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Effect of Docosahexaenoic Acid Rich Tuna Orbital Oil on Acute Liver Injury Induced by Carbon Tetrachloride

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Docosahexaenoic acid (DHA) rich oil was obtained from blue fin tuna (*Thunnus thynnus orientalis*) orbital tissue with centrifugation of 12,000 rpm under vaccum (10^{-1} Torr) at 4℃. The effect of DHA rich oil (DHA content; 27.8%) on CCl_4-induced acute injury was investigated biochemically and histopathologically. Dosage of DHA rich oil on 24h before CCl_4-administration prevented significantly the increase of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GTP) values. No necrosis of hepatocytes was observed in rat livers treated with DHA oil on 24h prior to CCl_4-administration. These results suggested that DHA oil controls the accumulation of fat in the liver and prevented the liver injury.

Key words : docosahexaenoic acid, tuna orbital oil, CCl_4-induced liver injury

Introduction

As studies on the physiological activities and functions of docosahexaenoic acid (DHA) which is a rich in available functional lipid in preventing adult dieseases and as precursor of prostaglandin, the effect of DHA in improving learning ability (Lamtey and Walker, 1976; Yamamoto et al., 1987), namely developing intellectual power and preventing demtia, has attracted public attention, and therefore DHA is now regarded as a raw material for physiologically functional food.

Recently, Nakajima et al. (1994, 1995) reported that the DHA oil controlled the accumulation of fat in the liver and prevented the decrease of liver function. As a result, it is expected that DHA might be useful in treating and preventing the abnormal function of liver injury. In this study, the effect of tuna orbital tissue rich in DHA (DHA oil) on acute liver injuries induced by carbon tetrachloride (CCl_4) was investigated by means of serum-biochemical and histopathological examination in rats.

Material and Methods

1. Preparation of DHA rich oil from tuna orbital tissue

For the preparation of DHA rich oil from tuna orbital tissue, 8 Kg of fresh blue fin tuna heads were cut surrounding the eyes and 500 g of orbital tissue was collected. To the orbital tissue, 2.5 ℓ of water was added and the mixture was homogenized under vacuum (10^{-1} Torr) at 4℃. The homogenate was then centrifuged (12,000 rpm, 20 min.) at 4℃, and the orbital lipid layer was separated as DHA oil (Suetsuna et al., 1994).

2. Analysis of fatty acid in tuna orbital tissue by gas chromatography

One hundred milligram of lipid was mixed with 10% NaOH-methanol solution to a final volume of 1 mℓ. Then the mixture was vigorously shaken under heating for 30 min. to completely dissolve the lipids, and was cooled. The fatty acid compositions were determined as methyl ester by gas-chromatography (GLC) (Prevot and Mordret, 1976). Analysis of fatty acid methyl esters was perfomed with a Shimazu GC-14A gas chromatography on a 10% DEGS column (Shimalite W 60~80 mesh, 2 m×3 mm). The flow rate of N_2 gas was 40 mℓ/min, and the column temperature was programed from 210℃ to 230℃ at rate of 3℃/min.

3. Effect of DHA rich oil on experimental liver injury in rats

Six male Winster rats (Japan SLC Inc.) weighing 200 ± 10 g were subjected to each experimental group ecti-

vely. After fasting for 24 h, 50% CCl_4 (2 mℓ per kg of rat, Nakarai Chemicals Co.) dissolved in olive oil (Nakarai Chemicals Co.) was given intraperitoneally (i. p.). DHA oil (20 mℓ/kg of rats) was given orally 1h and 24h before CCl_4-administration. The rats that received CCl_4 were sacrificed 8h and 24h later. The rat sera obtained by centrifugation of blood were measured for glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total bilirubin (T. Bil), leucine amino peptidase (LAP), alkaline phosphatase (ALP), total protein (TP) and albumin (ALB) with a Wako kit (Wako Chemicals Co.). The rat livers were prepared for electron microscopic observation with an electron microscope (Hitachi HS-9 type).

4. Electron microscopic observatic of the liver tissues rat by electron microscope

Affer quick soacking of the left side of the liver in 2.5 % glutalaldehyde-0.1M phosphate buffer solution, it was devided into cubes or of 1 mm^3 and then pre-fixed for 2hr at 5℃. The sections were rinsed by 0.1 M phosphate buffer solution for overnight and post-fixed for 2hr in 1 % osmium tetroxide-0.5M phosphate buffer solution supplomented with 4% sugar. The post-fixed sections were dehydrated by methanol, substituted with propylene oxide, wrapped with Epok-812 and polymerized with heat, and ultra thin sections were made with microtome. After double-staining of the sampres with uranyl acetate and lead citrate, it was photographed by electron microscope (Hitachi HS-9 type) of penetration type under condition of accelated voltage, 75 Kv.

