行政院國家科學委員會專題研究計畫　成果報告

青蔥萃取物及相關黃酮化合物影響細胞內膽固醇累積之研究

計畫類別：個別型計畫
計畫編號：NSC92-2320-B-041-004-
執行期間：92 年 08 月 01 日至 93 年 07 月 31 日
執行單位：嘉南藥理科技大學生活應用與保健系

計畫主持人：王柏森

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報告類型：精簡報告

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中　華　民　國 93 年 11 月 3 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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執行單位：嘉南藥理科技大學
報告類型：精簡報告

中　華　民　國　　93 年　10 月　29 日

一、中文摘要

ABCA1 是一 220kDa 大小的醣蛋白。目前已知 ABCA1 在細胞排除細胞內磷脂質與膽固醇過程中，扮演重要角色。在本實驗中，我們探討青蔥萃取液(WOE)與其主要類黃酮成分（quercetin 與 kaempferol）是否可以影響 ABCA1 在巨噬細胞中的表現，進而影響細胞內膽固醇含量。目前結果顯示，脂多醣體可以抑制巨噬細胞表現 ABCA1，而青蔥萃取液、quercetin、kaempferol 則抑制此作用。另一方面，以青蔥萃取液、quercetin、kaempferol 預先處理 24 小時後，可以減少氧化態低密度脂蛋白（oxLDL）增加巨噬細胞內膽固醇。

關鍵詞：青蔥萃取物、巨噬細胞、ABCA1

Abstract

ABCA1 is a glycoprotein with an 220 kDa molecular weight. It has been reported that ABCA1 required for the efflux of phospholipids and cholesterol from cells. In this study, we attempt to clarify the effect of Welsh onion extract (WOE) and its major flavonoid derivatives (quercetin and kaempferol) on the expression of ABCA1 in RAW 264.7 macrophage cells, and that content of intracellular cholesterol. As results shown, ABCA1 expression was decreased by lipopolysaccharide (LPS) in RAW 264.7 macrophage cells. And, this inhibitory effect of LPS on ABCA1 expression could be blocked by WOE, quercetin and kaempferol. On the other hand, the increasing effect of oxidized low density lipoprotein (oxLDL) on intracellular cholesterol content could be inhibited by pretreatment of WOE, quercetin and kaempferol for 24 hr in macrophages.

Keywords: Welsh onion extract, macrophage, ABCA1

二、緣由與目的

It is a well known fact that vegetables belonging to Allium species, especially garlic and onion, have been used in cardiovascular disease prevention and attracted a great deal of attention. In Taiwan, Welsh onion (Allium fistulosum L., Alliaceae) is an important flavoring vegetable crop and used for cooking. And, a number of sulfur-containing amino acids and peptides are found in allium plants such as onion and garlic that have physiologic functions. In HepG2 cells, S-propyl cysteine decreased the secretion of apolipoprotein B100. The compound reduced the secretion of newly synthesized triacylglycerol and cholesterols（1）. It has also been reported that Welsh onion or garlic extracts can modulate rat aortic vascular tone (2, 3) and inhibit platelet aggregation (4, 5). Furthermore, numerous laboratory and clinical investigations have confirmed garlic with the well-known properties (reduction of hyperlipidemia, hypertension; prevention of thrombus formation) that may be useful in the prevention, and treatment of atherosclerotic cardiovascular diseases.

It has been established that an inverse correlation between HDL cholesterol level and cardiovascular mortality （6）. The HDL-mediated transport of cholesterol from extra-hepatic tissues to the liver, a process called reverse cholesterol transport, is believed to play a critical role in cholesterol homeostasis. Furthermore, it has been also known that HDL and apoA can enhance cholesterol efflux from macrophage foam cells （7）. Numerous ATP-binding cassette (ABC) transporters are expressed in monocyte-derived macrophages and are subject to sterol-dependent regulation. Many studies also have suggested that the ABCA1 facilitate the efflux of cholesterol onto lipid-deficient apolipoproteins. ABCA1 has been identified as a key regulator of macrophage cholesterol efflux and HDL-mediated reverse cholesterol transport. And, cholesterol loading induced ABCA1 expression in macrophages, and can be reversed by HDL（8, 9）. On the other hand, different pathways involving high-density lipoprotein (HDL) and apoA promote cellular cholesterol efflux have been described. Nuclear hormone receptors

including PPAR/RXR heterodimers are recognized as direct or indirect regulators of ABCA1 expression and are discussed as potential targets for pharmacological intervention in cardiovascular disease.

