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行政院國家科學委員會補助專題研究計畫成果報告

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※探討魚油抑制 GST-P(+)肝前癌細胞形成及影響癌細 ※

※胞生長之機轉 ※

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共同主持人：

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- □赴國外出差或研習心得報告一份
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- □國際合作研究計畫國外研究報告書一份

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中文摘要

研究結果顯示，飲食油脂之含量及種類與癌症發生有密切之相關。富含亞麻油酸之高玉米油飲食，可促進乳癌及大腸直腸癌形成。相反的，魚油，富含超長鏈 n-3 多元不飽合脂肪酸 Eicosapentaenoic acid (EPA)及 Docosahexaenoic acid (DHA)，則可抑制乳癌及大腸直腸癌發生。在飲食油脂與肝癌形成的相關研究方面，本實驗室研究資料證實，與高玉米油飲食比較下，高魚油飲食可有效抑制 Diethylnitrosamine (DEN;誘發劑)-Phenobarbital (PB;促進劑)模式下誘發之 placental glutathione S-transferases (GST-P)(+) 肝前癌細胞形成，但真正機轉並不清楚。故本計劃主要研究目的是從魚油對肝前癌細胞形成的影響及對癌細胞生長相關蛋白質之調節，探討魚油抑制肝前癌細胞的形成可能機制。Peroxisome proliferator-activated receptor (PPAR)-α可被 PPAR-α的活化劑 (例如: WY-14,643，Clofibrate，EPA，DHA)活化後，將進而調節對 PPAR 有反應的基因 (PPAR-response genes) mRNA 的轉錄。然而文獻顯示，雖然魚油和 Clofibrate 皆可減少 GST-P (+)肝前癌細胞，但 Clofibrate 會增加 basophilic 肝前癌細胞生成及最終導致肝癌形成。這可能因爲不同之 PPAR-α活化劑，對 PPAR-response genes mRNA 轉錄的強弱不同而影響其誘發肝癌形成能力。研究魚油對調節肝前癌細胞的形成及其在肝細胞影響 PPAR-response genes mRNA 轉錄。

英文摘要

Data have been shown that the amount and the type of dietary fat related to carcinogenesis. The high corn oil diet which is rich in linoleic acid could cause breast and colon cancer. In contract, fish oil, which is high in in n-3 fatty acid, eicosapentaenoic acid (EPA) and decosahexaenoic, could inhibit carcinogenesis. Data from our laboratory have shown that dietary fish oil could decrease rat placental glutathione S-transferases (GST-P) (+) liver foci synthesis from the diethylnitrosamine-phenobarbital (PB) model when compared with dietary corn oil which is rich in linoleic acid. The mechanism of fish oil of inhibiting liver foci synthesis may be involved in the stimulation of hepatic detoxification system, inhibition of prostaglandin E_2 synthesis, the enhancement of glutathione-related oantioxidant capacity. However, the mechanisms of chemoprevention by fish oil are not yet understood. In order to understand the mechanisms of fish oil inhibiting liver foci, the objective of this proposal is to explore the modulation of fish oil in liver foci synthesis and cell cycle related protein expression. Peroxisome proliferator-activated receptor (PPAR)-α could be activated by their ligands or activators such as WY-14,643, Clofibrate, EPA and DHA. Activated PPAR-α could not only regulate PPAR-response genes mRNA expression but also antagonize signaling through an array of important pathway, including STATs, AP-1 and NF-kB. The different potent of PPAR-α activators could affect the induction of PPAR response gene expresssions and may influence the liver carcinogenesis. Therefore there may be other mechanisms involved in the inhibiting liver carcinogenesis by fish oil. In order to realize effect of fish oil in DEN-PB induced liver foci synthesis, we explore the ability of fish oil in activated PPAR-α. To understand the mechanism of fish oil inhibiting liver cancer could help us to explore the role of dietary fat in carcinogenesis.

