以活體內及活體外方式給予β-胡蘿蔔素對大白鼠初代肝細胞之生存利及抗氧化系統之影響

The Effect of β-Carotene Supplementation on the Cell Viability and Antioxidative System of Primary Rat Hepatocytes

中文摘要

β胡蘿蔔素是一種普遍存在於深色蔬菜及水果中的脂溶性營養素。由近年來的研究顯示:於不同的實驗模式中，β胡蘿蔔素的抗氧化性質亦有差異，因此本實驗欲探討，以活體內及活體外方式給予β胡蘿蔔素對於大白鼠初代肝細胞之生存力及抗氧化系統之影響。

Wistar 品系雄性大白鼠，控制組 8 隻，餵予 AIN-76 標準飲食配方，β胡蘿蔔素組(BC)3 隻，於 AIN-76 標準飲食配方中添加 0.1%β胡蘿蔔素，進行 3 週的餵食後，分別進行兩組實驗。(壹)控制組及 BC 組大白鼠各 3 隻，以兩階段膠原蛋白酶灌流技術，取得大白鼠初代肝細胞，培養至 12-14 小時，更換有或無添加 0.1 mM $FeCl_3$ 新鮮培養液，進行 0,1,2 及 3 小時的培養。(貳)控制組大白鼠 5 隻，以兩階段膠原蛋白酶灌流法，取得肝細胞，進行(A) 長時間處理實驗:培養液有或無添加 10^{-5} M β-胡蘿蔔素 0,24,48 及 72 小時的培養。(B)短時間處理實驗:肝細胞以 10^{-5} M β-胡蘿蔔素處理 48 小時後，換上含有 0.1 mM $FeCl_3$ 的培養液，做 0,1,3,5, 及 7 小時的培養。並於不同時間點收集肝細胞，分析細胞生存力(以 lactate dehydrogenase leakage 表示，簡稱 LDH leakage)、脂質過氧化(以 thiobarbituric acid reactive substances 表示，簡稱 TBARS) 、麩胱甘肽(glutathione, GSH)濃度及 GSH 代謝相關酵素:glutathione peroxidase (GSH Px) 、glutathione reductase(GSH Rd) 、glutathione S-transferase(GST)等的活性。

實驗結果:在以活體內方式給予β-胡蘿蔔素的實驗中，控制組及 BC 組其 TBARS 值隨 0.1 mM $FeCl_3$ 處理時間的增長而增加，但 LDH leakage 值，GSH、CSSG 濃度則無顯著變化。以活體外方式給予β胡蘿蔔素的實驗(A)長時間處理實驗:肝細胞對β胡蘿蔔素的攝入量於 48 小時達最高量。控制組第 24 小時，β胡蘿蔔素組第 48 小時有最大量的總 GSH、還原性 GSH；而 GSSG、GSH/GSSG ratio 則隨培養時間的增加而有上升的趨勢。至於 GSH Px, GSH Rd, GST 活性方面，控制組及β胡蘿蔔素組均是在 24 小時有最大活性，之後有逐漸下降的趨勢。在(B)短時間處理實驗:控制組 TBARS 及 LDH leakage 會隨 0.1 mM $FeCl_3$ 誘導時間的增加而顯著上升，β-胡蘿蔔素則顯著抑制此種上升的現象，且細胞內β-胡蘿蔔素含量顯著下降。在 0.1 mM $FeCl_3$ 加入至 7 小時時，控制組還原型 GSH 濃度及 GSH/GSSG ratio 均顯著下降，而 GSH Px、GSH Rd、GST 活性有下降的趨勢；β-胡蘿蔔素

組則除 GST 活性有下降趨勢外，還原型 GSH 濃度 GSH/GSSG ratio、GSH Px 及 GSH Rd 活性均無明顯變化。
由本研究得知，活體外給予β-胡蘿蔔素可減少肝細胞因 0.1 mM $FeCl_3$ 所誘導的脂質過氧化傷害。

英文摘要

β-Carotene, a fat-soluble nutrient, commonly exists in dark pigmented vegetables and fruit. Recently, studies have indicated that the antioxidative effect of β-Carotene varies with different experimental models. The purpose of this study was to investigate the effects of β-Carotene supplementation on the cell viability an dantioxidative system of rat hepatocytes in vivo and in vitro.
During a 3-week period, eight Wistar male rats were fed with AIN-76 standard diet, and three rats were fed with AIN-76 standard diet with 0.1% β-Carotene added(BC group). In the experiment(1):Primary rat hepatocytes were obtained from the control or BC group of three by the two-step collagenase perfusion technique. After the cell were cultured for 12-14 hours, the medium was renewed with frish medium with or without 0.1 mM $FeCl_3$, and the culturing was continued for 0,1,2 and 3 hours. In the experiment(2): Primary rat hepatocytes were obtained from the control group of five by the two-step collagenase perfusion for either [A] long-term treatments: supplementation with or without 10^{-5} M of β-Carotene for 0,24,48, and 72 hours or [B] short-term treatments:with or without the addition of 10^{-5} M of β-Carotene for 48 hours followed by the addition of 0.1 mM $FeCl_3$, for 0,1,3,5 and 7 hours. Hepatocytes were collected at different time fo rthe analyses of cell viability (LDH leakage), lipid peroxidation (TBARS), glutathione (GSH) levels, and the activities of enzymes related to GSH metabolism enzymes: glutathione peroxidase (GSH Px), glutathione reductase (GSH Rd), and glutathione S-transferase (GST).
Results showed: in the *in vivo* study, the TBARS values increased with the duration of added 0.1 mM $FeCl_3$ for both the control and BC groups. However, there was no significant difference in the LDH leakage value or the concentrations of GSH and GSSG. In the *in vitro* long-term study, the uptake of β-Carotene by the hepatocytes reached the maximum at 48 hours. At 24 hours, the biosynthesis of total GSH and reduced GSH in the control group reached the maximum. While in the BC group, total GSH and reduced GSH levels reached the maximum at 48 hours.
The GSSG level and the GSH/GSSG ratio tended to be increased over of the culturing time. The activities of GSH Px, GSH Rd and GST, were increased to the maximum at 24 hours in both groups. In the short-term *in vitro* study, TBARS production and LDH leakage in the control group were obviously elevated with the treatment of 0.1 mM $FeCl_3$. In BC group, the TBARS and the LDH leakage were significantly lower than

control group, and cellular β-Carotene levels were significantly reduced. The reduced GSH concentration and the GSH/GSSG ratio of the control group were decreased significantly, while the activities of GSH Px, GSH Rd, and GST tended to reduce at 7 hours of the addition of 0.1 mM $FeCl_3$. In the BC group, except for the decreasing tendency of GST activity, no apparent change occurred in the reduced GSH concentration, the GSH/GSSG ratio, and the activities of GSH Px, and GSH Rd. The data reveal that *in vitro* supplement action of β-Carotene can reduce lipid peroxidation and damages on hepatocytes induced by 0.1 mM $FeCl_3$.