However, whether Welsh onion extract or flavonoid-derivatives can modulate intracellular cholesterol accumulation through ABCA1 is still unknown. Now, we will investigate whether Welsh onion extract and flavonoid-derivatives can influence ABCA1 activities or their expressions.

三、結果與討論

Effect of Welsh Onion Extract, quercetin and kaempferol on ABCA1 expression in RAW macrophages.

To evaluate the changes of ABCA1 protein during inflammatory processes in macrophages, we examined the effect of LPS on the expression of ABCA1 in RAW 264.7 macrophages by immunoblot. Figure 1 shows the ABCA1 protein expression in macrophages after 3-24 hr incubation with or without LPS. After 24 hr treatment, macrophages that were incubated in medium containing 1 μg/mL LPS showed a obviously lower expression of ABCA1 protein than did cells incubated in medium without LPS. Next, we compared the magnitude of the effects of Welsh onion extract (WOE) on ABCA1 protein expression in macrophages. As Figure 2 shown, after 24 hr treatment, ABCA1 protein expression was increased in macrophages with increasing concentration of WOE (0.2 – 1.0 mg/mL). At 1.0 mg/ml, WOE also increased ABCA1 protein with increasing incubation periods (3- 24 hr).

Furthermore, as Figure 3 shown, after 12 hr treatment, LPS obviously decreased the mRNA of ABCA1 in macrophages. This inhibitory effect of LPS on ABCA1 mRNA could be block by 1 mg/ml WOE. As Figure 4 shown, the effect of Quercetin (20 μM) and kaempferol (30 μM) on ABCA1 mRNA increasing were superior to WOE

Effect of Welsh Onion Extract, quercetin and kaempferol on intracellular cholesterol content.

As shown in Figure 5E, oxLDL (20 μg/ml) obviously increased intracellular cholesterol content after 2 hr incubation. Pretreatment with WOE (0.5 mg/ml), quercetin (20 μM) and kaempferol (30 μM) for 24 hr could decrease oxLDL-induced intracellular cholesterol accumulation in macrophages (Figure 5F, 5G and 5H).

四、計畫成果自評

青蔥為國人日常使用的蔬菜之一，而國外的相關研究文獻不多見。本實驗數據初步證實青蔥與與其主成分類黃酮 quercetin 及 kaempferol 可調節巨噬細胞膽固醇排除器 ABCA1 之蛋白及 mRNA 表現，且運用螢光標定方式觀察膽固醇排出，則是新的發現。

