行政院國家科學委員會補助專題研究計畫 ■ 成果報告
□期中進度報告

青蔥萃取物與相關黃酮類化合物影響脂蛋白清除受體功能之研究

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執行單位：嘉南藥理科技大學　生活應用與保健系

中　華　民　國　92　年　10　月　28　日

一、中文摘要

SRB-1 與 CD36 分別是 82 與 88kDa 的脂蛋白清除受體。目前已知 SRB-1 與 CD36 在巨噬細胞轉形為泡沫細胞過程中，扮演重要角色。在本實驗中，我們探討青蔥萃取液(WOE)與其主成分類黃酮(Quercetin, kaempferol)是否可以影響 SRB-1 與 CD36 在巨噬細胞中的表現。目前結果發現，脂多醣體(LPS)可以減少 SRB-1 蛋白及增加 CD36 蛋白，而青蔥萃取液(WOE)則可抑制脂多醣體的作用。另一方面，Quercetin 加強 WOE 增加 SRB-1 蛋白的作用。且 Quercetin 及 Kaempferol 可以抑制脂多醣體減少 SRB-1 mRNA，而 WOE 及 Quercetin 則可抑制脂多醣體增加 CD36 mRNA。

關鍵詞：SRB-1、CD36、巨噬細胞、青蔥、類黃酮

Abstract

SR-B1 and CD36 are lipoprotein scavenger receptors with 82 and 88 kDa molecular weight, respectively. It has been reported that SR-B1 and CD36 play important role in the transformation process of foam cell formation by macrophage. 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 SR-B1 and CD36 in RAW 264.7 macrophage cells. The results showed the expression of SR-B1 decreased and of CD36 increased after stimulated with lipopolysaccharide (LPS) in RAW 264.7 macrophages for 24 h. And, WOE suppresses LPS-decreased SR-B1 and increased CD36 expression by dose-dependent manner. On the other hand, incubation of macrophages with 20 uM Quercetin enhanced WOE-increased SR-B1 production after 24 hr. And, after 12 hr treatment, Quercetin and Kaempferol also blocked LPS-decreased SR-B1 mRNA in cells. In addition, WOE and kaempferol also inhibited LPS-promoted CD36 mRNA expression in cells.

Keywords: SR-B1, CD36, macrophage, Welsh onion, flavonoid

二、緣由與目的

Goldstein et al have first reported a receptor on macrophages that mediated the uptake and degradation of oxidized LDL and produced massive intracellular cholesterol deposition (1). Currently, it is known that this receptor belongs to a large family of scavenger receptors, all mediating the uptake of modified LDL (2). These scavenger receptor pathways include scavenger receptor A (SR-A), CD-36, and macrosialin (2-4).

Acton et al. (5) provided the first evidence that scavenger receptor class B1 (SR-B1), a member of CD-36 family, bind HDL and can mediate the selective uptake of HDL. Then, 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 can enhance cholesterol efflux from macrophage foam cells (6).

CD-36 has also been proved to be highly regulated in macrophage during differentiation (7) and to be present in lipid-loaded macrophages in atherosclerotic lesions (8). This scavenger receptor is enhanced by IL-4 (9), macrophage colony-stimulating factor (7), modified LDL (10), and peroxisome proliferator activated receptor ligands (11). Unlike SR-B1, CD-36 is more broadly expressed and has been proved to play an important role in lipoprotein and lipid metabolism (12). Although a role for CD-36 in atherogenesis in humans has not yet been established, multiple line of evidence suggest CD-36 plays a role in lipid accumulation and macrophage foam cell formation in vivo and in vitro.

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. It has been reported that Welsh onion or garlic extracts can modulate rat aortic vascular tone (13, 14) and inhibit platelet aggregation (15, 16). Numerous laboratory and clinical investigations have confirmed garlic with the well-known properties (reduction of hyper-lipidemia, hypertension; prevention of thrombus formation) that may be useful in the prevention, and treatment of atherosclerotic cardiovascular diseases. However, whether Welsh onion and its flavonoid-derivatives can modulate the expression of macrophage scavenger receptors is still unknown. Now, we will investigate whether Welsh onion extract and its flavonoid-derivatives can influence SR-B1 and CD36 expression in macrophages.