計畫緣由與目的

近年來癌症在大部分已開發國家已漸居死亡原因的榜首，台灣亦不例外。無論從流行病學調查或是動物模式實驗，結果都顯示飲食中油脂含量及種類與癌症發生有密切的相關(Miller et al., 1990)。一般而言，高油脂飲食較低油脂飲食易誘發及促進癌症形成。例如，高油脂飲食即被證實可以促進致癌物質 (carcinogens)誘導乳癌形成 (initiation stage)及增進癌症促進劑 (tumor promoters)對已誘發細胞 (initiated cells)生長而造成腫瘤之形成 (reviewed in Welsch, 1987)。飲食油脂中脂肪酸種類亦會影響癌症形成，許多研究指出，脂肪酸可增進或抑制癌症促進劑作用，進而調節癌症形成 (Rose et al., 1994; Welsch, 1992)。雖然許多實驗已證實，與玉米油相比較，魚油可抑制乳癌以及結腸癌的形成，但是對魚油抑制癌症形成之機轉至今仍未完全清楚瞭解。在飲食油脂與肝癌形成的相關研究方面，本實驗室研究資料證實，高魚油飲食 (10% by weight)在與高玉米油飲食 (15% by weight)相比較下，魚油可有效抑制 Diethylnitrosamine (DEN;誘發劑)-Phenobarbital (PB;促進劑)模式下γ-glutamyl transpeptidase (+) 及 placental glutathione S-transferases (GST-P)(+)肝前癌細胞之形成 (Ko et al., 2000; Lii et al., 2000; Chen et al., 1997)。雖然結果顯示魚油抑制肝前癌細胞的形成可能與減少 prostaglandin E_2 生成，改變肝臟解毒系統，Glutathione 相關之抗氧化能力有關，但真正機轉並不清楚 (Ko et al., 2000; Lii et al., 2000)。故本計劃主要研究目的是從魚油對肝前癌細胞形成的影響，探討魚油抑制肝前癌細胞的形成可能機制。

在研究 peroxisome proliferators (PPs)誘發老鼠肝癌之實驗中，引起注意的是，GST-P (+)肝前癌細胞並不能用於評估 PPs 誘發肝癌形成之能力。因爲 DEN-PP 處理模式沒有 GST-P (+)肝前癌細胞之形成 (Nishimura et al., 1995; Yokoyama et al., 1993)，但卻可促進 basophilic 肝前癌細胞形成，並增加肝臟腫瘤生成數目及發生率 (Cattley, et al., 1994)。PPs 指一群可誘發 Peroxisome proliferation 之化學物質，包括有 fibrate class of hypolipidemic drugs ([4-chloro-6(2,3-xylidino)-2-pyrimidinylthio]acetic acid (WY-14,643)，clofibrate and fenofibrate)，antidiabetic thiazolidinedione drugs，plasticizers (phthalate esters) herbicides (e.g., lactofen)，leukotriene antagonists (e.g., LY-171883)，及溶劑 (e.g.,trichloroethylene)(reviewed in Krey et al., 1997)。PPARs 可被 PPAR 的活化劑 (ligands 或 activators)活化後，將進而調節對 PPAR 有反應的基因 (PPAR-response genes) mRNA 的轉錄 (mRNA transcription)(Reviewed in Cattley et al., 1998)。目前所知的 PPAR-response genes，包含有 acyl-CoA oxidase (ACO)，liver fatty acid binding protein (L-FABP)、adipocyte lipid binding protein (aP2)、cytochrome P450 4A1 (CYP 4A1)(Aldridge et al., 1995)、hydroxymethylglutaryl-CoA synthase、apolipoprotein AI and CIII 等。PPAR 的活化劑，除了已知的 peroxisome proliferators 外，近來由於 binding assay 的發展，一些脂肪酸 (例如: LA，linolenic acid [LNA], arachidonic acid [AA]， EPA，DHA)及 eicosanoids (prostaglandin J_2，8-hydroxyeicosatetraenoic acid，leukotriene B4 等) (Kliewer et al., 1997; Krey et al., 1997)也被證實具有活化 PPARs 的能力，並可調節 PPAR-response gene mRNA 的轉錄。用 Glucocorticoid receptor –PPAR 模式顯現 EPA 及 DHA 對 PPAR-α的活化力比 AA 強 (Gustagsson et al., 1994)。也有實驗證實，老鼠肝實質細胞 (hepatic parenchymal cells) PPAR-α

