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Organotropic Chemopreventive Effects of *n*–3 Unsaturated Fatty Acids in a Rat Multi-organ Carcinogenesis Model

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Organotropic chemopreventive effects of *n*–3 unsaturated fatty acids were studied using a multi-organ carcinogenesis model in male rats. Rats were treated with diethylnitrosamine (DEN), N-methyl-N-nitrosourea (MNU), N-butyl-N-4-hydroxybutylnitrosamine (BBN), 1,2-dimethylhydrazine (DMH) and dihydroxy-di-*n*-propylnitrosamine (DHPN) during the first 7 weeks, and then given unsaturated fatty acid (UFAs), docosahexaenoic acid (*n*–3, $C_{22:6}$) (DHA), eicosapentaenoic acid (*n*–3, $C_{20:5}$) (EPA), linoleic acid (*n*–6, $C_{18:2}$) (LA) or oleic acid (*n*–9, $C_{18:1}$) (OA) at a dose of 1.0 ml/rat, 3 times a week by gavage for the consecutive 30 weeks. All rats were fed a low LA basal diet throughout the experiment and a calorie-restricted basal diet during the period of UFAs feeding administration. DHA significantly reduced tumor size and numbers in the large intestine as compared to OA treatment. Furthermore, DHA showed a tendency to inhibit carcinogenesis in the small intestine and lung. EPA also showed a tendency to inhibit intestinal carcinogenesis. On the other hand, LA showed a tendency to inhibit lung carcinogenesis, but to promote large intestinal carcinogenesis. However these UFAs did not influence preneoplastic and neoplastic lesion development in the liver, kidney, and urinary bladder. Levels of the administered fatty acids were clearly increased in the serum and organs. In contrast, arachidonic acid (AA) levels in the large and small intestines and liver were markedly decreased by treatment with DHA and EPA. Decreased levels of AA in the large intestine correlated well with tumor incidence, although the number of glutathione S-transferase-positive ($GST\text{-}P^+$) foci showed an inverse correlation with AA levels. The data thus provide evidence that an organotropism exists with regard to the influence of UFAs on carcinogenesis, which correlates with reduction of tissue AA levels in the target organs.

Key words: Organotropic chemopreventive effects — Rat — *n*–3 Unsaturated fatty acids — Docosahexaenoic acid — DHA

Epidemiological studies have indicated that a high fat diet is associated with increased incidences of colon, prostate and mammary cancers.[1)] From animal studies, unsaturated fatty acids (UFAs) are known to modify carcinogenesis of various organs.[2)] Fish oils rich in docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) were shown to inhibit colon, mammary gland and pancreas tumor development in rats.[3–8)] For practical use of DHA and EPA as chemopreventive agents, it is important to know whether their action is limited to target organs without adverse promotion or carcinogenic effects in other organs. However, the results from the majority of studies have been limited to single organotropism, because the studies were conducted using carcinogens featuring a single target organ or a very limited range of target organs. Therefore, it is probable that inhibitory or even adverse promoting effects in other organs may have been overlooked.[9–12)]

In the present study, for elucidating the organotropic inhibitory effects of three different UFAs, DHA, EPA and linoleic acid (LA), in comparison with oleic acid (OA), an established multi-organ carcinogenesis model using neoplasia and preneoplasia as end-point lesions was chosen.[13, 14)] OA is considered to be an ineffective fatty acid for carcinogenesis. The target organs included were small and large intestines, liver, lung, kidney, urinary bladder and forestomach. Furthermore, levels of various fatty acids including arachidonic acid (AA) in the serum, liver and intestines were measured to look for any correlation to modification influence on tumor development.