三、結果與討論

Effect of Welsh Onion Extract on SR-B1 and CD36 protein expression in RAW macrophages

To evaluate the changes in SR-B1 expression on the cell surface, we examined the effect of LPS on the expression of SR-B1 in RAW 264.7 macrophages by immunoblot. Figure 1 shows the SR-B1 expression in macrophages after 2-24 hour time course incubation with and without LPS. After 24 hr treatment, macrophages that were incubated in medium containing 1 ug/mL LPS showed a obviously lower expression SR-B1 than did cells incubated in medium without LPS. Next, we compared the magnitude of the effects of Welsh onion extract (WOE) on SR-B1 and CD36 expression on macrophages. Figure 2 showed WOE at 0.5 and 1 mg/mL increased SR-B1 expression in macrophages. And, 200 ng/mL LPS obviously enhanced 0.5 and 1 mg/mL WOE-increased SR-B1 expression to 130 and 150 %, respectively, of cells cultured with WOE alone. WOE did not affect CD36 expression in RAW cells. But, 0.5 and 1 mg/mL WOE suppressed LPS-induced CD36 expression to 50 and 10 %, respectively, of cells cultured with LPS alone.

Effect of quercetin and kaempferol on SR-B1 and CD36 expression in Macrophages

To evaluate further evidence that quercetin or kaempferol identified in Welsh onion extract is involved in SR-B1 and CD36 expression in RAW 264.7 macrophages. Figure 3 showed at 20 uM quercetin enhanced WOE-increased SR-B1 protein expression in RAW cells. Furthermore, Figure 4 showed quercetin (20 uM) and kaempferol (30 uM) blocked LPS-decreased SR-B1 mRNA after 12 hr treatment. On the other hand, WOE (0.5 mg/mL) and quercetin (20 uM) blocked LPS-increased CD36 mRNA after 12 hr treatment.

四、計畫成果自評

青蔥為國人日常接觸的食品之一，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 可調節巨噬細胞脂蛋白清除受體 SR-B1 與 CD36 蛋白及 mRNA 表現，則是新的發現。

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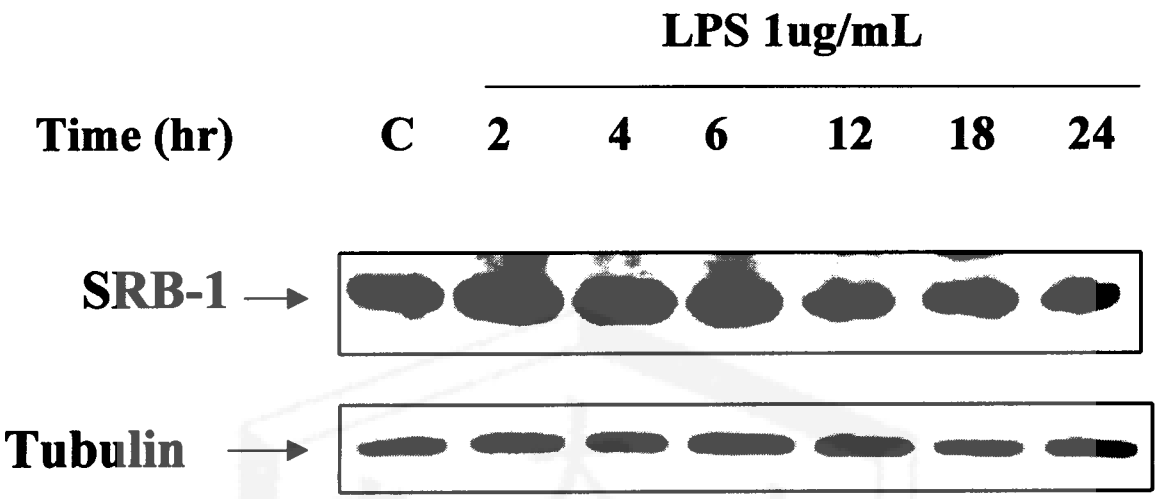