Results and Discussion

1. Preparation of DHA rich oil

Five hundred grams of orbital tissue was homogenized and the orbital oil homogenate was further centrifuged with cooling under vacuum (12,000 rpm, 4℃, 10 ^{1} Torr). Two hundred grams of DHA oil could be obtained at 40 % yield from the orbital tissue of tuna head. As shown in Table 1, the lipids containing 27.8% DHA and 8.3% EPA with respectivery times of 32~36 and 16~20, respectivery time (min) 32~36 and 16~20 was transparent and had no flavor and odor.

Table 1. Fatty acid composition of tuna obital oil

Fatty acid	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:1}$	$C_{20:4}$ + $C_{22:1}$	$C_{20:5}$	$C_{22:6}$
Area (%)	2.6	7.4	7.3	3.7	12.8	2.8	4.3	7.3	8.3	27.8

It was epidemiologically known that the incidence of myocardial infraction and thrombosis is lower in Eskimos and Japanese than in Westerners (Dyerberg et al., 1975 ; Dyerberg and Bang, 1979). Studies concerning n-3 polyunsaturated fatty acids (PUFA) in fish oils have been focused on lipids rich eicosapentaenoic acid (EPA), and therefore DHA has been produced merely as a by-product of EPA hitherto. Lipids in marine products contain high levels of n-3 PUFA, and therefore they readily deteriorate via peroxides, resulting in the development of undesirable changes such as off-flavor, discoloration, and production of toxic substance in fatty foods (Rizvi and Acton, 1982). In our experiment, it was possible to economically obtain lipids rich in DHA from fish heads which are available as by-products in the fishery processing. Further more, it seems possible that DHA oil without any deterioration and more safety than being extracted in such solvents as ethanol and acetone can be obtained without any deteriorat by a simple, which is more safer than extractic with, solwerts such as by a simple centrifugation, cooling and vacuuming procedure.

2. Effect of DHA rich oil on CCl_4-induced liver injury in rats

Rats that received 50% CCl_4 (2 mℓ/kg) intraperitocally were sacrificed 8h and 24h. As shown in Table 2, remarkable increases in hepatic GOT and GTP were observed in CCl_4+D.W. group compared with olive oil+D.W. group. DHA oil (20 mℓ/kg) was given orally (P. O.) on 1h and 24h before CCl_4-administration, and each rat was sacrified 8h and 24h after CCl_4-administration. Pretreatment with DHA oil on 24h before CCl_4-administration (CCl_4+ DHA group) prevented increase of hepatic GOT (decrease rate; 48%) and GTP (decrease rate; 57%) significantly. On the other hand, pretreatment with DHA oil

Table 2. Effect of DHA oil on several hepatic parameters in the rats treated with CCl_4

Group	Olive oil+D.W.		CCl_4+D.W.		CCl_4+DHA	
Items	8h	24h	8h	24h	8h	24h
GOT (U/ℓ)	95 ± 13	131 ± 19	2717 ± 667	6580 ± 2079	2143 ± 342	3422 ± 467*
GPT (U/ℓ)	24 ± 3	43 ± 6	1313 ± 240	2251 ± 730	1127 ± 507	968 ± 225*
T. Bil (mg/dℓ)	0.14 ± 0.05	0.27 ± 0.08	0.20 ± 0.14	0.19 ± 0.07	0.13 ± 0.05	0.14 ± 0.05
LAP (U/ℓ)	188 ± 6	163 ± 6	202 ± 12	231 ± 23	201 ± 14	216 ± 35
ALP (U/ℓ)	605 ± 39	840 ± 85	796 ± 67	1055 ± 112	763 ± 60	1034 ± 182
TP (g/dℓ)	5.81 ± 0.17	5.07 ± 0.10	4.43 ± 0.16	3.01 ± 0.24	3.40 ± 0.24	4.50 ± 0.26*
ALB (g/dℓ)	2.51 ± 0.07	2.17 ± 0.05	2.01 ± 0.09	1.43 ± 0.06	1.67 ± 0.17	2.13 ± 0.13*

DHA oil was given p. o. 1h and 24 h before CCl_4-administration i. p.
Rats (6 animals per group) were sacrificed 8 h and 24 h after CCl_4-administration.
Each value represents the mean ± S. E.
*Statistically significant difference from the CCl_4+D.W. (distilled water) group at $p<0.05$.
Abbreviation : Glutamic oxaloacetic transaminase (GOT), Glutamic pyruvic transaminase (GPT), Total bilirubin (T. Bil), Leucine amino peptidase (LAP), Alkaline phophatase (ALP), Total protein (TP), Albumin (ALB).