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MATERIALS AND METHODS

Experiment 1 Male 5-week-old F344 rats (Charles River Japan, Inc., Atsugi) were divided into 3 groups. Group 1 rats were initially treated with a single dose of diethylnitrosamine (DEN, 100 mg/kg, i.p.) followed by 6 doses of N-methyl-N-nitrosourea (MNU, 20 mg/kg, i.p.) and administration of N-butyl-N-4-hydroxybutylnitrosamine (BBN, 0.05% in drinking water) during weeks 1 and 2, then 6 doses of 1,2-dimethylhydrazine (DMH, 60 mg/kg, i.p.) and exposure to 2,2′-dihydroxy-di-*n*-propylnitrosamine (DHPN, 0.1% in drinking water) during weeks 3 to 5 (Fig. 1). All carcinogens were purchased from Tokyo Chemical Industry Co., Ltd., Tokyo. Starting 1 week later, animals were given ethyl ester of DHA (*n*−3, $C_{22:6}$), EPA (*n*−3, $C_{20:5}$) or LA (*n*−6, $C_{18:2}$) (97% purity, Sagami Chemical Research Center, Sagamihara) 3 times a week by gavage at a dose of 1.0 ml/rat for the following 30 weeks. Group 2 received ethyl ester of OA (*n*−9, $C_{18:1}$) (98% purity, Wako Pure Chemical Industries, Ltd., Osaka) using the same protocol in group 1. Group 3 animals were treated with DHA, EPA, LA or OA without the prior carcinogenic regimen. Since LA is known to affect carcinogenesis in several organs, LA content in the basal diet was reduced.[3, 4] Animals were fed a modified AIN93M basal diet, in which soy bean oil (LA approximately 55% of total fatty acids content) was replaced by rape seed oil (LA approximately 20.93%) resulting in an LA content of 2 to 0.8% of total fatty acids, throughout the experimental period (Table I).

During the period of UFAs administration, animals were fed a calorie-restricted modified AIN93M basal diet so as to adjust the calorie intake equivalent to the amount given by fatty acids (6−8 kcal/g for 2-day-period), by reducing the corn starch content (Table II). This adjustment facilitates avoidance of modification of carcinogenesis by overdose of fatty acids.

The liver, lungs and kidneys were fixed in buffered formalin and processed for paraffin embedding. The liver was immunostained for placental-form glutathione S-trans-

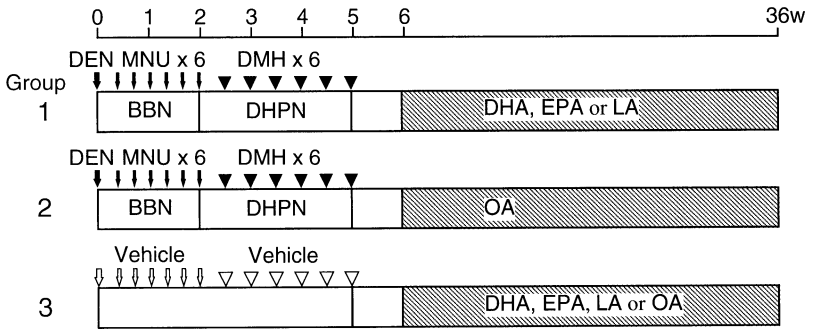

Fig. 1. Five-week-old male F344 rats were used. DEN, diethylnitrosamine (100 mg/kg, i.p.); MNU, N-methyl-N-nitrosourea (20 mg/kg, i.p., 6 times every other day); DMH, 1,2-dimethylhydrazine (60 mg/kg, s.c., 6 times every 3 days); BBN, N-butyl-N-4-hydroxybutylnitrosamine (0.05% in drinking water); DHPN, 2,2′-dihydroxy-di-*n*-propylnitrosamine (0.15% in drinking water); ▭, modified AIN93M basal diet (soy bean oil was replaced by rape seed oil); ▨, calorie adjusted modified AIN93M basal diet (by reducing corn starch). Unsaturated fatty acids were given at 1 ml/rat, 3 times weekly.

