction of ACF by the heterocyclic amine, 2-amino-1-methyl-6-phenylimidazo[*4,5-b*]pyridine (PhIP), was also inhibited by DHA treatment[4]. Furthermore colon cancer multiplicity was significantly decreased in another study[2,3]. In the mammary gland, development of tumors was also reduced by a low dose of DHA or eicosapentaenoic acid (EPA) treatment after carcinogen (DMBA) injection[6]; however, in a clinical trial with familial adenomatous polyposis (FAP) patients a high risk group for colorectal cancer, it was without major influence[7]. The three FAP patients were administered concentrated DHA in fish oil capsules (2.2 g of DHA-TG and 0.6 g eicosapentaenoic acid (EPA) per day) for one or two years. The patients with FAP developed endometrial cancer after 12 months, colon cancer after 24 months and lung cancer after 12 months, respectively[7].

It is well established that a chemical may act as a tumor inhibitor in one organ and as a promoter in others[8–10]. It is

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Table 1. Fatty Acid Contents for a Rat in Dosing Solvent (mg)

Groups		16:0 Palmitic acid	18:0 Stearic acid	18:1 Oleic acid	18:2 Linoleic acid	20:1 Gadoleic acid	20:4(n-6) AA	20:5 EPA	22:5 DPA	22:6 DHA	Other FA	tocopherol	Total
1,6	128 mg 97% purify DHA-E	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	120.0	4.0	4.0	128.0
2,7	404 mg 30% purify DHA-TG	56.8	13.6	74.0	5.2	10.0	8.0	32.8	12.0	113.2	74.4	4.0	404.0
3	128 mg 30% purify DHA-TG	17.6	4.2	22.9	1.6	3.1	2.5	10.2	3.7	35.1	23.1	4.0	128.0
4	4 mg tocopherol	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	4.0

AA: arachidonic acid. EPA: eicosapentaenoic acid. DPA: docosapentaenoic acid. FA: Fatty acid.

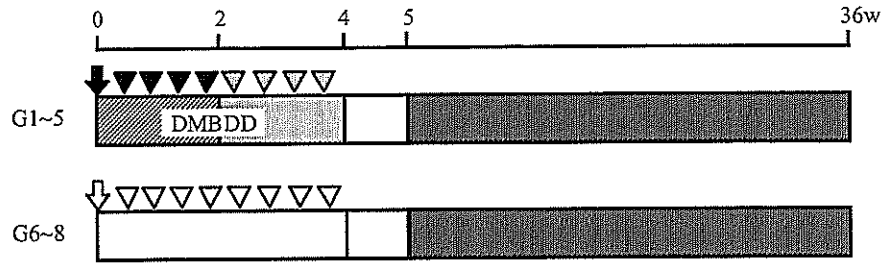

Fig. 1. Experimental protocol for the medium-term multi-organ carcinogenesis model. Animals: male F344/DuCrj rats, 6 weeks old; ▼, DEN, 100 mg/kg body wt. i.p.; ▼, MNU, 20 mg/kg body wt. i.p.; ▽, DMH, 40 mg/kg body wt. s.c.; ▨, BBN, 0.05% in drinking water; ▤, DHPN, 0.1% in drinking water; ⇩▽, saline injection; ▩, G1 and 6, 128 mg 97% purity DHA-E, G2 and 7, 404 mg 30% purity DHA-TG, G3, 128 mg 30% purity DHA-TG, G4, 4 mg tocopherol, G5 and 8, no treatment.

therefore important to examine modification potential not in a single organ, but rather in the whole body. This requires *in vivo* experimental models which can detect effects in a wide spectrum of organs, and for this purpose several multi-organ wide-spectrum initiation models have been established[11–14]. The medium-term approach has clear benefits for the examination of modifying effects of chemicals in multiple organs in a single experiment within a relatively short experimental period[15–17] and is based on the proven good agreement between the multi-organ carcinogenesis model and long-term experimental results[18].

The ethyl ester formed by DHA (DHA-E) has been used in many chemoprevention studies[1–6], and DHA-TG has been used in a clinical trial study[7]. Therefore, we thought it important to investigate the difference in the modifying effects on carcinogenesis of DHA-E and DHA-TG. In the present study, we investigated the post-initiation-phase modifying activity of DHA-E and DHA-TG at the whole organ level using a rat medium-term multi-organ carcinogenesis model developed in our laboratory[8,15,19,20]. Furthermore, a tocopherol group was included as a comparative control.