Fig 1

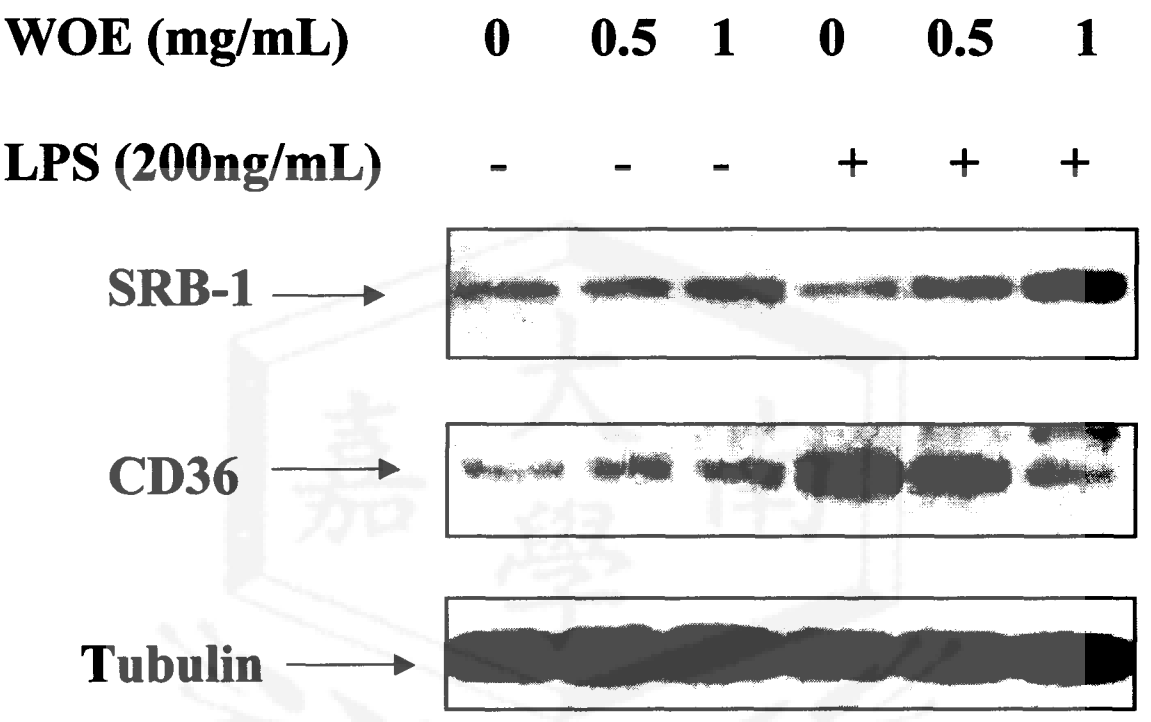

Fig 2

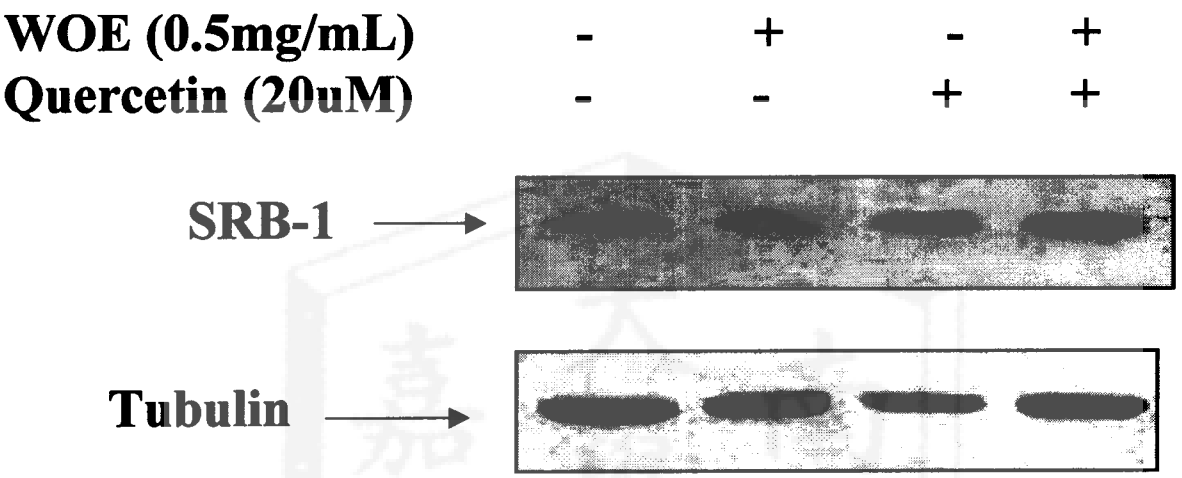

Fig 3

WOE(0.5mg/mL)	-	-	+	-	-