Table I. Comparison of Constituent Fatty Acids in the Diets (%)

16:0 Palmitic acid	18:0 Stearic acid	18:1 (*n*−9) Oleic acid	18:1 (*n*−7) Vaccenic acid	18:2 Linoleic acid	18:3 Linolenic acid	20:4 Arachidonic acid	20:5 EPA	22:5 DPA	22:6 DHA	Others
Modified AIN93M basal diet										
4.52	2.17	56.37	3.36	20.93	7.45	0.00	0.00	0.00	0.00	5.20
Calorie adjusted modified AIN93M basal diet										
4.84	1.86	56.04	2.79	21.75	7.80	0.00	0.00	0.00	0.00	4.92
Conventional basal diet (CE2)										
15.6	1.77	20.38	1.42	48.13	3.61	0.32	2.37	0.18	2.70	4.06

Constituent fatty acids in the diet. The LA content is reduced to a half in the modified AIN93M basal diet by replacement with rape seed oil. DPA, docosapentaenoic acid.

Table II. Diets

Ingredient	Modified AIN93M basal diet (%)	Calorie adjusted modified AIN93M basal diet (%)
Casein	14.0	14.0
Corn starch	46.6	36.7
α-Corn starch	5.5	6.4
α-Potato starch	10.0	6.0
Sucrose	10.0	8.0
Fat (Rape seed oil)	4.0	4.0
Vitamin mix	1.0	1.0
Mineral mix	3.5	3.5
Others	0.4	0.4
Energy value	360 kcal/100 g	300 kcal/100 g

ferase-positive (GST-P$^+$) foci, a marker lesion of rat liver preneoplasia, then quantitatively measured using an image analyzer (IPAP system, Sumika Technoservice Co., Ltd., Osaka), GST-P$^+$ foci larger than 0.2 mm in diameter were counted in sections on slide glass.[8, 9] The large intestine was inflated with the buffered formalin then opened longitudinally and spread on filter papers. The urinary bladder was also inflated with buffered formalin. Values of group 1 treated with DHA, EPA and LA were compared to those treated with OA in group 2 in terms of incidence and/or multiplicity (number of lesions/rat) of preneoplastic and neoplastic lesions in the intestine, liver, kidney, lung and urinary bladder. Statistical analysis was performed with the χ^2 test for incidence and Student's *t* test for multiplicity.

Experiment 2 Male 5-week-old F344 rats were treated with ethyl ester of DHA, EPA, LA or OA, at 1.0 ml/rat every other day for 4 weeks as in experiment 1, then sacrificed. The serum, and part of the liver, small and large intestines were immediately taken, wrapped in aluminum foil and frozen in liquid nitrogen. The intestine was washed with saline to remove mucus and stool before freezing. Samples were stored at −80°C until use.

Lipid extraction and fatty acid analysis Total lipids were extracted from lyophilized tissues as reported previously.[15] Briefly, 1.5 ml of a chloroform-methanol mixture (1:2) and 0.5 ml of distilled water, followed by chloroform (0.5 ml), were added to each tumor (about 100 mg). The residual lipids in the aqueous phase were extracted twice with 0.5 ml of chloroform. The extracts were pooled and evaporated to dryness at 37°C under nitrogen. Methyl esters of fatty acids were formed by adding 1 ml of methanolic hydrochloric acid (8%) to lipid extracts in Teflon-coated screw-capped culture tubes for 60 min at 100°C. They were then extracted three times with *n*-hexane, the organic phase was evaporated under nitrogen and the extracts were stored at −4°C. Residues were dissolved in 50 μl of *n*-hexane and the fatty acid methyl esters were isolated by thin layer chromatography (#5721, Merck, Darmstadt, Germany), developed with *n*-hexane:diethyl ether:acetic acid, 80:20:1,v/v/v, and rendered visible with primurin (Sigma Chemical Co., St. Louis, MO) solution to locate their positions for further extraction with *n*-hexane. Gas liquid chromatography of tumor samples was performed as reported previously.[15]