可被 EPA 活化，但 LA，LNA 和 AA 則無此作用 (Ren et al., 1997)。 然而與 WY-14,643 相比較，EPA 對 PPAR-α的親和力較低，故對 ACO 以及 CYP 4A1 mRNA 的轉錄量較少 (Krey et al., 1997)。脂肪酸對 PPAR 的活化能力，將脂肪酸與基因調節連接在一起，是一個相當重要的發現，因爲這可解釋爲何飲食油脂可調控細胞的生長，分化，進而影響一些疾病的發展，例如，癌症的形成。

近來研究也已趨向探討 PPAR-response genes 與細胞生長，分化 (differentiaiton)及癌症形成 (carcinogenesis)之間的關係，例如 ACO (Gonzalez et al., 1998)、CYP4A1 和 L-FABP (reviewed in Bentley et al., 1993)則與老鼠類肝癌生成有關。長期給予大鼠 PPAR-α強效力之 ligands，例如 WY-14,643 或 Clofibrate，可導致肝癌 (Gonzalez et al., 1998)。然而也有文獻指出如投予小鼠與 PPAR-α親和力較弱之活化劑，LA，AA 以及 perfluorinated decanoate (脂肪酸衍生物)等，則不會影響 DEN 處理後之鼠類肝癌形成 (Klaunig et al., 1990)。以往研究報告亦發現，魚油 (Ko et al., 2000)和 WY-14,643 或 Clofibrate (Yokoyama et al., 1993)皆可減少 GST-P (+)肝前癌細胞，但 WY-14,643 或 Clofibrate 會增加 basophilic 肝前癌細胞生成及最終導致肝癌形成 (Cattley et al., 1994; Reddy et al., 1978)。這可能因爲不同之 PPAR-α活化劑，對 PPAR-response genes mRNA 轉錄的強弱不同而影響其誘發肝癌形成能力。 概要而言，爲探究飲食中魚油對調節肝癌形成所扮演角色及相關機制，本計劃假說魚油經活化 PPAR-α干擾 AP1 與 GPE1 結合能力，而減少 GST-P (+)肝前癌細胞形。但因 EPA 及 DHA 爲較弱之 PPAR-α活化劑故無法類似 WY-14,643 或 clofibrate 有效率的誘發大量 PPAR-response genes 表現而導致肝癌形成。藉由探討飲食中魚油調節肝癌形之相關機轉，將進一步增加我們對飲食油脂調控癌症生成之瞭解。

結果與討論

所有不同實驗組老鼠之進食量、體重及肝臟重皆沒有顯著差異 (data not shown)。與餵食玉米油組老鼠相比較，餵食魚油組老鼠肝臟磷脂質中 EPA 及 DHA 顯著增加而 AA 顯著減少。餵食 clofibrate 組老鼠肝臟磷脂質脂肪酸分布與餵食玉米油組老鼠相似 (Table 1)。與以往實驗結果一致，與餵食玉米油組老鼠相比較，餵食玉米油組老鼠肝臟中 GSH Foci 顯著減少，而餵食 clofibrate 組老鼠肝臟中 GSH Foci 顯著增加。餵食玉米油組老鼠肝臟中 Catalase 活性顯著增加。老鼠肝臟中 CYP 2B1 mRNA 表現不受油之影響且不同脂肪酸在肝細胞中 CYP4A1 蛋白質量無顯著不同。

計畫成果自評

本計畫在探討魚油與 Liver Foci 形成之關係。研究在探討魚油是否經由活化 PPAR 來影響 Genes 表現。本研究結果顯示魚油活化 PPAR 來影響 Genes 表現能力比玉米油及 Clofibrate 強。本研究結果亦顯示，魚油抑制 Liver Foci 形成。由以往研究結果得知，Proxisome Proliferators 可誘發老鼠肝癌形成。由於本研究結果得知魚油可活化 PPAR，但卻可抑制 Liver Foci 形成，故對於魚油與肝癌形成之關係及其相關機轉仍需繼續探討。

參考文獻

Cattley, R.C., DeLuca, J., Elcombe, C., Fenner-Crisp, P., Lake, B.G., Marsman, D.S., Pastoor, T.A., Popp, J.A., Robison, D.E., Schwetz, B., Tugwood, J and Wahli,W. (1998) Do peroxisome proliferation compounds pose a hepatocarcinogenic hazard to humans? Regul. Toxicol. Pharmacol., 27,47-60.