Materials and Methods

Animals

Male F344 rats, aged 5 weeks, were obtained from Charles River Japan Inc. (Kanagawa Japan), and housed five to a plastic cage with wood chips for bedding in an air-conditioned room at 22 ± 2°C with a 12-h light: 12-h dark cycle. They were maintained on Oriental MF diet (Oriental Yeast Co., Tokyo, Japan) and tap water *ad libitum*. The study was started after 1 week of acclimatization.

Chemicals

N-Diethylnitrosamine (DEN), *N*-methyl-*N*-nitrosourea (MNU), 1,2-dimethylhydrazine (DMH) and *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN) were obtained from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan) and dihydroxy-di-*N*-propylnitrosamine (DHPN) was obtained from Nacalai Tesque Co. (Osaka, Japan). The DHA dosing solution was supplied by Nippon Suisan Kaisha, Ltd. (Tokyo, Japan). DHA naturally exists in fish oil as a triglyceride (DHA-TG). DHA-E was chemically synthesized from DHA-TG by removing other fatty acids such as oleic acid and EPA. The contents in the dosing solution used in the present study are shown in Table 1. They were stored in sealed ampules under anaerobic conditions at –20°C in the dark.

Experimental methods

Medium-term multi-organ carcinogenesis study

The experimental protocol is shown in Fig. 1. The animals were randomly allocated to 8 groups of 10 – 20 animals. Those in groups 1 to 5 received the combined carcinogen treatments, consisting of a single *i.p.* injection of 100 mg/kg body wt. of DEN, four *i.p.* injections of 20 mg/kg body wt. of MNU, four *s.c.* injections of 40 mg/kg body wt.

Table 2. Final Body and Organ Weights Data

Groups	DMBDD	Treatment	Effective no. of rats	Body wt.[a] (g)	Liver wt.[a] (g)	Liver wt.[a] (%, b.w.)	Kidneys wt.[a] (g)	Kidneys wt.[a] (%, b.w.)
1	+	128 mg 97% DHA-E	19	312.4 ± 14.8[b]	6.60 ± 0.45	2.11 ± 0.08	2.00 ± 0.44	0.64 ± 0.15
2	+	404 mg 30% DHA-TG	17	318.6 ± 15.5	7.01 ± 0.51	2.23 ± 0.11[c][d]	3.12 ± 3.15	1.00 ± 1.00
3	+	128 mg 30% DHA-TG	19	306.8 ± 20.5[c]	6.53 ± 0.59	2.13 ± 0.09	2.03 ± 0.65	0.67 ± 0.25
4	+	4 mg Tocopherol	18	314.8 ± 24.7	6.74 ± 0.54	2.14 ± 0.10	1.97 ± 0.22	0.62 ± 0.06
5	+	no treatment	19	324.6 ± 18.2	6.83 ± 0.47	2.10 ± 0.08	2.03 ± 0.36	0.63 ± 0.11
6	–	128 mg 97% DHA-E	10	356.6 ± 18.2	7.56 ± 0.71	2.13 ± 0.24	1.93 ± 0.16	0.54 ± 0.02
7	–	404 mg 30% DHA-TG	10	373.0 ± 11.7	7.80 ± 0.24	2.09 ± 0.06	2.05 ± 0.11	0.55 ± 0.03
8	–	no treatment	10	369.5 ± 14.9	7.61 ± 0.36	2.06 ± 0.05	2.00 ± 0.11	0.54 ± 0.03

a) Mean ± SD.
b), c) Significantly different from group 5 at $P<0.05$ and 0.01, respectively.
d) Significantly different from group 4 at $P<0.05$.

of DMH, together with 0.05% BBN for 2 weeks and then 0.1% DHPN for 2 weeks (both given in the drinking water), during the initial 4 week period for multiple initiation (DMBDD treatment) as described previously[21–23]. Animals in groups 1 to 5 were then given intragastric injections, 1 ml of 128 mg/ml of 97% purity DHA-E, 404 mg/ml of 30% purity DHA-TG, 128 mg/ml of 30% purity DHA-TG, each with 4 mg/ml of tocopherol, or tocopherol alone or distilled water, 3 times a week from 1 week after completion of the DMBDD treatment to the end of the experiment. Animals in groups 6 to 8 were given 128 mg/ml 97% purity DHA-E, 404 mg/ml 30% purity DHA-TG and distilled water as a solvent control without DMBDD treatment from week 5. The treatment times per week and concentration of DHA dosing solution were decided according to a trial study[7]. Animals were weighed once a week in the initial 14 weeks, then once every 2 weeks until the end of the study period, at week 36, when all surviving animals were sacrificed by exsanguination under ether anesthesia and subjected to complete necropsy.

