行政院國家科學委員會專題研究計畫　成果報告

以糖尿病鼠模式來探討綠藻對於抑制高血糖和降低動脈粥狀硬化危險因子的作用及其改善病鼠抗氧化酵素之關聯性 (2/2)

計畫類別：個別型計畫
計畫編號：NSC92-2320-B-041-003-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：嘉南藥理科技大學醫藥化學系

計畫主持人：程中玉

報告類型：完整報告

處理方式：本計畫可公開查詢

中　華　民　國 93 年 9 月 29 日

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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※ 乙糖尿病鼠模式來探討綠藻對於抑制高血糖和降 ※
※ 低動脈粥樣化危險因子的作用及改善病鼠抗氧化 ※
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共同主持人：

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□國際合作研究計畫國外研究報告書一份

執行單位：嘉南藥裡科技大學

中　華　民　國　93 年　9 月　25 日

摘要:

綠藻曾被證實具有改善糖尿病鼠的高血糖現象但其中的作用機轉尚未研究過。經 streptozocin (STZ)誘發的的糖尿病鼠在接受綠藻的餵食之後再進行血糖利用的基本機轉 Lipogenesis, glucose uptakes, non-esterified fatty acids 等的試驗。接受綠藻餵食的糖尿病鼠肝臟的 glucose uptakes 有明顯的增加，血清中的 non-esterified fatty acids 也比較低，這可能是綠藻減輕高血糖情形的機轉之一。

Abstract

Aims/hypothesis. Chlorella, a type of unicellular fresh water growth algae, has been a popular foodstuff in Japan and Taiwan. There are many studies have shown some beneficial effects of Chlorella administration, including in decreasing blood glucose in alloxan-induced and streptozocin (STZ)-induced diabetic animals. However, the mechanisms in influencing blood glucose homeostatsis by *Chlorella pyrenoidosa* treatment have not been studied.

Methods. Streptozocin-induced mice (n=8) were treated with 100 mg/kg of Chlorella (oral) 60 min prior to soluble insulin (2.5 IU/kg, i.p.) and at 60 min intervals thereafter for insulin sensitivity test. Plasma insulin levels were determined by EIA. Lipogenic rates were measured followed Chlorella or H_2O 1 hr prior. Lipogenesis were measured by incorporating ^3H-H_2O into lipids in brown (BAT) and white (WAT) adipose tissues. Glucose uptakes were measured by extracting ^3H -2-deoxy-$_D$-[1,2-^3H] glucose level in the liver and soleus muscles. Fasting serum non-esterified fatty acids (NEFA) were measured by enzymatic hydrolysis commercialized assay kits.

Results. Chlorella enhanced the hypoglycemic effects of exogenous insulin in STZ mice. Neither basal nor insulin-stimulated lipogenesis in BAT and WAT were affected by administration of Chlorella. Similar results were observed in plasma insulin levels in either group. However, *Chlorella* treatment increased glycogen ^3H-glucose counts in the liver of STZ mice (3382±890 dpm) compared to that in H_2O-treated-STZ mice (1665±431 dpm, $p<0.01$). The same treatment reduced fasting NEFA levels in STZ mice (0.91 ± 0.06 mmol/L) compared to H_2O-treated STZ mice (1.18 ± 0.14 mmol/L, $p<0.05$, t-test).

Conclusion. The current results suggest that hypoglycemic effects of Chlorella may be due to an enhancement of glucose uptake in the liver. The improved insulin sensitivity after Chlorella treatment could be due to is lowering NEFA level, since insulin sensitivity usually blunted by elevated NEFA in diabetes.

Introduction:

Chlorella, a type of unicellular green algae, has been a popular foodstuff in Japan and Taiwan. It has a yield and a good quality of protein (Morvimura & Tamiya, 1954), lipid

soluble vitamins, choline, and all the minerals in an available form (Shino *et al.*, 1967). Administration of *Chlorella* has been shown to play some biochemical functions, such as promoting the growth rate of animals (Ishibashi, 1972), boosting immune function (Tanaka *et al.*, 1984; Singh *et al.*, 1998), preventing stress-induced ulcer (Tanaka *et al.*, 1997), and influencing the lipid contents of the liver and serum in ethionine treated rats (Wang et al., 1980) and in cholesterol contained diet treated rabbits (Sano & Tanaka, 1987; Sano et al., 1988). In addition, acute administration of *Chlorella* produced a significant hypoglycemic effect in alloxanized rats (Rodriguez-Lopez & Lopez-Quijada, 1971) and STZ mice (Shih, 2001). However, the possible mechanisms of its hypoglycemic effects have not been investigated.

Blood glucose is utilized, under the influence of insulin, by tissues or stored as glycogen in the liver and muscles. Excessive glucose can be also stored as triglyceride via a process of lipogenesis in adipose tissues. Thus the aims of this study are to investigate the primary mechanism(s) of hypoglycemic effects of *Chlorella* by measuring blood glucose and insulin level, hepatic and muscle glucose uptake, adipose tissue lipogenesis and fasting non-esterified fatty acid levels. STZ diabetic animal model has previously been used by other investigators to study hyperglycemia-induced clinical and/or cellular conditions and anti-diabetic drug (Dresner LS *et al*., 1997; Marette *et al*, 1999; Liu *et al*., 2000).