24h before CCl_4-administration prevented decrease of hepatic TP and ALB ($p<$) significantly, and only the increase in TP and ALB was observed in CCl4 + D.W. group compared with olive oil+D. group.

As shown histopathologically in Fig. 1, the vacuolation of liver induced by lipid denaturalization was observed in the CCl_4+D.W. group (Photograph a.) and pretreatment with DHA oil on 24h before CCl_4-administration (CCl_4+DHA group) prevent the vacuolation of rat liver (Photograph b.) That is to say, morphological changes such as the necrosis of hepatocytes, infiltration of inflammatory cells occured in the liver not pretreated with DHA. However, no necrosis of hepatocytes was observed in rats treated with DHA oil 24h prior to CCl_4-administration. These results suggested that DHA oil is protective against CCl_4-induced acute liver injury.

Nakajima et al. (1994, 1995) inuestigaled the effect of dietary DHA oil on liver lipid composition and lipid drops in rat liver. They shouted that the serum total cholesterol, HDL-cholesterol and glucose levels of rats fed a DHA oil (rich in n-3 PUFA) diet was lower than that of rats fed a corn oil (rich in n-6 PUFA) diet, and DHA oil prevent furthermore the growing of lipid drop in rat hepatocytes. The DHA oil is applicable to health foods and physiologically functional foods. In addition, it may be used as starting materials for producing medicines such as DHA esters. It has been expected that DHA oil is physiologically effective on improving learning ability (memory and brain functions), and on cardiovascular disease, hypertension, and atherosclerosis, as related to prostanoid metabolism (Ke et al., 1975), and on acting as an antiallergosis (Weber et al., 1991) or an anti-inflammation (Nakamura et al., 1994). Timmer-Bosscha (1989)

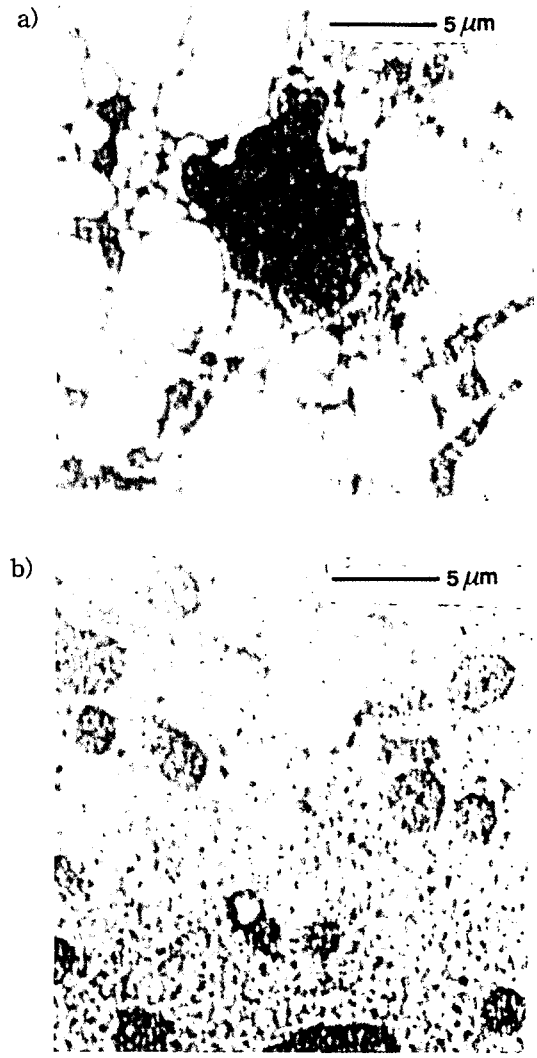

Fig. 1. Electron micrograph (a) of the rat liver 24h after CCl_4-administration, and electron micrograph (b) of the rat liver pretreated with DHA oil 24h before CCl_4-administration.

reported the effect of DHA as anti-tumor agents aginst mammary cancer, colon cancer and lung cancer, and Bush et al. (1991) on improving of the retinal reflex (suppression of visual acuity decrease). In addition to these physiological effects, we concluded from this experiment that DHA oil has an effect on the prevention of liver function *in vivo*.

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