All experimental procedures were performed in accordance with the in-house guideline for the Care and Use of Laboratory Animals at DIMS Institute of Medical Science.

Aberrant crypt foci assay

Nine or 10 rats for each treatment with DMBDD initiation and 5 rats each without DMBDD were analyzed for colon ACF. The colon was removed, slit open from the anus to the cecum along the longitudinal axis, flattened between sheets of filter paper, and fixed in buffered 10% formalin. Then it was stained with 0.2% methylene blue solution by the procedure of Bird[24] to observed aberrant crypts. The number of aberrant crypt foci per colon, the number of aberrant crypts in each focus, and the location of each focus was determined by microscopy.

Histopathological examination

At necropsy, the brain, liver, kidneys, spleen, heart, lungs, thymus, testes and adrenals were excised and weighed, and the relative percentage organ weights were calculated on the basis of final body weights. These and the other major organs including small and large intestines were fixed in 10% buffered formalin, and routinely processed. Paraffin-embedded sections were stained with hematoxylin and eosin for histopathological examination. Liver slices fixed in 10% buffered formalin were also prepared for quantitative assessment of immunohistochemically demonstrated glutathione *S*-transferase placental form (GST-P) positive foci, as previously described[25]. GST-P positive foci larger than 0.2 mm in diameter and the total areas of the liver sections examined were quantitated using a video image processor (SPICCA-II, Nippon Avionics, Tokyo, Japan) and the data expressed as numbers and areas (mm^2) per unit area of the liver section (cm^2).

Statistical analysis

The significance of intergroup differences in numerical data obtained for body and organ weights was assessed using the two-tailed Student's t-test. Insufficient homogeneity of variance was corrected with respect to the degrees of freedom according to the method of Welch. The significance of differences in the incidences of histopathological findings between treated and control groups was evaluated using Fisher's exact probability test.

Results

No post-initiation treatment-related clinical signs or mortalities were noted in any of the groups in the current experiment. Eight rats were found dead in the course of study, one in group 1, three in group 2, one in group 3, two in group 4 and one in group 5, and the deaths were all considered to have been caused by the DMBDD treatment.

The average body weights of rats in the DMBDD treated groups were significantly less than in the non-DMBDD initiated groups, throughout the study period. After DMBDD initiation, 30% DHA-TG was associated with retardation of body weight increase from week 7. The body weights in the other DMBDD treated groups were not

