嘉南藥理科技大學專題研究計畫成果報告

探討 Chlorella 對於糖尿病鼠之血脂及血糖之影響

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研究背景及實驗目的:

糖尿病可依其對胰島素之倚賴性而分為胰島素倚賴型（第一類型）及非胰島素倚賴型（第二類型）糖尿病。傳統口服降血糖藥, 如 sulphonylureas 藉由提高胰島素分泌而達到其降低血糖之功效,但低血糖之產生則常為糖尿病患者在控制血糖上的一項困擾。糖尿病常見的併發症之一是 atherosclerosis 及心血管疾病(Kannel & McGee, 1979)。高濃度的脂質過氧化物被發現存於 atherosclerotic lesions and plasma 中(Wang *et al.*, 1989)。在許多的研究報導中指出 atherosclerosis 與 low density lipoprotein (LDL)的過氧化有關, 此與血中抗氧化功能降低有極密切的關係(Paget *et al.*, 1998; Hussein *et al.*, 1997; Toborek *et al.*, 1995)。脂質過氧化(lipid peroxidation)可能是糖尿病併發的血管疾病的一大關鍵(Wolff *et al.*; 1990; Faure *et al.*, 1993; Peuchant *et al.*, 1997)。糖尿病引發的高血糖伴隨著高濃度的 malondialdehyse (MDA, 為脂質被氧化後的產物),如以 insulin 將血糖控制在正常範圍中, 發現糖尿病患者血液中的 MDA 會明顯的比未治療前低(Peuchant *et al.*, 1997; Mukherjee *et al.*, 1998)。過度產生的含氧自由基(i.e. superoxide, hydrogen peroxide (H_2O_2), & hydroxyl radical 可能是來自於 protein glycation (Gillery *et al.*, 1988; Baynes, 1991)或者是 glucose autoxidation (Hunt *et al.*, 1990)。在糖尿病鼠的紅血球中, 可將 superoxide 轉變成 H_2O_2 的 superoxide dimutase (SOD)及可將 H_2O_2 轉變為 H_2O 的 catalase 和 glutathion peroxidase (GPx)的活性是明顯的低於正常老鼠的值(Loven *et al.*, 1996; Wohaieb & Godin, 1987)。另外的非酵素性的抗氧化系統, 如 Vitamine E, glutathion etc., 亦有較低的傾向(Vandewouder *et al.*, 1987; Uzel *et al.*, 1987; Bono *et al.*, 1987)。

綠藻曾在 1971 年被證實可降低由 alloxan 引發的糖尿病鼠(屬於 IDDM)的血糖(Rodriguez-Lopez & Lopez-Quijada, 1971)。綠藻也可能具有一些抗氧化的能力,曾有報導指出綠藻可降低肝臟脂質過氧化物 MDA 的功能(Singh *et al.*, 1998)。因此, 此研究計畫的目的是想要藉由綠藻及其萃取物對糖尿病鼠的治療來觀察是否可藉由降低血糖濃度進而影響可能引發糖尿病的 atherosclerosis 的因子, 即紅血球中的 SOD 和 GPx 的活性來產生正面的改善功效。綠藻本身即是一種食品,如果可改善糖尿病的高血糖及可能引發糖尿病的 atherosclerosis 的因子,那麼綠藻也許可以被用於糖尿病的飲食調節。除此之外, 綠藻萃取物也許可望被研發成另一新的血糖調節物。綠藻萃取物之純化分離將是下一個階段的目標。而進一步的工作則是尋找其作用機轉,這需視綠藻其萃取物對糖尿病鼠的影響是在改善高血糖情況進而作用在引發 atherosclerosis 的因子或者兩者都有受到調節。

研究方法:

1. Animals: Insulin-dependent diabetic (IDDM) and non-insulin dependent diabetic (NIDDM) rats can be induced by injecting streptocozocin (STZ) to 5-6 weeks and neonatal rats, respectively. Both models and control animals will be included in this study. About 3-5 weeks after the injection of STZ to the rats, their blood glucose will be checked prior to the

following experiments.