Cattley, R.C., Kato, M., Popp, J.A., Teets, V.J. and Voss, K.S. (1994) Initiator-specific promotion of
hepatocarcinogenesis by WY-14,643 and clofibrate. Carcinogenesis, 15, 1763-1766.

Chen, H.W., Ko, J.J., Lii, C.K., Ou, C.C. and Sheen, L.Y. (1997) Corn oil enhances gamma-glutamyl transpeptidase-positive foci development in the presence of phenobarbital during hepatocarcinogenesis in rats. Nutr. Cancer, 28, 252-257.

Chu, S., Huang, Q., Alvares, K., Yeldandi, A.V., Rao, M.S. and Reddy, J.K. (1995) Transformation of mammalian cells by overexpressing H2O2-generating peroxisomal fatty acyl-CoA oxidase. Proc. Natl. Acad. Sci. USA, 92, 7080-7084.

Gonzalez, F.J., Peters, J.M. and Cattley, R.C. (1998) Mechanism of action of the nongenotoxic peroxisome proliferators: role of the peroxisome proliferator-activator receptor α. J. Natl. Cancer Insti. 90, 1702-1709.

Keller, H., Dreyer, C., Medin, J., Mahfoudi, A., Ozato, K. and Wahli, W. (1993) Fatty acids and retinoids control lipid metabolism through activation of peroxisome proliferator-activated receptor-retinoid X receptor heterodimers. Proc. Natl. Acad. Sci.USA, 90, 2160-2164.

Klaunig, J.E., Weghorst, T.R. and Weghorst, C.M. (1990) Liver tumor promoting ability of corn oil gavage in B6C3F1 male mice. Cancer Lett., 50, 215-219.

Kliewer, S.A., Sundseth, S.S., Jones, S.A., Brown, P.J., Wisely, B., Koble, C.S., Devchand, P., Wahli, W., Willson, T. and Lenhard, J.M. (1997) Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ. Proc. Natl. Acad. Sci. USA, 94, 4318-4323.

Kliewer, S.A., Forman, B. M., Blumberg, B., Ong, E.S., Borgmeyer, U., Mangelsdorf, D.J., Umesono, K. and Evans, R.M. (1994) Differential expression and activation of a family of murine peroxisome proliferator-activated receptors. Proc. Natl. Acad. Sci. USA, 91, 7355-7359.

Kliewer, S.A., Umesono, K., Noonan, D.J., Heyman, R.A. and Evans, R.M. (1992) Convergence of

9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors. Nature, 358:771-774.

Krey, G., Braissant, O., L'Horset, F., Kalkhoven, E., Perroud, M., Parker, M.G. and Wahli, W. (1997) Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by co-activator-dependent receptor ligand assay. Mol. Endocrinol., 11, 779-791.

Lii, C.K., Ou, C.C., Liu, K.L.; Liu, J.Y., Lin, W.L. and Chen, H.W. (2000) Suppression of altered hepatic foci development by a high fish oil diet compared with a high corn oil diet in rats. Nutri. Cancer, 38, 50-59.

Miller, A.B. (1990) Diet and cancer. A review. Acta Oncol., 29, 87-95.

Nishimura, S., Yokoyama, Y., Nakano, H., Satoh, K., Kano, H., Sato, K. and Tsuchida, S. (1995) Decreased expression of glutathione S-transferases and increased fatty change in peroxisomal enzyme-negative foci induced by clofibrate in rat livers. Carcinogenesis, 16, 1699-1704.

Reddy, B.S., Burill, C. and Rigotty, J. (1991) Effect of diets high in omega-3 and omega-6 fatty acids on initiation and postinitiation stages of colon carcinogenesis. Cancer Res., 51, 487-491.