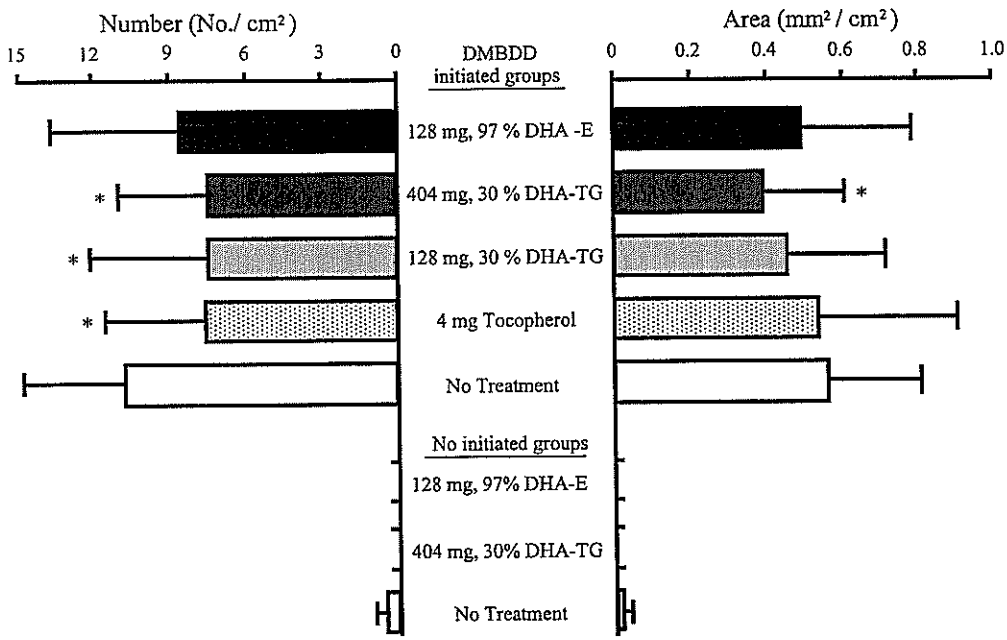

Fig. 2. Areas and numbers of GST-P positive foci in rat livers. * P<0.05 versus DMBDD initiated no treatment group.

Table 3. Number of ACF in Rats Treated with and without DHA during the Post-Initiation Stage

Groups	DMBDD	Treatment	Effective no. of rats	ACF / Colon			AC / Focus
				< 4 crypts	4 crypts≤	Total	
1	+	128 mg 97% DHA-E	9	27.1 ± 6.9	6.2 ± 3.9	33.3 ± 9.7	2.5 ± 0.3
2	+	404 mg 30% DHA-TG	9	27.0 ± 14.3	6.2 ± 6.1	33.2 ± 18.3	2.3 ± 0.4
3	+	128 mg 30% DHA-TG	9	26.6 ± 7.5	7.9 ± 4.6	34.4 ± 11.6	2.5 ± 0.4
4	+	4 mg Tocopherol	9	25.9 ± 16.9	5.2 ± 3.8	31.1 ± 19.3	2.4 ± 0.3
5	+	no treatment	10	26.8 ± 10.1	8.2 ± 5.7	35.0 ± 13.5	2.7 ± 0.4
6	–	128 mg 97% DHA-E	5	0.3 ± 0.5	0	0.2 ± 0.5	0.4 ± 0.9
7	–	404 mg 30% DHA-TG	5	0.3 ± 0.5	0.2 ± 0.5	0.4 ± 0.6	1.4 ± 0.2
8	–	no treatment	5	0.3 ± 0.5	0	0.2 ± 0.5	0.2 ± 0.5

AC: aberrant crypts.

significantly changed.

A significant increase in relative liver weight and a tendency to increase in relative kidney weights were noted in group 2 (Table 2).

Quantitative analysis of GST-P positive foci (Fig. 2) showed the numbers and areas were significantly decreased by the 404 mg/ml 30% DHA-TG treatment. The numbers were also suppressed by 128 mg/ml 30% DHA-TG and tocopherol alone.

No significant difference was observed in ACF between DHA and/or tocopherol treatment groups and the DMBDD alone group (Table 3).

Histopathological examination revealed hyperplastic and neoplastic lesions in various organs/tissues in the rats initiated with the five carcinogens (Tables 4, 5). However, no DHA treatment-related alteration in their incidences was evident. No proliferative lesions were noted in any of the rats given DHA and tocopherol without DMBDD treatment.

Discussion

The present investigation of the modifying potential of DHA in a rat medium-term multi-organ carcinogenesis model found no modifying effects on lesion development in any organ. Decreases of number and/or area of GST-P positive foci in the liver given 404 mg and 128 mg 30% DHA-TG were demonstrated, but similar results were obtained with tocopherol alone, so the latter was considered responsible, in line with its reported inhibitory potential[26,27].

The effect of dietary sardine oil including 28.5% DHA on rat hepatocarcinogenesis was examined with administration in the initiation and post-initiation period[28]. The sardine oil inhibited the number of DEN-induced GST-P positive foci when administered in the initiation period, but enhanced the area of GST-P positive foci when administered in the post-initiation period. However, in another study, fish oil inhibited AOM-induced GST-P positive foci in the post-

Table 4. Incidences of Neoplastic Lesions in the Large and Small Intestines

Groups	DMBDD	Treatment	Effective no. of rats	Small intestine		Large intestine	
				Adenoma	Adenocarcinoma	Adenoma	Adenocarcinoma
1	+	128 mg 97% DHA-E	20	0	1 (5)	1 (5)	1 (5)
2	+	404 mg 30% DHA-TG	20	2 (10)	4 (20)	0	1 (5)
3	+	128 mg 30% DHA-TG	20	1 (5)	1 (5)	2 (10)	1 (5)
4	+	4 mg Tocopherol	20	1 (5)	1 (5)	1 (5)	1 (5)
5	+	no treatment	20	2 (10)	2 (10)	1 (5)	1 (5)
6	–	128 mg 97% DHA-E	10	0	0	0	0
7	–	404 mg 30% DHA-TG	10	0	0	0	0
8	–	no treatment	10	0	0	0	0

