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行政院國家科學委員會專題研究計畫成果報告

β-胡蘿蔔素對大白鼠初代肝細胞之生存力及抗氧化系統之影響

β-carotene affects the cell viability antioxidative system of primary rat hepatocytes

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一、中文摘要

本研究主要探討攝取 0.1g/kg β-胡蘿蔔素 6 週之大白鼠，在大白鼠初代肝細胞中其抗氧化情形。以 0.05~0.2 mM $FeCl_3$ 培養 30 或 60 分鐘誘發氧化傷害，評估細胞抗氧化酵素及脂肪過氧化情形。研究結果顯示：SOD 活性顯著降低(p =0.0023)，CAT 活性顯著升高(p =0.0001)，脂肪過氧化產物(malondialdehyde)濃度顯著升高(p =0.0001)。在此研究結果得知：在促氧與抗氧化平衡上 β-胡蘿蔔素還是有趨向抗氧化之功能。

關鍵詞：β-胡蘿蔔素、抗氧化酵素、大白鼠初代肝細胞、脂肪過氧化

英文摘要

The ability of β-carotene to protect against oxidative stress and lipid peroxidation was assessed. Primary rat hepatocytes cultures were oxidatively stressed by exposure to ferric chloride ($FeCl_3$). Activities of the antioxidant enzymes superoxide dismutase dismutase (SOD; *EC* 1.15.1.1), catalase (CAT; *EC* 1.11.1.6) and glutathione peroxidase (GSH-Px; *EC* 1.11.1.9) were measured as indices of oxidative stress. Rats fed 0.1g β-carotene per1 kg diet for 6 weeks, then the primary rat hepatocytes incubated with 0.05 ~0.2 mM $FeCl_3$ for 30min or 60 min exhibited decreased SOD activity (p = 0.0023), and increased CAT activity (p =0.0001) and increased malondialdehyde concentration (p =0.0001) compared with a β-carotene- free diet. The rat primary hepatocytes incubated with 0.05~0.2 mM $FeCl_3$ for 60 min exhibited increased GSH-Px activity compared with a β-carotene- free diet. These results indicate that β-carotene act as a shift in the prooxidant-antioxidant balance toward antioxidant activity.

Keyword: β-carotene: Antioxidant enzymes: Rat Hepatocytes: Lipid peroxidation

二、緣由與目的

Numerous epidemiological studies support a strong inverse relationship between consumption of β-carotene-rich fruits and vegetables and the incidence of some degenerative diseases. β-carotene is widely used as a precursor of vitamin A, as a food colorant, and as a food additive. Most work has focused on β-carotene as a potential anticarcinogen. Because free radical-induced damage to mammalian tissues is believed to contribute to the aging process and to the development of some degenerative diseases (Canfield et al. 1992), it has been proposed that dietary carotenoids serve as antioxidants in tissues (Thurnham 1994).

β-carotene has been shown to function as an antioxidant in many *in vitro* systems (Krinsly, 1991). A plausible mechanism for the potential anticarcinogenic effects of β-carotene is its ability to scavenge reactive oxygen species that cause oxidative DNA damage. However, two recent major intervention trials, one in Finland (Heinonen & Albanes, 1994) and one in the USA (Omenn *et al.* 1996) unexpectedly demonstrated an increased risk of lung cancer in smokers who were given high-doses of β-carotene supplements.

Moreover, the products of β-carotene directly responsible for the prooxidant activity have not yet been identified. Improved knowledge of the prooxidant role of in vitro and in vivo carotenoids will assist in tests regarding their potential to influence biological processes in humans (Palozza 1998). Moreover, the role of β-carotene in antioxidative efficacy is still controversial because of the antioxidative efficacy of β-carotene in vivo. Very little work has been conducted investigating the effects of β-carotene supplementation in vivo on the cell viability and antioxidatve system of primary rat hepatocytes.

We have previously shown the efficacy of β-carotene in inhibiting $FeCl_3$-induced oxidative stress in a cellular model system using primary rat hepatocytes. In this study we examined the ability of β-carotene to protect against $FeCl_3$-induced oxidative stress in primary rat hepatocytes.