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Figure 1

LPS («g/ml)	0	0.002	0.02	0.2	1	1	1	1
Time (hr)	24	24	24	24	24	12	6	3

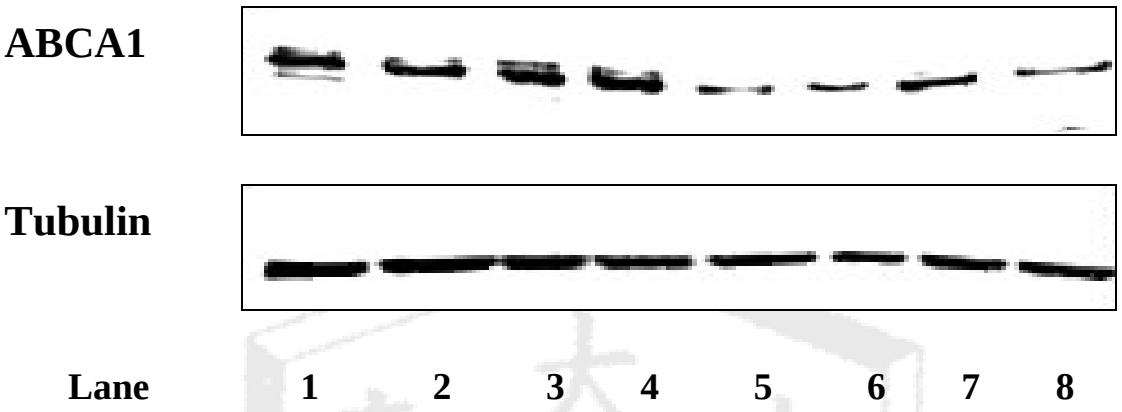


Figure 2

WOE (mg/ml)	0	0.1	0.2	0.5	1	1	1	1
Time (hr)	24	24	24	24	24	12	6	3

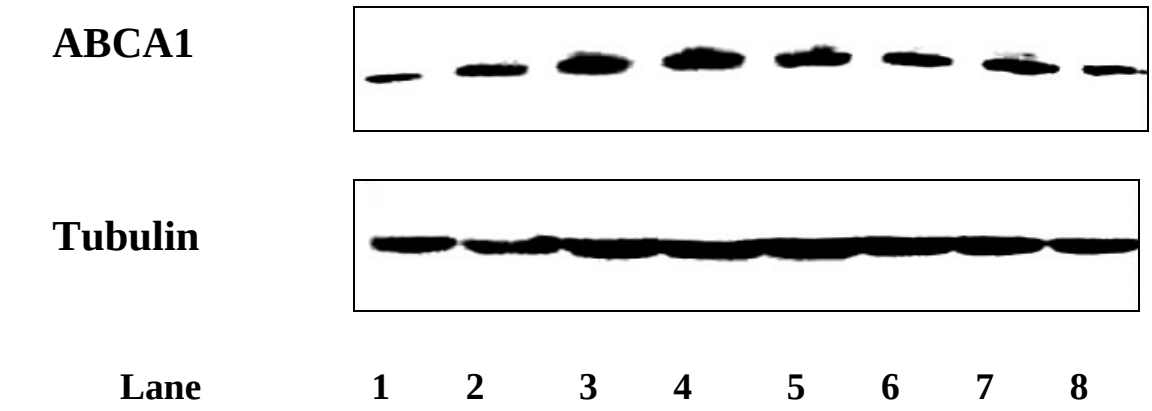


Figure 3

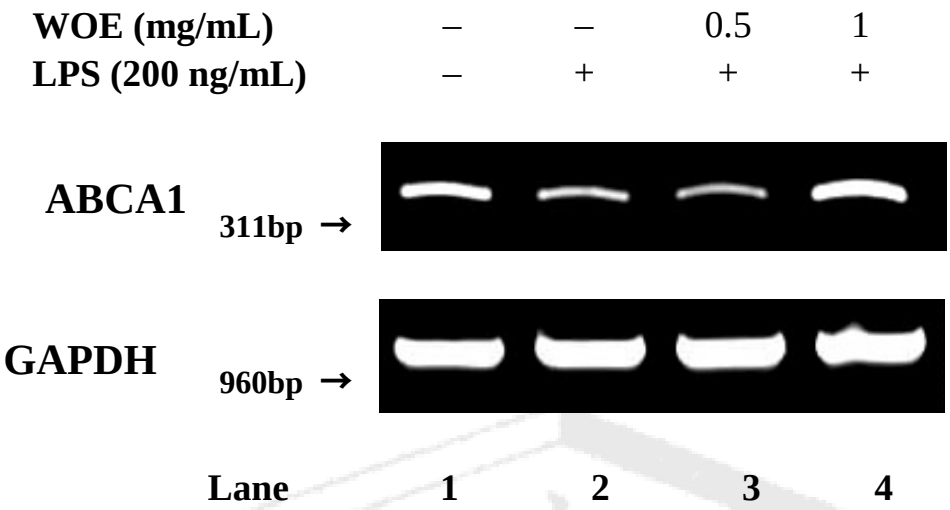