RESULTS

Experiment 1 The combined incidence of adenomas and carcinomas of the large intestine in the DHA treatment group, 3/10 (30%), was significantly decreased as compared to the OA treatment group, 12/16 (75%). Similarly, the number of combined adenomas and carcinomas per rat in the DHA treatment group, 0.40±0.66, was significantly decreased as compared to the OA value of 1.38±1.32 ($P<0.05$). Incidences and multiplicity of the tumors in rats treated with EPA tended to decrease (Table III). In the small intestines, the incidences and multiplicities of carcinomas in the DHA and EPA-treated groups showed a tendency to decrease as compared to the value for the OA treatment group (Table IV). In the liver, numbers and areas of GST-P$^+$ foci per unit area (cm^2) were not different between treatment and control groups (Table V). In the lung, incidences of adenomas in DHA and LA treatment groups tended to decrease as compared to the OA treatment group (Table V). Incidences and multiplicities of kidney lesions including renal epithelial cell hyperplasias and adenomas,[16] transitional cell carcinomas and mesenchymal-type nephroblastomas[17] in treatment groups did not show any difference as compared to the OA treatment group (Table VI). In the urinary bladder, lesions including simple hyperplasias, papillary or nodular hyperplasias (PNH) and papillomas[9, 14] in the DHA and EPA treatment groups did not show significant difference as compared to the OA group (Table VII).

Experiment 2 Levels of fatty acids in the serum, small and large intestines and liver after oral treatment with UFAs were measured. These fatty acids were well absorbed into the blood. However, total fatty acids in the serum in groups treated with DHA, EPA and LA were significantly decreased as compared with the untreated control group (917, 810, 925 μg/ml, respectively, vs. 2410 μg/ml, $P<0.001$). EPA level in the serum of the group treated with OA was very low, but markedly increased after treatment with EPA. Moreover, treatments with EPA and DHA caused remarkable decreases in LA and AA levels ($P<0.001$) (Table VIII). In the large intestine, however, total fatty acids were at similar levels (DHA, 45.5; EPA, 44.1; LA, 44.0 and untreated control, 55.5 mg/g tissue) (Table IX). AA level was significantly decreased by treatment with DHA and EPA ($P<0.05$). Conversely, treatment with LA caused a significant increase of AA ($P<0.05$).

Table III. Effects of DHA, EPA and LA on Tumor Induction in the Large Intestine

Group		Treatment	No. of rats	No. of rats with			No. of tumors/rat		
				AD	CAR	AD+CAR	AD	CAR	AD+CAR
1. Ca.	→	DHA	10	1 (10)[a)]	3 (30)	3 (30)*	0.10±0.30[b)]	0.30±0.46	0.40±0.66*
		EPA	9	5 (56.6)	2 (22.2)	5 (55.6)	0.67±0.67	0.22±0.42	0.89±2.59
		LA	16	9 (56.3)	11 (68.8)	15 (93.8)	1.13±1.15	1.19±1.20	2.32±1.69
2. Ca.	→	OA	16	7 (43.8)	8 (50)	12 (75)	0.75±1.07	0.63±0.70	1.38±1.32
3. —		DHA	5	0	0	0	0	0	0
		EPA	5	0	0	0	0	0	0
		LA	5	0	0	0	0	0	0
		OA	5	0	0	0	0	0	0

AD, adenomas; CAR, carcinomas; AD+CAR, adenomas and carcinomas combined; Ca., treatment with carcinogens.
a) ( ), percentage incidence.
b) Mean±SD.
* $P<0.05$ as compared to group 2.