Materials and Methods

All other chemicals used were of reagent grade. Male Wistar rats (National Taiwan University Hospital Animal Center), weighing about 160 g procedures followed the Guide for the Care and Use of Laboratory Animals National Science Council, Taiwan (National Science Council 1994). Rats were randomly divided into two groups of six rats each and fed for 6 wks. The two groups were fed AIN-76 diets with or without 0.1g/kg β-carotene. Rat hepatocytes were isolated using a two-step collagenase perfusion as described by (Bonney et al. 1974). After isolation, hepatocytes were resuspended in L-15 culture medium (pH 7.6), containing 18 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES),0.2% BSA, 5 μg/ml each of insulin and transferrin, 5ng/ml selenium as sodium selenite, 1 mg/ml galactose, 1 μM dexamethasone, 100 IU/ml penicillin, and 100 μg/ml streptomycin, in a final volume of 0.5 $\times 10^6$ cells/ml. Five milliliters of the cell suspension were plated onto collagen-precoated 60-mm plastic tissue culture dishes (Falcon Labware, Franklin Lakes NJ, USA) with a total of 2.5 $\times 10^6$ cells in each dish.

Lipid peroxidation product was assayed according to an improved thiobarbituric acid (TBAR) reactive substances (malodialdehyde, MDA) fluorometric method at 553 nm with excitation at 515 nm (Yagi, 1987). 1,1,3,3-Tetramethoxypropane was used as the standard.

CAT activity in primary rat hepatocytes was determined on the same day as harvesting using the method of Baudhuin *et al.*(1964). The remaining supernatant fractions were stored at -20℃until SOD activity determined by the method of McCord & Fridovich (1969), and GSH-Px activity by the method of Lawrence and Burk (1976) were measured. Total protein was determined in each RPH using the method of Lowry *et al.* (1951).

Determination of β-carotene in primary rat hepatocytes or liver

Statistical analysis

Differences among the treatment group means were assessed using a two-way ANOVA method (SAS Institute Inc., Cary, NC). Group means were considered to be significantly different at $p < 0.05$ as determined by Duncan's new multiple range test.

Results and Discussion

The feed efficiency was not significantly different between the with and without β-carotene diets. The β-carotene content of the liver tissue and primary rat hepatocytes isolated from rats fed AIN-76 diet with 0.1g/kg β-carotene were 0.0478±0.004μg/mg protein (0.532 ± 0.088 nmol/g tissue) and 0.0178 ± 0.003 μg/mg protein respectively.

The SOD activity of feeding β-carotene diet was significantly less than the β-carotene-free diet ($p = 0.0023$) when primary rat hepatocytes incubated with 0.05~0.2 mM $FeCl_3$ for 30 min and 60 min (Fig. 1). The SOD activity of the β-carotene diet was also significantly less than the β-carotene-free diet when primary rat hepatocytes were incubated without $FeCl_3$.

The CAT activity and MDA concentrations of the β-carotene diet were significantly greater than the β-carotene-free diet when the primary rat hepatocytes were incubated with 0.05~0.2 mM $FeCl_3$ ($p = 0.0001$) (Fig. 2, Fig. 4). The CAT activity and MDA concentrations of the β-carotene diet were also significantly greater than the β-carotene-free diet when the primary rat hepatocytes were incubated without $FeCl_3$.

The GSH-Px activity of the β-carotene diet was not significantly different than the β-carotene-free diet when the primary rat hepatocytes were incubated with 0.05~0.2 mM $FeCl_3$ for 30 min ($p > 0.05$) (Fig. 3). The GSH-Px activity was also not affected with or without the β-carotene diet when the primary rat hepatocytes were incubated 0.05 mM $FeCl_3$ or without $FeCl_3$. But the GSH-Px activity of feeding β-carotene diet were significantly increase than β-carotene-free diet when the primary rat hepatocytes were incubated 0.1~0.2 mM $FeCl_3$ for 60 min ($p < 0.05$) (Fig. 3).