Methods:

Mice (n=8-10, age 3 weeks old) received 60 mg/kg (i.p.) of Streptozocin (STZ) in citrate buffers (10 mM, pH 4.8) as STZ mice (Dresner *et al* 1997) or buffer only as sham STZ mice.

Plasma insulin level.

Blood samples were taken 20 min after the drugs or saline. Plasma was stored at -20℃ for further assay. Plasma insulin was determined by using EIA system assay kits (Amersham Life Science RPN 2567).

Adipose tissue lipogenic rate.

Fatty acid synthesis was measured in vivo by following the incorporation of [^{3}H]-H_2O into adipose tissue fatty acids (Mercer & Trayhurn, 1983). Fed mice were given *Chlorella* or saline 1h prior to one dose of insulin (2.5 IU kg^{-1} body weight i.p) and 30 min later then received 0.5 mCi [^{3}H]-H_2O i.p (Amersham Life Science). Animals were killed 60 min after receiving the tritiated water, free fatty acids were collected and dry to calculate the [^{3}H]-H_2O incorporated

rate..

Glucose uptake assay.

Glucose uptake was measured in vivo by incorporating 2-deoxy-$_{D}$-[1,2-^{3}H] glucose (Amersham Life Science) into glycogen content of the liver and soleus muscles Meszaros K *et al* 1987)..

Non-esterified fatty acid assay.

Following an overnight fast, *Chlorella* or water was administered by oral gavage 60 min prior to blood samples were collected and serum was prepared for non-esterified fatty acid assay, suing commercialized assay kits.

Results

Insulin levels and lipogenic rate in normal ICR mice & STZ mice Slightly lower insulin levels were observed in H_2O-treated STZ mice. Administration of *Chlorella* did not affect plasma insulin in either normal ICR or STZ mice (see fig. 1). However, there was a dramatic increase in plasma insulin levels in glybenclamide-treated mice, expectedly ($p<0.005$). *Chlorella* was shown to enhance the hypoglycemic effects of exogenous insulin in early results. Therefore it is rational to investigate whether insulin-stimulated lipogenetic rates were also increased after *Chlorella* treatment. STZ mice had a slightly, but significantly, higher lipogenic rate than normal ICR mice in brown adipose tissue ($p<0.05$). However the rates in white adipose tissue were much higher in STZ mice than normal ICR mice ($p<0.005$). Insulin-stimulated lipogenic rate in brown adipose tissue (figure 2a) and in white adipose tissue (figure 2b) were only slightly induced at the time mice were killed for assay in *Chlorella*-treated ICR and STZ mice when compared to their respected controls

ICR mice. The uptake was slightly increased in ICR mice after *Chlorella* treatment (see fig. 3a), however the same treatment significantly increased the uptake in STZ mice ($p<0.01$). Although the glucose uptake in the liver was not significantly affected by the treatment of *Chlorella* in ICR mice, the uptake in soleus muscles was statistically significantly higher in *Chlorella*-treated ICR mice than that in controls ($p<0.05$,). However, *Chlorella* treatment did not produce a similar outcome in STZ mice as that seen in ICR mice (see fig. 3b). There was no changed in non-esterified fatty acid levels after *Chlorella* treatment in ICR mice (see fig 4).

However, the level was significantly lower in *Chlorella*-treated STZ mice compared to H_2O-treated STZ mice ($p<0.05$). Glybenclamide treatment produced a slightly but not statistically higher level in ICR mice.

Discussion:

The data of *Chlorella* on glucose tolerance test, basal glucose test and comparative insulin sensitive test observed in normal mice suggest that *Chlorella* has the potential effects to lower blood glucose level. The results of basal glucose and insulin levels from *Chlorella*-treated STZ mice are consistent with early finding by Rodriguez-Lopez & Lopez-Quijada (1971). Thus, *Chlorella* seems to influence the blood glucose via pathways other than inducing release of insulin from the pancreas.

The hypoglycemic effects of *Chlorella* are demonstrated not via an enhancement of insulin secretion from the pancreas. Since there is no any other investigators have ever demonstrated any other possible mechanisms, the next question is how *Chlorella* works to produce its effects in hyperglycemic STZ mice? Thus changes in lipogeneic rates in adipose tissues cannot be accounted for the hypoglycemic effects of STZ mice treated with *Chlorella*. This can be possibly explained by our previously findings have shown that chronic *Chlorella* administration did not increase body mass significantly in high fat diet-fed rats (Shih & Shih, 2002). Lipogenesis is one of the pathways that blood glucose utilized by the peripheral tissues. Since long-term treatment of certain hypoglycemic agents, e.g. insulin and sulfonyluears, tend to increase body weight and to cause insulin resistance in diabetes, *Chlorella* may be favor in conjunctional control of blood glucose in diabetes than these hypoglycemic drugs.

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