Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmacology 554 (2007) 191–195
www.elsevier.com/locate/ejphar

Influence of Crocetin on experimental atherosclerosis in hyperlipidamic-diet quails

Shu-Ying He [a], Zhi-Yu Qian [b,*], Na Wen [b], Fu-Tian Tang [c], Guang-Lin Xu [d], Cheng-Hua Zhou [b]

[a] School of Life Science and Technology, China Pharmaceutical University, Nanjing, 210009, China
[b] Department of Pharmacology, China Pharmaceutical University, Nanjing, 210009, China
[c] Laboratory of Pharmacology and Toxicology, School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510080, China
[d] College of Life Science, Nanjing Normal University, Nanjing 210097, China

Received 28 May 2006; received in revised form 23 September 2006; accepted 27 September 2006
Available online 19 October 2006

Abstract

Antioxidants have been expected to have potential as antiatherogenic agents. Crocetin is a natural carotenoid antioxidant isolated from *Gardenia jasminoids* Ellis. Therefore, in the present study, we investigated the inhibitory effect of Crocetin on experimental atherosclerosis in quails. The atherosclerosis model was established by feeding hyperlipidamic diet to quail and Crocetin (25, 50, 100 mg/kg/day) was administered by oral gavage. At the 9th week, serum lipids, malondialdehyde and nitric oxide were measured, and Hematoxylin–Eosin (H&E) stains was used to investigate the histopathological changes of aorta. Results showed that Crocetin could reduce the levels of serum total cholesterol, triglyceride, low density lipoprotein cholesterol and inhibit the formation of aortic plaque. Crocetin could also reduce malondialdehyde and inhibit the descending of nitric oxide in serum. The results suggested that Crocetin could inhibit the formation of atherosclerosis in quails, which might be related to the hypolipidemic effects along with the antioxidative properties of Crocetin.

Keywords: Atherosclerosis; Crocetin; Lipids; Malondialdehyde; Nitric oxide; (Quail)

1. Introduction

The pathogenesis of atherosclerosis involves endothelium dysfunction, infiltration of monocytes, activation of monocytes into macrophages, and smooth muscle cell proliferation (Hessler et al., 1979). Recent studies have demonstrated that oxidized low density lipoprotein (Ox-LDL) plays an important role in the initiation and progression of atherosclerosis (Wu et al., 1998). Minimally modified LDL is capable of inducing gene expression in endothelial cells that may result in the acceleration of atherogenesis (Steinberg et al., 1989; Cushing et al., 1990; Lusis and Navab, 1993). By further modification in the intima, Ox-LDL is taken up by the scavenger receptors of macrophages, gradually leading to the formation of foam cells and fibrous plaques (Parthasarathy et al., 1986). Immunochemical studies have demonstrated that Ox-LDL is present in the atherosclerotic lesions of animals and humans (Palinski et al., 1989; Herttuala et al., 1994; Holvoet and Collen, 1997).

Dysfunction of the endothelium is a hallmark of the early atherosclerotic lesion, and Ox-LDL activates endothelial cells, leading to an alteration of the functional and structural integrity of the endothelial barrier. Ox-LDL causes an increased permeability of the endothelium, and stimulates the expression of adhesion molecules on the endothelial surface, allowing monocytes to attach and transmigrate into the subendothelial space. Further lipid accumulation induces foam cells formation. In addition, Ox-LDL promotes smooth muscle cell proliferation and migration. These processes play key roles in atherogenesis, so Ox-LDL is an important lipoprotein in atherosclerosis (Ross, 1993).

Oxidative stress, specifically the oxidation of LDL, has long been suspected of having a critical role in the development of atherosclerosis, in consequence of which antioxidants have

* Corresponding author. Tel.: +86 25 83271322.
E-mail address: heshuying66@sohu.com (S.-Y. He).

0014-2999/$ - see front matter © 2006 Published by Elsevier B.V.
doi:10.1016/j.ejphar.2006.09.071

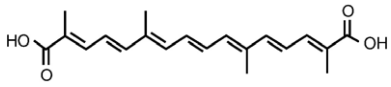

Fig. 1. Chemical structure of Crocetin.

been expected to have potential as antiatherogenic agents. Such agents would be able to inhibit the oxidative modification of LDL that leads to the accumulation of cholesterol in atherosclerotic lesions (Rosenson, 2004).

Since the relationship between Ox-LDL and the progression of atherosclerosis has become more fully understood, the antioxidants, which decrease the level of Ox-LDL contemporarily, have been expected to have potential as antiatherogenic agents. Crocetin, extracted and purified from *Gardenia jasminoids* Ellis in our lab, has been reported to have antioxidative effects and hypolipidemic effects (Zheng et al., 2005). In the present studies, we investigated the antiatherosclerotic effect of Crocetin in vivo and its possible mechanisms against atherosclerorosis.