In conclusion, this study has shown the efficacy of β-carotene in inhibiting $FeCl_3$-induced oxidative stress in a cellular model system using primary rat hepatocytes. The effects of β-carotene supplementation in vivo and in vitro on the cell viability and antioxidative system of primary rat hepatocytes. These results indicate that β-carotene act as a shift in the prooxidant-antioxidant balance toward antioxidant activity.

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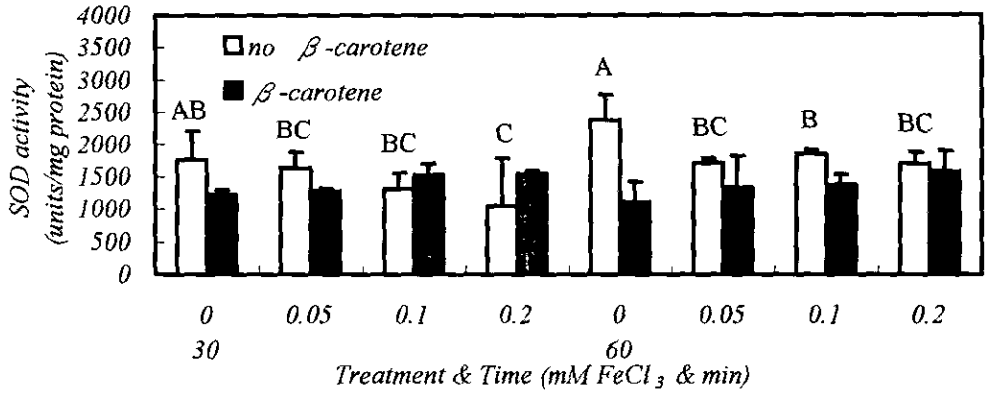

Figure 1. Effect of $FeCl_3$ on superoxide dismutas (SOD) activity in rat primary hepatocytes from rat fed AIN-76 diet with or without 0.1g/kg β-carotene

a. Values are mean ±SD of triplicate culture dishes.

b. Values with different superscripts in the same color bar are significantly different from one another at $p < 0.05$ as determined by Duncan's multiple range test. a, b, c: β-carotene diet. A, B, C: β-carotene-free diet.

c. The SOD activity are significantly different ($p = 0.0023$) between feeding β-carotene diet and β-carotene-free diet.

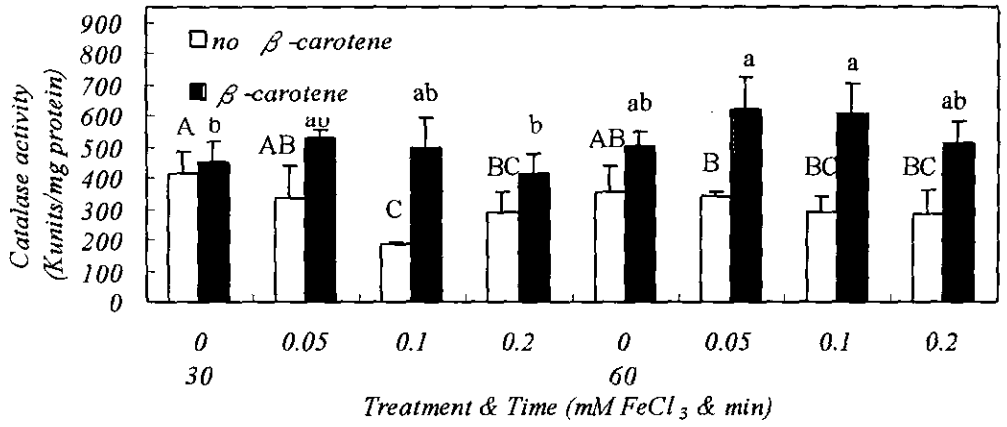

Figure 2. Effect of $FeCl_3$ on catalase (CAT) activity in primary rat hepatocytes isolated from rat fed AIN-76 diet with or without 0.01% β-carotene

a. Values are mean ±SD of triplicate culture dishes.

b. Values with different superscripts in the same color bar are significantly different from one another at $p <$ 0.05 as determined by Duncan's multiple range test. a, b, c: β-carotene diet. A, B, C: β-carotene-free diet.

c. The CATactivity are significantly different ($p = 0.0001$) between feeding β-carotene diet and β-carotene-free diet.