Table 5. Incidences of Preneoplastic and Neoplastic Lesions in Other Organs in DMBDD Treated Groups

Organ / Findings		DMBDD treatment				
		128 mg 97% DHA-E	404 mg 30% DHA-TG	128 mg 30% DHA-TG	4 mg Tocopherol	No treatment
No. of rats examined		20	20	20	20	20
Spleen:	Hemangioma	0	0	0	0	1 (5)
Thyroids:	Follicular cell hyperplasia	13 (65)	17 (85)	10 (50)	12 (60)	10 (50)
	Follicular cell adenoma	4 (20)	10 (50)	9 (45)	5 (25)	6 (30)
	Follicular cell carcinoma	5 (25)	7 (35)	6 (30)	5 (25)	5 (25)
Nasal cavity:	Hyperplasia	17 (85)	19 (95)	17 (85)	20 (100)	20 (100)
	Adenoma	2 (10)	0	0	1 (5)	0
Lung:	Alveolar hyperplasia	20 (100)	20 (100)	20 (100)	20 (100)	20 (100)
	Adenoma	8 (40)	6 (30)	8 (40)	10 (50)	8 (40)
	Adenocarcinoma	1 (5)	4 (20)	2 (10)	3 (15)	4 (20)
Tongue:	Squamous cell hyperplasia	0	0	2 (10)	1 (5)	0
	Papilloma	0	0	0	1 (5)	0
Esophagus:	Squamous cell hyperplasia	18 (90)	17 (85)	19 (95)	20 (100)	17 (85)
	Papilloma	0	2 (10)	0	0	1 (5)
Stomach:	Squamous cell hyperplasia	10 (50)	8 (40)	12 (60)	12 (60)	13 (65)
	Squamous cell papilloma	3 (15)	1 (5)	2 (10)	0	3 (15)
	Squamous cell carcinoma	0	1 (5)	0	1 (5)	0
Liver:	Hepatocellular adenoma	3 (15)	1 (5)	2 (10)	1 (5)	5 (25)
	Hepatocellular carcinoma	0	0	0	1 (5)	0
Kidneys:	Atypical tubules	11 (55)	13 (65)	7 (35)	10 (50)	9 (45)
	Renal cell hyperplasia	0	0	1 (5)	0	0
	Transitional cell hyperplasia	6 (30)	8 (40)	4 (20)	4 (20)	8 (40)
	Renal cell adenoma	1 (5)	1 (5)	3 (15)	1 (5)	1 (5)
	Nephroblastoma	5 (25)	7 (35)	6 (30)	4 (20)	10 (50)
	Transitional cell carcinoma	0	3 (15)	0	0	1 (5)
Urinary bladder:	Simple hyperplasia	12 (60)	12 (60)	14 (70)	13 (65)	11 (55)
	PN hyperplasia	4 (20)	2 (10)	4 (20)	3 (15)	3 (15)
	Papilloma	0	0	0	0	1 (5)
	Transitional cell carcinoma	1 (5)	1 (5)	0	1 (5)	0
Other site:	Histiocytic sarcoma	0	0	0	1 (5)	0
	Leiomyosarcoma	0	0	0	1 (5)	1 (5)
	Malignant lymphoma/ leukemia	1 (5)	1 (5)	1 (5)	1 (5)	0

initiation stage[29]. These results suggest that the effects of fish oil appear to be dependent on the types of carcinogens. In the present study, DHA did not enhance hepatocarcinogenesis initiated with five carcinogens.

Some previous studies indicated the chemopreventive effect of DHA on colon[1–4], mammary glands[6] and pancreas carcinogenesis[5] in rats. DHA exerted significant inhibitory effects on implanted tumor growth and metastasis to the lungs in a subcutaneously implanted and highly metastatic colon carcinoma model[30]. However, no chemoprevention was observed for rat colon and other organ carcinogenesis with DHA treatment in the present study. The reason for the discrepancy with the many previous studies which showed chemopreventive effects on colon carcinogenesis and ACF development[1–4,27,31,32], may be due to the different number of treatment times per week. DHA was injected five times a