2. 葡萄糖耐受性測試 (Glucose Tolerance test): 此實驗可用來簡單區別第一類及第二類型糖尿病, endogenous insulin 對 glucose challenge 之反應, 以及藥物是否有改善第二類型糖尿病之功效。受測試的動物需經過 12 小時以上的斷食, 先測第一次的血中葡萄糖濃度然後再給予一劑量的葡萄糖 (1g/kg, i.p.), 之後每隔 30 分鐘測一次的血中葡萄糖濃度, 直到血中葡萄糖濃度恢復至第一次的血中葡萄糖濃度的範圍。測定藥物時, 藥物則於測完第一次的血中葡萄糖濃度後給投予, 30 分鐘之後再測一次的血中葡萄糖濃度, 之後才給予一劑量的葡萄糖, 之後步驟如同上述。

3. 基礎血糖測試 (Basal Glucose Level): 此實驗可用來測驗藥物是否會造成低血糖。受測試的動物在測完第一次的血中葡萄糖濃度然後給予一劑量的藥物, 60 分鐘後再測一次的血中葡萄糖濃度, 之後每 20 分鐘測一次的血中葡萄糖濃度, 直到給藥後 180 分鐘。

4. 胰島素敏感性測試 (Insulin Sensitivity Test): 此實驗可用以測驗藥物是否會加強 exogenous insulin 之作用而達到降血糖效果。受測試的動物在測完第一次的血中葡萄糖濃度然後再給予一劑量的胰島素, 之後每 60 分鐘測一次的血中葡萄糖濃度, 直到血中葡萄糖濃度恢復至第一次的血中葡萄糖濃度的範圍。測定藥物時, 藥物則於測完第一次的血中葡萄糖濃度後給投予, 60 分鐘之後再測一次的血中葡萄糖濃度, 之後才給予一劑量的胰島素, 之後步驟如同上述。

結果

Conventional oral hypoglycemic drug (glybenclamide 2.5 mg/kg) prevented blood glucose raise after a single dose of glucose (1 mg/kg) challenge at time of 30 min. Low dose (75 mg/kg) of acute *Chlorella pyrenoidosa* also suppressed the glucose challenge, but this was not seen in the high dose (100 mg/kg) of Chlorella treatment in normal mice.

Although acute treatment of 100 mg/kg Chlorella did not produce any significant effects on blood glucose level after acute glucose challenge, sub-chronic (two weeks) treatment of Chlorella reduced blood glucose level at time 90 min after glucose treatment. Therefore I chose dose of 100 mg/kg as the standard dose for further experiments.

Hypoglycemic effects of glybenclamide were seen 90 min after the acute treatment. The treatment of Chlorella only produced a transient decreasing effect on BGL at time 90 min in high dose (100 mg/kg) but not in low dose (75 mg/kg). Thus, Chlorella does not produce a significant hypoglycemic effect as that of glybenclamide.

The basal glucose levels (BGL) of STZ mice were much higher than normal and shunt STZ

mice, respectively. The BGL decreased to near the level of normal mice 90 min after a single dose of Chlorella (100 mg/kg) in STZ mice. The hypoglycemic effects sustained for the whole period of experimental time (i.e. 180 min after the initial treatment).

In normal mice, both doses of Chlorella and glybenclamide prolonged the hypoglycemic effects of exogenous insulin for at least 60 min.

Untreated STZ mice did not response to insulin injections whereas those Chlorella-treated STZ mice produced a dramatically change in their blood glucose after the treatment of insulin. The hypoglycemic effects of insulin were maintained for 180 min (from time 60 to 240 min).

討論

Results indicate that *Chlorella pyrenoidosa* has the capacity of lowering blood glucose level. The mechanisms involved in this observed effect are still unknown. This is consistent with the study of Lopez & Lopez-Quijada (1971). The future work will be followed this aspect.

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