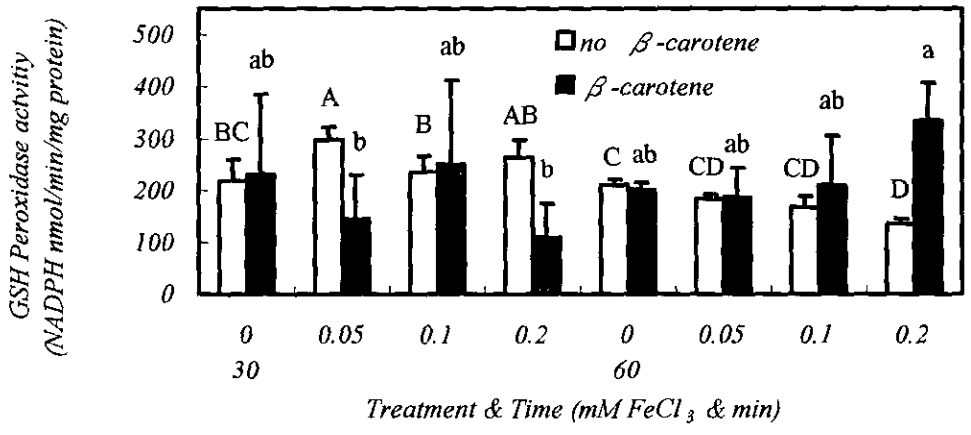

Figure 3. Effect of $FeCl_3$ on glutathione peroxidase (GSH-Px) activit in primary rat hepatocytes isolated from rat fed AIN-76 diet with or without 0.01% β-carotene

a. Values are mean ±SD of triplicate culture dishes.

b. Values with different superscripts in the same color bar are significantly different from one another at $p <$ 0.05 as determined by Duncan's multiple range test. a, b, c: β-carotene diet. A, B, C, D: β-carotene-free diet.

c. The GSH-PX activity are no significantly different ($p > 0.05$) between feeding β-carotene diet and β-carotene-free diet.

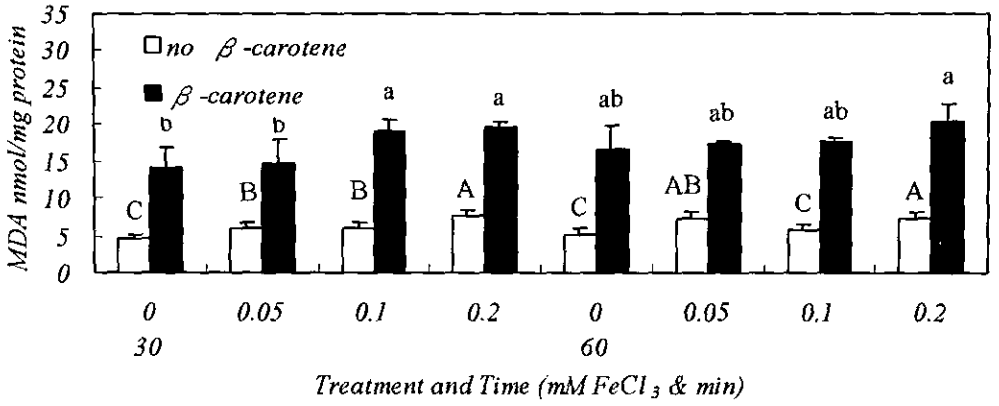

Figure 4. Effect of $FeCl_3$ on thiobarbituric acid reactive substances (malondialdehyde, MDA). in primary rat hepatocytes isolated from rat fed AIN-76 diet with or without 0.01% β-carotene.

a. Values are mean $\pm$SD of triplicate culture dishes.

b. Values with different superscripts in the same color bar are significantly different from one another at $p <$ 0.05 as determined by Duncan's multiple range test. a, b: β-carotene diet. A, B, C: β-carotene-free diet.

c. The concentration of MDA are significantly different ($p = 0.0001$) between feeding β-carotene diet and β-carotene-free diet.