Table IV. Effects of DHA, EPA and LA on Tumor Induction in the Small Intestine

Group		Treatment	No. of rats	No. of rats with			No. of tumors/rat		
				AD	CAR	AD+CAR	AD	CAR	AD+CAR
1. Ca.	→	DHA	10	1 (10)[a)]	4 (40)	5 (50)	0.10±0.32[b)]	0.40±0.52	0.50±0.53
		EPA	9	0 (0)	3 (33.3)	3 (33.3)	0	0.33±0.50	0.33±0.50
		LA	16	3 (18.8)	4 (25)*	6 (37.5)	0.19±0.40	0.25±0.45*	0.44±0.63
2. Ca.	→	OA	16	1 (6.3)	10 (62.5)	11 (68.8)	0.06±0.25	0.69±0.60	0.75±0.58
3. —		DHA	5	0	0	0	0	0	0
		EPA	5	0	0	0	0	0	0
		LA	5	0	0	0	0	0	0
		OA	5	0	0	0	0	0	0

AD, adenomas; CAR, carcinomas; AD+CAR, adenomas and carcinomas combined; Ca., treatment with carcinogens.
a) ( ), percentage incidence.
b) Mean±SD.
* $P<0.05$ as compared to group 2.

Table V. Effects of DHA, EPA and LA on Lesion Induction in the Liver and Lung

Group		Treatment	No. of rats	Liver GST-P$^+$ foci		No. of rats with lung lesions	
				No./cm^2	Area (mm^2)/cm^2	HPL	AD
1. Ca.	→	DHA	10	4.67±1.15[a)]	0.39±0.12	10 (100)[b)]	3 (30)
		EPA	9	4.48±1.84	0.49±0.30	9 (100)	4 (44.4)
		LA	16	3.07±1.93*	0.40±0.33	10 (100)	3 (18.8)
2. Ca.	→	OA	16	3.99±2.39	0.32±0.35	16 (100)	10 (62.5)
3. —		DHA	5	0	0	0	0
		EPA	5	0	0	0	0
		LA	5	0	0	0	0
		OA	5	0	0	0	0

GST-P$^+$ foci, glutathione S-transferase-positive foci larger than 0.2 mm in diameter; HPL, hyperplasias; AD, adenomas; Ca., treatment with carcinogens.
a) Mean±SD.
b) ( ), percentage incidence.
* $P<0.05$ as compared to DHA treatment group.

From Tables III and IX, decrease in tumor incidence correlated closely with reduction of AA levels in the large intestine (r=0.987, P=0.012) (Fig. 2). On the other hand, the tumor incidence (number of tumors/rat) showed an inverse correlation with total amounts of $n-3$ UFAs (DHA + EPA + linolenic acid + docosapentaenoic acid) (r=−0.780, P=0.296).

In the small intestine, total amounts of fatty acids were rather similar irrespective of treatment with different UFAs (DHA, 70.7; EPA, 25.9; LA, 45.0 and untreated

Table VI. Effects of DHA, EPA and LA on Lesion Induction in the Kidney

Group	Treatment	No. of rats	No. of rats with			No. of lesions/cm^2		
			RCH/RCA	NB	TCH/TCC	RCH/RCA	NB	TCH/TCC
1. Ca. →	DHA	10	5 (50)[a)]	5 (50)	5 (50)	0.25±0.29[b)]	0.14±0.15	0.14±0.15
	EPA	9	5 (55.6)	6 (66.7)	3 (33.3)	0.22±0.24	0.26±0.22	0.10±0.16
	LA	16	7 (43.8)	7 (43.8)	11 (68.8)	0.19±0.24	0.13±0.16	0.23±0.18
2. Ca. →	OA	16	11 (68.8)	9 (56.3)	5 (31.3)	0.30±0.28	0.19±0.19	0.11±0.18
3. —	DHA	5	0	0	0	0	0	0
	EPA	5	0	0	0	0	0	0
	LA	5	0	0	0	0	0	0
	OA	5	0	0	0	0	0	0

RCH/RCA, renal cell hyperplasias and/or renal cell adenomas; NB, nephroblastomas; TCH/TCC, transitional cell hyperplasias and/or transitional cell carcinomas; Ca., treatment with carcinogens.
a) ( ), percentage incidence.
b) Mean±SD.