2. Methods

2.1. Preparation of Crocetin

Crocetin, a carotenoid compound, was isolated from *Gardenia jasminoids* Ellis in our laboratory (98%, HPLC, chemical structure shown as Fig. 1).

2.2. Establishment of atherosclerotic quail model and prophylaxis effect of Crocetin on experimental atherosclerosis (Zhang, 1998)

Male quails (100±10 g, 5–6 weeks, provided by animal experimental center of China Pharmaceutical University) were divided into six groups at random: Normal group, receiving normal diet; Model group, receiving hyperlipidamic diet which containing 14% lard, l6% groundnut oil, 1% cholesterol, 79% commercial basal diet; Crocetin (25) group, Crocetin (50) group, Crocetin (100) group, receiving hyperlipidamic diet plus Crocetin (25, 50, 100 mg/kg/day) respectively; Zhibituo group (Zhibituo is hypolipidemia drug with lovastatin as one of its components. It was originated from Traditional Chinese Medicine and therefore has few side effects.), receiving hyperlipidamic diet plus Zhibituo (1 g/kg). Crocetin and Zhibituo were intragastrically administered once daily for 9 weeks. This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the United States National Institutes of Health.

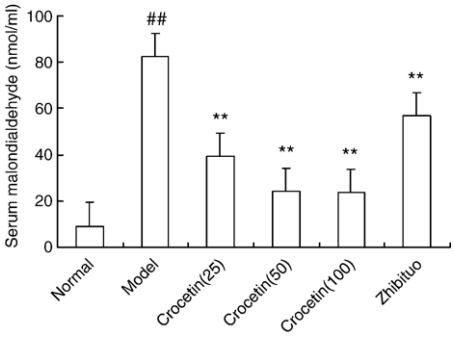

Fig. 2. Effect of Crocetin on serum malondialdehyde level in atherosclerotic quails. Quails (100±10 g) were divided into six groups in random: normal group, model group, Crocetin groups and Zhibituo group. The serum malondialdehyde was investigated at the 9th week. The results are shown as the mean±S.D. (n=12). $^{\#\#}P<0.01$ compared with normal group; $^{**}P<0.01$ compared with model group.

2.3. Measurement of body weight and other biochemical parameters

The body weight of each quail was determined at the beginning of experiment and every two weeks until the end of the experiment. At the 9th week, fasting blood samples were collected followed by centrifugation at 1500 *g*, 4–8 °C for 10 min. Serum total cholesterol, triglycerides, high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol were measured using enzymatic method employing commercial kit supplied by Daiichi Pure Chemicals Co., Ltd. (Japan). Atherosclerosis index was calculated by the formula: atherosclerosis index = (serum total cholesterol − HDL-C) / HDL-C. Serum malondialdehyde and nitric oxide were measured using the commercial kit supplied by NanJingJuLi Biological Engineering Co., Ltd. (China). Serum malondialdehyde content was measured by thiobarbituric acid reactive substances. Serum nitric oxide content was assessed according to the Griess method

Table 1
Effects of Crocetin on serum triglyceride and cholesterol levels in atherosclerotic quails

Group	Triglyceride mmol/L	Total cholesterol mmol/L	LDL-C mmol/L	HDL-C mmol/L	VLDL-C mmol/L	Atherosclerosis index
Normal	1.75±0.33	5.78±0.82	1.23±0.25	4.15±0.70	0.40±0.14	0.40±0.08
Model	3.22±1.20 [a]	41.79±4.22 [a]	13.29±4.57 [a]	5.10±1.55	23.40±2.78 [a]	8.04±3.10 [a]
Crocetin (25)	1.63±0.86 [b]	29.57±9.76 [b]	5.82±3.89 [b]	3.81±0.83 [c]	19.93±6.68	6.61±1.65
Crocetin (50)	1.36±0.35 [b]	27.95±7.68 [b]	4.27±1.42 [b]	4.00±0.69 [c]	19.68±6.29	6.14±1.97
Crocetin (100)	1.12±0.53 [b]	24.74±9.15 [b]	4.04±2.30 [b]	4.02±0.75 [c]	16.69±7.85 [c]	5.18±2.43 [c]
Zhibituo	1.89±1.05 [c]	30.58±10.73 [b]	8.29±5.21 [b]	4.63±1.28	17.67±6.78 [c]	5.66±1.99 [c]

Quails (100±10 g) were divided into six groups in random: normal group, model group, Crocetin groups and Zhibituo group. The levels of serum lipids were investigated at the 9th week. The results are shown as the mean±S.D. (n=12).

[a] $P<0.01$ compared with normal group.

[b] $P<0.01$.

[c] $P<0.05$ compared with model group.