LPS(200ng/mL)	-	+	+	+	+
Quercetin(20uM)	-	-	-	+	-
Kaempferol(30uM)	-	-	-	-	+

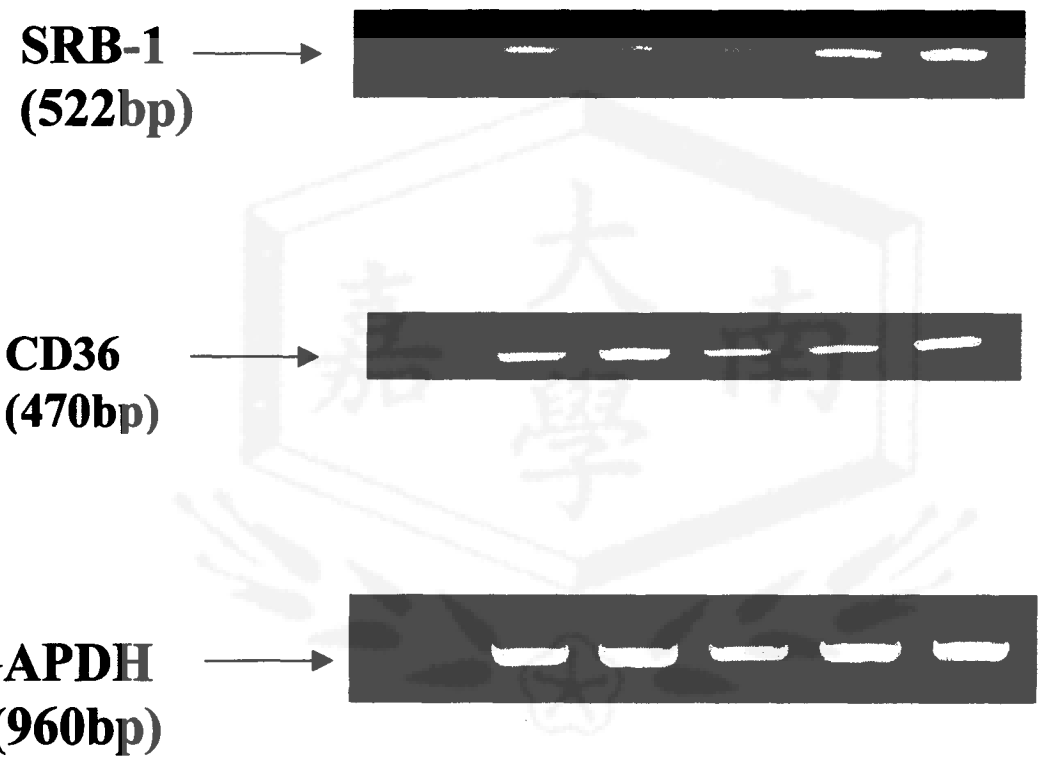

Fig 4