Table VII. Effects of DHA, EPA and LA on Lesion Induction in the Urinary Bladder and Forestomach

Group	Treatment	No. of rats	Number of rats with urinary bladder lesions			No. of rats with forestomach lesions
			PNH	PAP	PNH+PAP	HPL
1. Ca. →	DHA	10	6 (60)[a)]	2 (20)	7 (70)	1 (10)
	EPA	9	6 (66.7)	0	6 (66.7)	3 (33.3)
	LA	16	11 (73.3)	7 (46.6)*	12 (80)	4 (25)
2. Ca. →	OA	16	6 (40)	3 (20)	8 (53.3)	1 (6.3)
3. —	DHA	5	0	0	0	0
	EPA	5	0	0	0	0
	LA	5	0	0	0	0
	OA	5	0	0	0	0

PNH, papillary or nodular hyperplasias; PAP, papillomas; HPL, hyperplasias; Ca., treatment with carcinogens.
a) ( ), percentage incidence.
* P<0.05 as compared to EPA treatment group.

Table VIII. Serum Levels of Fatty Acids

Treatment	No. of rats	Fatty acids (μg/ml)					
		Total	OA	LA	AA	EPA	DHA
DHA	10	916.5±208.5$^{***, a)}$	191.0±76.3**	101.2±32.3***	37.6±7.7***	73.2±13.0	169.0±35.0***
EPA	7	809.8±456.5***	148.8±64.7**	57.8±26.9***	37.8±15.4***	163.6±47.9***	17.9±8.3**
LA	7	924.6±324.3***	198.5±51.0**	163.1±38.2***	49.6±13.9***	94.2±32.0**	57.4±16.0
Untreated control	5	2409.6±678.7	599.8±345.8	355.4±111.6	299.3±144.7	37.9±30.1	72.9±26.8

a) Mean±SD.
** P<0.01, *** P<0.001 as compared to untreated control.

Table IX. Levels of Fatty Acids in the Large Intestine

Treatment	No. of rats	Fatty acid (mg/g)							
		Total	OA	LA	AA	EPA	DHA	Total $n-3$ UFAs	Total $n-6$ UFAs
DHA	10	45.52±15.79[a)]	20.24±7.79	4.07±1.31	0.44±0.08*	0.31±0.04	1.62±0.44***	2.72±0.89	4.10±1.85
EPA	7	44.06±27.52	20.14±5.89	3.51±1.00	0.51±0.18*	2.19±1.77*	0.26±0.12	3.38±2.93	3.53±1.55
LA	7	44.02±22.21	18.54±9.71	9.94±4.75	1.19±0.24*	0.04±0.08	0.15±0.04	1.13±0.62	9.16±5.73
Untreated control	5	55.49±14.42	25.05±7.71	4.43±1.16	0.79±0.07	0.06±0.05	0.13±0.08	1.22±0.41	5.31±1.22

a) Mean±SD.
* $P<0.05$, *** $P<0.001$ as compared to untreated control.

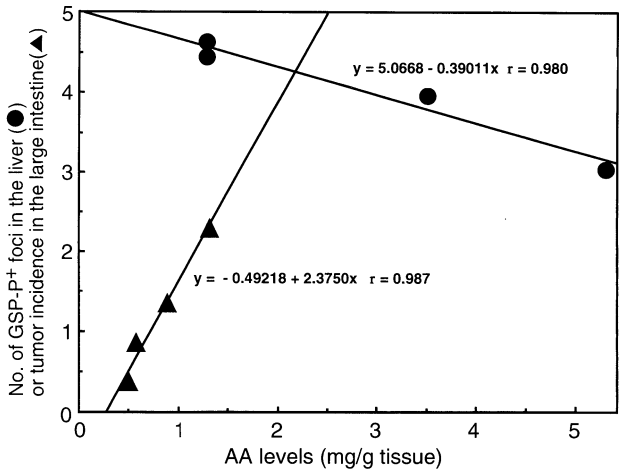

Fig. 2. Correlation of number of GST-P$^+$ foci in the liver (●) or tumor incidence (number of tumors/rat) in the large intestine (▲) with AA levels in the liver or the large intestine, respectively.

control, 64.0 mg/g tissue). AA levels were significantly decreased by treatment with DHA and EPA ($P<0.01$), and increased by administration of LA (Table X). However, no significant correlation of tumor incidences (number of tumors/rat) with the AA levels was found ($r=0.418$, $P=0.656$).