Figure 4

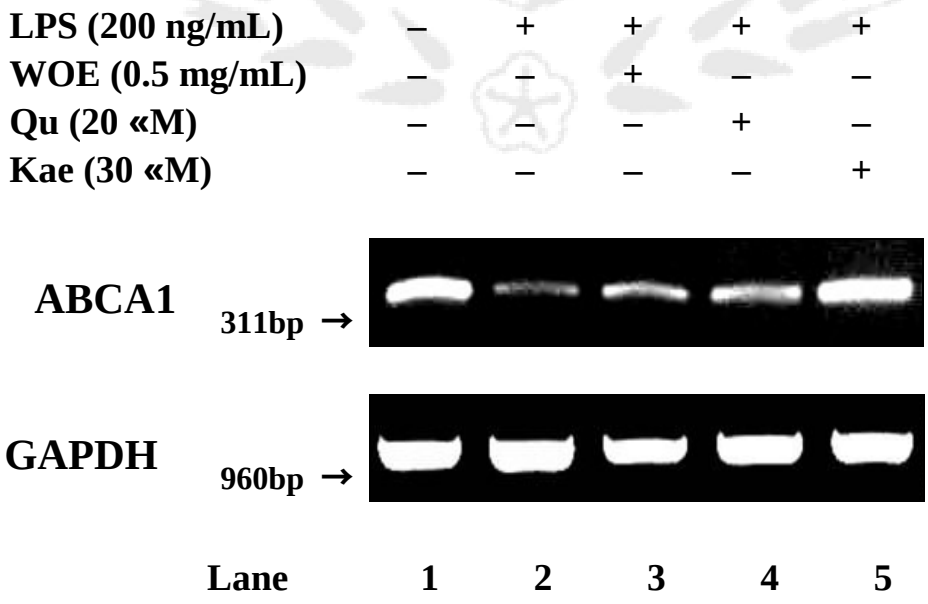
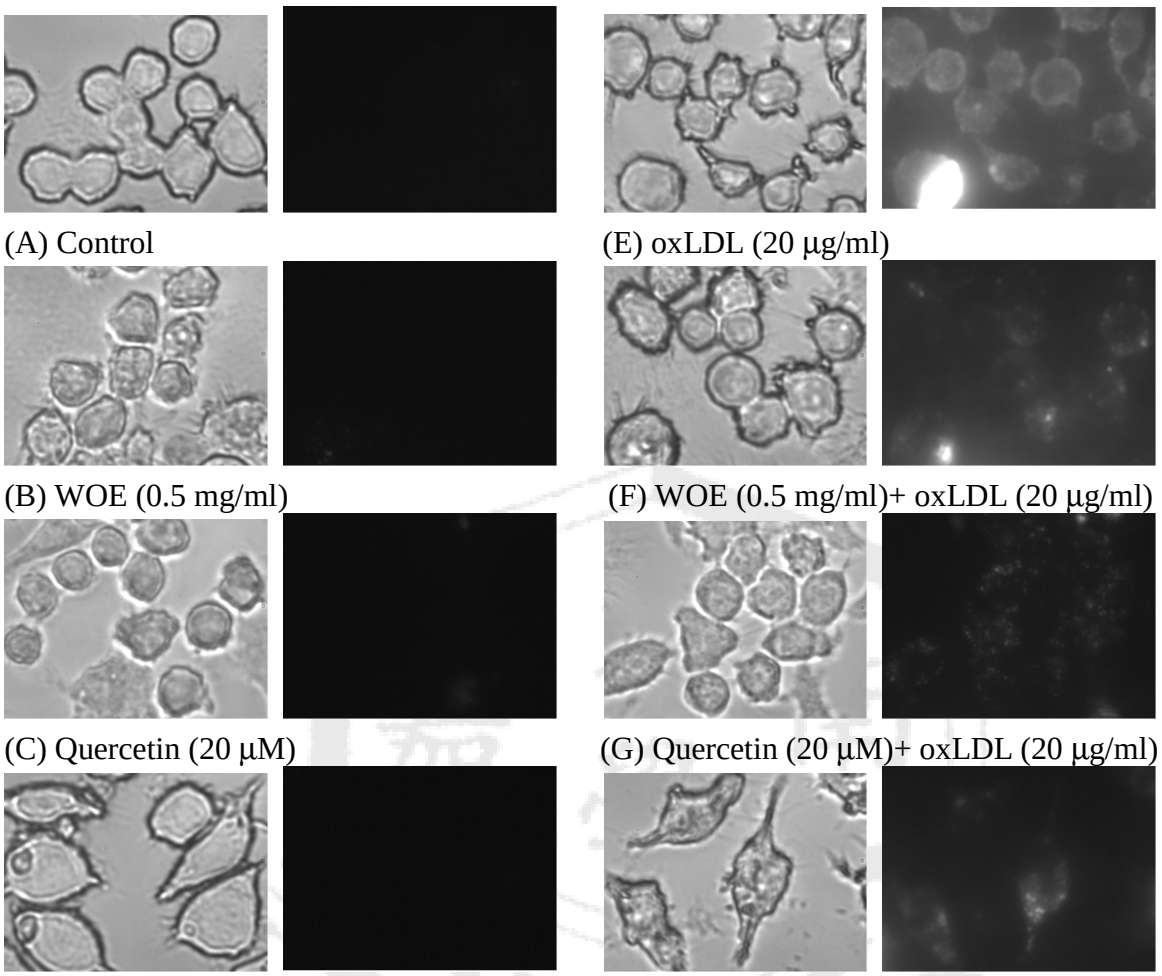


Figure 5



(A) Control (E) oxLDL (20 μg/ml)

(B) WOE (0.5 mg/ml) (F) WOE (0.5 mg/ml)+ oxLDL (20 μg/ml)

(C) Quercetin (20 μM) (G) Quercetin (20 μM)+ oxLDL (20 μg/ml)

(D) Kaempferol (30 μM) (H) Kaempferol (30 μM)+ oxLDL (20 μg/ml)