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Anti-Atherogenic Effects of 3,4-Dihydroxy Hydrocinnamides

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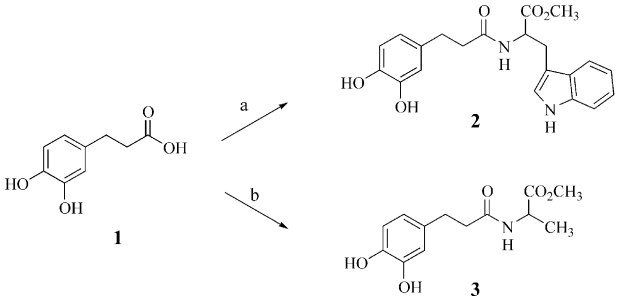
Abstract—3,4-Dihydroxy hydrocinnamides **2** and **3** exhibited the anti-atherogenic activities by inhibiting the formation of aortic fatty streak in high cholesterol-fed rabbits.

Atherosclerosis is characterized by the massive accumulation of fibrous plaques in the large arteries, resulting in thrombosis and occlusion of the artery.[1] In the early stage of atherosclerosis, an aggregation of foam cells so called fatty streak, is distributed sparsely in the innermost layer of the artery wall. As atherosclerosis progresses, the fatty streak advances into the complex and occlusive lesions called fibrous plaques that protrude into the arterial lumen, obstruct the flow of blood, and cause various complications.[2] It has been reported that elevated plasma cholesterol causes the deposition of macrophages and foam cells on the wall of blood vessels, leading to plaque formation and eventually to atherosclerosis.[3] A series of hydroxylated hydrocinnamides were found to possess cholesterol-lowering effects in the cholesterol-fed mice.[4] Among these compounds, 3,4-dihydroxy hydrocinnamides **2** and **3** were selected for further studies on the development of a potent anti-atherogenic agent. This paper deals with the anti-atherogenic evaluation of compounds **2** and **3** in high cholesterol-fed rabbits.

3,4-Dihydroxy hydrocinnamides **2** and **3** were prepared by condensation of 3,4-dihydroxy hydrocinnamic acid (**1**) with L-tryptophan methyl ester hydrochloride or L-alanine methyl ester hydrochloride, respectively, utilizing 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), 1-hydroxybenzotriazole (HOBT), and triethylamine in methylene chloride (Scheme 1).[5,6]

In order to evaluate the anti-atherogenic effects of the compounds **2** and **3**, the atherosclerotic lesions in the aorta were measured in high cholesterol-fed rabbits. The aortic fatty streak lesions were measured after feeding a high cholesterol diet supplemented with 0.05% (wt/wt in diet) of the test compounds (Fig. 1).[7] The fatty streak lesions of thoracic aorta in each group were easily identified by staining with oil red O. to Broad and fused. The fused fatty streak lesions were found in control rabbits supplemented with the 1% cholesterol diet alone, while small plaques were sparsely observed in the groups supplemented with compounds **2** and **3**. The percentage area occupied by atherosclerotic lesions on the inner surface between the first and sixth intercostal arteries was significantly reduced in the compounds **2** ($24.6 \pm 5.3\%$, $p < 0.001$) and **3** ($16.1 \pm 3.6\%$, $p < 0.001$), as compared to control group ($53.5 \pm 6.5\%$).



Scheme 1. (a) L-Tryptophan methyl ester hydrochloride, EDC, HOBT, Et_3N, CH_2Cl_2; (b) L-alanine methyl ester hydrochloride, EDC, HOBT, Et_3N, CH_2Cl_2.

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Table 1. Effects of compounds **2** and **3** supplementation on the plasma lipids in high cholesterol-fed rabbits

Groups	*N*	Plasma lipids (mg/dl)[a]					
		Total cholesterol		HDL-cholesterol		Triglyceride	
		0 weeks	8 weeks	0 weeks	8 weeks	0 weeks	8 weeks
Control	6	54±10	1091±89	34±12	96±38	49±17	125±23
Compound **2** (0.05%, wt/wt diet)	10	50±9	1072±39	32±11	98±21	43±8	104±29
Compound **3** (0.05%, wt/wt diet)	10	53±11	1075±38	37±13	81±31	53±13	116±17

[a]All values are expressed as mean±SD (mg/dl). No significant differences were observed among groups.

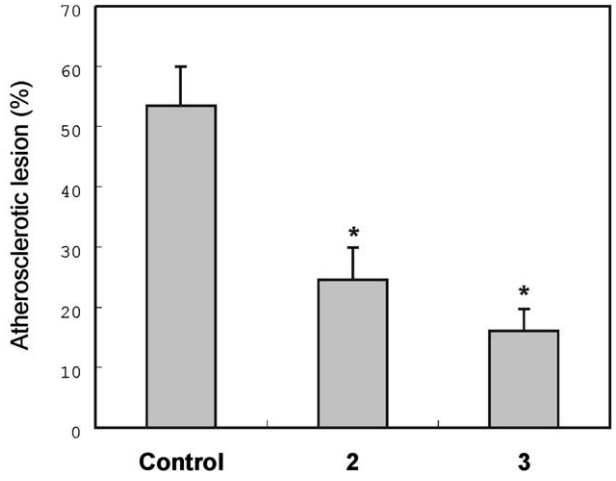

Figure 1. Effects of compounds **2** and **3** on the aortic fatty streak formations in rabbit model fed a high cholesterol diet for 8 weeks. A graph of atherosclerotic lesion size expressed as a percentage of the oil red-O positive area/measured internal surface in each group. Bars represent standard deviations. * is significantly different ($P<0.001$) from control group.