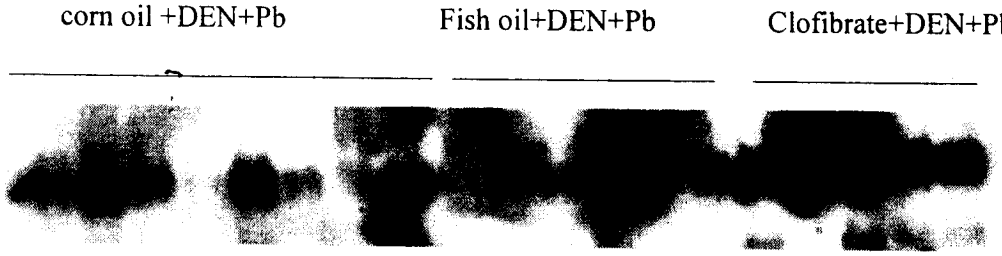
Ren, B., Thelen, A.P., Peters, J.M., Gonzalez, F.J. and Jump, D.B. (1997) Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor alpha. J. Biol. Chem., 272, 26827-26832.

Rose, D.P and Connolly, J.M. (1999) Omega-3 fatty acids as cancer chemopreventive agents. Pharmac. Ther., 83, 217-244.

Rose, D.P., Rayburn, J., Hatala, M.A.& Connolly, J.M. (1994) Effects of dietary fish oil on fatty acids and eicosanoids in metastasizing human breast cancer cells. Nutr. Cancer, 22:131-141.
Welsch, C.W. (1992) Relationship between dietary fatty and experimental mammary tumorigenesis: A Review and Critique. Cancer Res.(Suppl.)52:2040S-2048S.

Welsch, C.W. (1987) Enhancement of mammary tumorigenesis by dietary fat: review of potential mechanisms. Am. K. Clin. Nutr., 45:192-202.

Yokoyama, Y., Tsuchida, S., Hatayama, I. and Sato, K. (1993) Lack of peroxisomal enzyme inducibility in rat hepatic preneoplastic lesions induced by mutagenic carcinogens: contrasted expression of glutathione S-transferase P form and enoyl CoA hydratase. Carcinogenesis, 14,393-398.



Cytochrome 2B1 Northern blotting in rat liver

4A1 →

C CF wy CLA LA AA EPA EPA DHA DHA

Cytochrome 4A1 Western blotting in primary hepatocytes

Effects of n-6 、n-3 fats and clofibrate on area and number of rat

Diet	DEN	PB	Area , % (mm^2 / cm^2)	Number / cm^2	Number / cm^3
hepatic GGT - positive foci					
	Treatment			GGT – positive foci	
HCO	-	-	-	-	-
HFO	-	-	-	-	-
HCO+CF	-	-	-	-	-
HCO	±	-	-	-	-
HFO	±	-	-	-	-
HCO+CF	±	-	-	-	-
HCO	±	±	9.81 ± 8.76	1.26 ± 0.78 ^{ab}	9.34 ± 5.97
HFO	±	±	8.75 ± 12.50	1.06 ± 0.79 ^{b}	9.46 ± 9.85
HCO+CF	±	±	31.14 ± 45.67	2.22 ± 1.71 ^{a}	13.20 ± 11.49

Values are means ± SD. Groups that do not share the same letter (a, b) are significantly different from each other ($P < 0.05$).

n-6、n-3 油脂與 clofibrate 對大鼠肝臟 catalase 活性的影響

Diet	Treatment DEN	Treatment PB	catalase U / mg protein
HCO	-	-	14.2 ± 2.57 [b]
HFO	-	-	24.8 ± 3.69 [a]
HCO+CF	-	-	13.9 ± 3.19 [b]
HCO	±	-	16.3 ± 7.74
HFO	±	-	18.9 ± 2.23
HCO+CF	±	-	13.4 ± 5.86
HCO	±	±	19.0 ± 2.87 [a]
HFO	±	±	21.8 ± 5.64 [a]
HCO+CF	±	±	15.8 ± 4.19 [b]