β-胡蘿蔔素對大白鼠初代肝細胞之生存力,脂質過氧化及抗氧化酵素活性 的影響

The effects of β-carotene on the cellular viability, lipid peroxidation and activities of antioxidative enzymes in primary rat hepatocytes

中文摘要

許多研究結果顯示β-胡蘿蔔素具有正面的生物功能，如降低脂質過氧化生成以及 降低癌細胞的分裂等，故推論β-胡蘿蔔素具有降低 心血管疾病以及降低癌症發生的功 效。事實上，曾有研究者發現 β-胡蘿蔔素不具有抗氧化的效果，並且近年來的人體研 究亦發現 添加β-胡蘿蔔素反而增加心血管疾病與肺癌的發生，爲釐清β-胡蘿蔔素 之 抗氧化情形，本研究以體外實驗方式觀察β-胡蘿蔔素對大白鼠 初代肝細胞之細胞生存 力、抗氧化酵素活性以及脂質過氧化的影響 。 採用 Wistar 品系雄 性大白鼠，以兩階段膠原蛋白酉每的方式分離肝細胞，培養細胞 4 小 時後進行下列三階段實驗。第一，肝細胞在正常的情況之下持續培養 4 、10、16、22 、28 以及 40 小時，並在第 16 小時更換或不更換培養液 ，觀察細胞之生存力以及抗氧化酵 素活性的變化以決定後續實驗的 時間點。由本實驗結果將後續實驗的觀察時間點定爲 12 小時；第二 ，加入 0.05~2 mM FeCl3，觀察細胞之生存力以及抗氧化酵素活性的變化 以決 定後續實驗之氧化誘導劑的添加濃度。由本實驗結果決定以 0.1 mM 爲添加濃度；第三， 將β-胡蘿蔔素添加於培養液中，同時添加或 不添加 0.1 mM FeCl3，培養 12 小時之後觀察 細胞的生存力、脂質過氧 化以及抗氧化酵素活性的改變，實驗中加入α-tocopherol、 retinol、canthaxantnin 或α-carotene 以作比較。三階段實驗的生化分 析：以 lactate dehydrogenase leakage (LDH leakage)作爲細胞 生存力的指標；抗氧化酵素則以分析 GSH 代謝相關酵素：麩胱甘月太過 氧化酉每 (GSH peroxidase)、麩胱甘月太還原酉每 (GSH reductase) 以及麩胱甘月太硫轉移酉每 (GSH S-transferase) 活性 ；以 thiobarbituric acid-reactive substances (TBARS) 分 析 malondialdehyde (MDA) 生成。

結果顯示：FeCl3 降低細胞生存力以及抗氧化酵素活性，並且在 0.05~0.1 mM 之間隨濃 度呈現劑量效應。在 0.1 mM FeCl3 的存在下同時加入β- 胡蘿蔔素，發現抗氧化酵素的活 性更顯著降低，且細胞的生存力未獲 得改善，相同的實驗以 retinol 或α-tocopherol 代 替β-胡蘿蔔素時 發現細胞生存力明顯上升。在 0.1mM FeCl3 的存在下加入 10-8~10-5Mβ- 胡蘿蔔素，發現不同濃度對細胞的影響沒有差異。在不添加 0.1mM FeCl3 情形下，β-胡蘿 蔔素仍會降低抗氧化酵素活性，相同實驗以 canthaxanthin、retinol 或α-carotene 代替 β-胡蘿蔔素發現抗氧化 酵素活性不受影響。研究顯示β-胡蘿蔔素對 MDA 生成沒有明顯的 抑制 效果。 研究顯示，β-胡蘿蔔素會降低抗氧化酵素活性，亦發現β-胡蘿蔔素對脂 質過氧

化沒 有明顯的抑制效果。

英文摘要

There are many researches indicate thatβ-carotene has many biological functions, such as reducting lipid peroxidation and decreasing mitogenesis of cancer cells. Because of these observations, it has been suggested that people who consume more fruits and vegetables containing β- carotene have somewhat lower risk of cancer and cardiovascular disease. However, it has also been found thatβ-carotene has no effect on lipid peroxidation and may has adverse effect on the incidence of lung cancer and on the risk of death resulting from lung cancer and cariovascular diseases. In this present study , I examined the effect of β-carotene on the cellular activities of anti-oxidative enzymes, on the cellular viability and on the lipid peroxidation in primary rat hepatocytes. Primary rat hepatocytes were prepared from male, 8-week-old Wistar strain rats by two-step collagenase perfusion. The isolated cells were incubated in plating medium. After four-hour plating, the medium was removed and replaced by the same medium containing no fetal bovine serum (FBS). Then three experimental steps were followed. In the first step, the cells were incubated in normal condition for 4, 10, 16, 22, 28 and 40 hours with or without changing the culture medium at 16 hours. Changes of activities of the antioxidative enzymes and cellular viability were measured to describe time course of the cells. 12-hour plating was seclected as appropriate incubation time for subsequent experiments. In the second step, cells were incubated with 0.05~2 mM FeCl3 for 12 hours. Changes of activities of the antioxidative enzymes, cellular viability and lipid peroxidation were measured to describe dose effect of FeCl3. 0.1 mM was selected as suitable concentration for subsequent experiments. In the third step, cells were incubated with β-carotene and with or without 0.1 mM FeCl3 for 12 hours. The effect of β-carotene were observed by measuring the activities of the antioxidative enzymes, cellular viabilibty and the lipid peroxidation. α-tocopherol, retinol, canthaxanthin orα-carotene were added in the medium by the same protocol in contrast with β-carotene. Lactate de- hydrogense (LDH leakage) was analysed as index of cellular viability. GSH peroxidase, GSH reductase and GSH S-transferase were measured as indices of antioxidative enzymes. The analysis of thiobarbituric acid reactive sub- stances (TBARS) is index of malondialdehyde (MDA) production. The results indicate that cells incubated with 0.1 mM FeCl3 for 12 hours exhibited reduced the cellular viability and reduced the activities of anti- oxidative enzymes. Addingβ-carotene into 0.1 mM FeCl3 treated cells signi- ficantly reduced the activities of antioxidative enzymes. The effect of various concentration of β-carotene on cells was not significantly different from each others and it is indicated that the addition of β-carotene did not improve the cellular

viability. However, Addingα-tocopherol or retinol into 0.1 mM $FeCl_3$ treated cells significantly increased cellular viability.Incorporating β-carotene into non $FeCl_3$ treated cells, the decrease of activities of antioxidative enzymes was revealed. When canthaxanthin, retinol or α-carotene were applied instead of β-carotene, the activities of anti- oxidative enzymes were not different from control group. The result indicates that the addition of β-carotene had no effect on inhibition of lipid peroxi- dation. In summary, this study indicate thatβ-carotene has decreasing effect on the cellular activities of antioxidative enzymes. It also indicate that addition of β- carotene had no benefit on inhibition of lipid peroxidation.