In the liver, levels of total amounts of fatty acids were similar in groups treated with different UFAs (DHA, 30.9; EPA, 30.7; LA, 17.9 and untreated control, 26.6 mg/g tissue). AA levels were significantly decreased by treatment with DHA and EPA ($P<0.001$) and increased by LA ($P<0.05$) (Table XI). From Tables V and XI, values for GST-P$^+$ foci showed an inverse correlation with AA ($r=-0.980$, $P=0.022$) (Fig. 2).

Thus, treatment with fatty acids caused changes of fatty acid levels in the tissues examined. In particular, DHA treatments caused marked decreases of AA in the small and large intestines and liver.

DISCUSSION

The schedule described in the present study allowed the determination of inhibitory or promoting effects of UFAs in the same animals, as summarized in Fig. 1. It is important to consider the contents of dietary LA, which is known to modify carcinogenesis.[4)] Therefore, our modified AIN93M diet should be superior to those previously used in minimizing the influence of LA on carcinogenesis. Induction of tumors was clearly reduced by DHA and possibly by EPA in the large intestine, and a similar tendency was observed in the small intestine and lung. This is the first demonstration of such organotropic modifying effects of DHA and EPA in a rat multi-organ carcinogenesis system which allows us to study complex modification effects in one animal. Our results observed in the intestines are in line with previous findings using DHA.[5, 8, 18)] Although the effect was not statistically significant, EPA also reduced the combined incidence of adenomas and carcinomas of the intestine, in line with a previous report.[4)] However, larger numbers of animals would be required to determine the statistical significance of the observed effects of EPA. It should be noted LA tended to promote tumor induction in the large intestine, but conversely inhibit carcinoma and possibly adenoma yield in the small intestine. Further experimentation is needed to confirm this controversial result.

The current system allowed examination of modification influence in the intestines, liver, lung, kidneys, urinary bladder, and forestomach, but DHA, EPA and LA did not exert significant modifying effects on the development of preneoplastic and neoplastic lesions in organs other than the intestines, although DHA and LA exhibited a tendency to inhibit lung tumorigenesis. Accordingly, DHA and EPA can be concluded to be chemopreventive agents exclusively in the intestines, and particularly in the colon. Elucidation of the modifying effects of UFAs, such as linolenic acid ($n-3$, $C_{18:3}$) and AA which lie between LA and EPA in terms of their degree of saturation requires further investigation.

Table X. Levels of Fatty Acids in the Small Intestine

Treatment	No. of rats	Fatty acid (mg/g)							
		Total	OA	LA	AA	EPA	DHA	Total $n-3$ UFAs	Total $n-6$ UFAs
DHA	10	70.69±49.36[a)]	35.43±21.01	5.47±1.20	0.61±0.16**	1.31±1.06	3.89±1.64**	7.21±3.97*	6.21±1.39
EPA	7	25.93±7.24	8.78±2.16	2.95±1.87	0.61±0.13**	2.48±1.04**	0.26±0.09	3.94±1.27	2.70±0.61
LA	7	45.00±20.65	19.69±8.08	8.69±3.93	1.68±0.74	0.03±0.04	0.27±0.09	1.01±0.25	10.58±4.20
Untreated control	5	64.02±24.17	27.73±11.08	5.64±1.81	1.39±0.17	0.09±0.08	0.30±0.03	1.52±0.42	7.13±1.88

a) Mean±SD.
* $P<0.05$, ** $P<0.01$ as compared to untreated control.