On the other hand, the plasma lipid levels were analyzed after administration of the test compounds **2** and **3** for 8 weeks (Table 1).[7] The plasma total cholesterol levels had increased to almost 1100 mg/dl in all groups and showed no significant difference between groups during the experimental period. The triglyceride and HDL cholesterol levels of groups supplemented with compounds **2** and **3** were also not significantly different from those of control group.

In conclusion, 3,4-dihydroxy hydrocinnamides **2** and **3** significantly inhibited aortic fatty streak formation in high cholesterol-fed rabbits. The anti-atherogenic effects of **2** and **3** seem not to be closely related to plasma lipid levels. Further studies on mechanistic aspects for anti-atherogenic effects of **2** and **3** are underway.

Acknowledgements

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References and Notes

1. (a) Libby, P. *Nature* **2002**, *420*, 868. (b) Davies, M. J.; Thomas, A. N. *Engl. J. Med.* **1884**, *310*, 1137. (c) Rekhter, M. D.; Hicks, G. W.; Brammer, D. W.; Work, C. W.; Kim, J. S.; Gordon, D.; Keiser, J. A.; Ryan, M. J. *Circ. Res.* **1998**, *83*, 705.
2. (a) Brown, A. J. *Curr. Opin. Lipidol.* **2000**, *11*, 667. (b) Beisiegel, U.; St Clair, R. W. *Curr. Opin. Lipidol.* **1996**, *7*, 265. (c) Brown, B. G.; Maher, V. M. *Circulation* **1994**, *89*, 2928. (d) Guazzi, M. D.; Bussotti, M.; Grancini, L.; De Cesare, N.; Guazzi, M.; Pera, I. L.; Loaldi, A. *Circulation* **1997**, *96*, 1145.
3. Ross, R. *Nature* **1993**, *362*, 801.
4. These results were submitted for publication in the journal.
5. Compound **2**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.83 (s, 1H), 8,70 (s, 1H), 8.60 (s, 1H), 8.26 (d, $J=7.6$ Hz, 1H), 7.50 (d, $J=7.6$ Hz, 1H), 7.34 (d, $J=8.0$ Hz, 1H), 7.13 (d, $J=1.6$ Hz, 1H), 7.07 (t, $J=7.6$ Hz, 1H), 6.99 (t, $J=7.6$ Hz, 1H), 6.60 (m, 2H), 6.41 (d, $J=8.0$ Hz, 1H), 4.50 (m, 1H), 4.01 (q, $J=6.8$ Hz, 2H), 3.13 (dd, $J=14.4$, 6.4 Hz, 1H), 3.03 (dd, $J=14.4$, 8.0 Hz, 1H), 2.58 (dd, $J=8.8$, 6.0 Hz, 2H), 2.32 (t, $J=8.8$ Hz, 2H), 1.07 (t, $J=6.8$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 172.7, 172.3, 145.6, 143.9, 136.7, 132.7, 127.8, 124.3, 121.6, 119.3, 119.0, 118.7, 116.3, 116.1, 112.0, 110.2, 61.0, 53.8, 37.8, 31.1, 27.8, 14.5.
6. Compound **3**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8,61 (s, 1H), 8.23 (d, $J=7.2$ Hz, 1H), 6.58 (d, $J=8.0$ Hz, 1H), 6.54 (d, $J=1.6$ Hz, 1H), 6.40 (dd, $J=8.0$, 1.6 Hz, 1H), 4.22 (quint, $J=7.2$ Hz, 1H), 3.58 (s, 3H), 2.59 (m, 2H), 2.28 (m, 2H), 1.22 (d, $J=7.2$ Hz, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 173.9, 172.2, 145.6, 143.9, 132.7, 119.3, 116.3, 116.0, 52.4, 48.1, 37.7, 31.0, 17.6.
7. Male New Zealand White (NZW) rabbits weighing between 2.4 and 2.5 kg at the age of 3 months were used in the experiment. The rabbits were divided into three groups, which were supplemented with a 1% cholesterol diet (RC4, Oriental Yeast Co. Ltd., Tokyo, Japan; $n=6$), or a 1% cholesterol diet containing either 0.05% **2** ($n=10$) or 0.05% **3** ($n=10$) for 8 weeks. All rabbits were individually caged and maintained in a controlled facility at 20±2 °C , relative humidity (55±5%) and a strict 12-h light/dark cycle. First, for the analysis of plasma lipids, the blood samples (3 mL), with ethylenediamine-tetraacetic acid (EDTA) as an anticoagulant, were obtained from the marginal vein of the ear, and centrifuged at 8000g for 10 min. Collected plasma was analyzed in an automatic blood chemical analyzer (Hitachi 7020, Japan) and the plasma concentrations of total cholesterol, HDL-cholesterol, and triglyceride obtained (Table 1). Then, for the evaluation of aortic fatty streak lesions, all rabbits were anesthetized with thiopental sodium (Choongwae Pharma Co., Seoul, Korea) and sacrificed by exsanguinations from the femoral artery. Immediately after opening the thoracic cavity, the aorta was excised, and adventitial tissue grossly adhering to the aorta removed. The aorta was then dissected longitudinally. The portion, a segment between the first and the sixth intercostal arteries, was fixed in 10% neutral buffered formalin for 1 day. The aorta was then placed in absolute propylene glycol for 2 min and stained with oil red O for 4 h. After washing, the extent of the oil red O-positive area was measured and expressed as a percentage of the internal surface using a computer-assisted morphometry system (Image Pro Plus, MD, USA).