Table XI. Levels of Fatty Acids in the Liver

Treatment	No. of rats	Fatty acid (mg/g)							
		Total	OA	LA	AA	EPA	DHA	Total $n-3$ UFAs	Total $n-6$ UFAs
DHA	10	30.91±20.20[a)]	5.36±3.69	2.28±0.56	1.16±0.33***	3.77±3.41	9.27±6.50*	14.04±10.60	3.43±0.88
EPA	7	30.69±20.31	6.09±4.17	3.25±1.95	1.16±0.43***	8.23±5.42*	1.90±1.08	13.90±8.35	4.08±1.79
LA	7	17.92±9.53	2.96±0.80*	2.94±1.27	4.87±0.84*	0.07±0.05	1.49±0.18	1.46±0.78	6.50±3.74
Untreated control	5	26.63±4.31	5.13±0.97	2.48±0.47	3.21±0.55	0.62±0.09	2.38±0.43	3.65±0.65	5.74±1.00

a) Mean±SD.
* $P<0.05$, *** $P<0.001$ as compared to untreated control.

The present data highlight the necessity for choice of chemopreventive agents on the basis of adequate information regarding organotropism. Tumor-promoting effects of compounds must be taken into account. For example, it has been shown that naturally occurring and synthetic antioxidants inhibit liver carcinogenesis when administered during the initiation and post-initiation stage.[10–12] However, they were found to promote tumor development or even to be carcinogenic in other organs.[10, 12, 19, 20] For example, butylated hydroxyanisole, caffeic acids, catechol and *p*-methylcatechol were shown to be carcinogenic or to promote carcinogenesis in the rat forestomach, though these compounds markedly inhibited colon carcinogenesis.[12] With this in mind, appropriate experimentation with wide-spectrum assay protocols is necessary to clarify whether any particular compound might find practical application as a chemopreventive agent. The fact that DHA caused a reduction of intestinal tumors without any promoting influence elsewhere is important in this context.

The question of how far data obtained in rodents can be extrapolated to the human situation can not be answered in terms of the mechanisms of action. It should be noted that treatment with DHA and EPA significantly reduced levels of AA in the intestines and liver, as reported in the mouse.[15] Since AA is a key substance for the biosynthesis of leucotrienes, prostaglandins and thromboxane, which mediate inflammatory reactions, its decrease may correlate with a reduction of inflammatory reactions. In fact, tumor development was significantly reduced or showed a tendency for decrease only in the intestine, where non-steroidal anti-inflammatory agents are known to suppress tumor development.[21–23] On the other hand, GST-P$^+$ liver cell foci count showed an inverse correlation with AA levels in the liver (Fig. 2). In other words, increase in AA level correlated with decrease in the liver preneoplasia development, which is clearly in contrast to the situation in the large intestine. Hirose *et al.* reported that treatment with

Table XII. Summary of Modification Effects

Organ	Treatment group		
	DHA	EPA	LA
Large intestine	⬇	↘	↗
Small intestine	↘	↘	⬇
Liver	—	—	—
Lung	↘	↘	↘
Kidney	—	—	—
Urinary bladder	—	—	—
Forestomach	—	—	—

⬇, significant inhibition; ↘, tendency for inhibition; ↗, tendency for promotion; —, no effect.

LA caused a reduction of liver GST-P$^+$ foci development.[24] As judged from these results, LA may not be a hepatocarcinogen.

In the current study, AA contents in the intestines were markedly decreased by treatment with DHA and EPA and intestinal carcinogenesis was inhibited. The results are summarized in Table XII. Since reduction of fat intake and supplementation of fiber are not clearly associated with colon cancer prevention,[25] our results here may indicate that *n*−3 unsaturated fatty acids, DHA, may be promising for prevention of carcinogenesis, especially in high-risk populations with familial colon cancer association.[26] For general use, the agents would require safety approval based on long-term toxicity/carcinogenicity testing. Further development of appropriate *in vivo* animal assay systems to provide reliable information regarding organotropism and adverse effects is also critical for this purpose.

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