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ARTEPILLIN C ISOPRENOMICS: DESIGN AND SYNTHESIS OF ARTEPILLIN C ANALOGUES AS ANTIATHEROGENIC ANTIOXIDANTS

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1. INTRODUCTION

Artepillin C is a diprenyl-4-hydroxycinnamic acid derivative first isolated from *Baccharis* species[1] as a major constituent (>5%) in Brazilian propolis[2] and first synthesized by Uto[3]. Artepillin C is an isoprenyl-containing compound, which has various medicinal effects such as antibacterial,[4] antitumor,[5] apoptosis-inducing,[6] immunomodulating,[7] and antioxidative[8] activities. Therefore, it is useful for drug discovery to study of medicinal chemistry and biology of the isoprenyl-containing compounds, which we have termed "isoprenomics".

Low-density lipoprotein (LDL) is a major cholesterol carrier in the blood, and it is suggested that the oxidative modification of LDL has an important role in the development of atherosclerosis.[9, 10] The effective antioxidants, which prevent the oxidation of LDL, are beneficial in reducing atherosclerosis and coronary heart disease.

Herein, we discuss the design and synthesis of artepillin C analogues and their structure-activity relationship in terms of their inhibitory activity of LDL oxidation as new antiatherogenic antioxidants.

2. MATERIALS AND METHODS

2.1. Chemicals

Synthesis of artepillin C and its analogues was done in our laboratory and the detail of this is currently under preparation for submission. Probucol in a chemically pure form was obtained from a tablet used for a trial (Daiichi Pharmaceutical Co., Ltd, Otsuka

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Pharmaceutical Co., Ltd, and the Dow Chemical Company). 2-thiobarbituric acid (TBA) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 2-(N-morpholino)ethanesulfonic acid (MES) was obtained from Sigma Chemical Company (St. Louis, USA).

2.2. Preparation of Human LDL

A LDL (density, 1.006-1.063 g/mL) was fractionated from human plasma by ultracentrifugation (80,000 rpm, 1.0 hr) using the CS120 ultracentrifuge equipped with a RP80AT rotor (Hitachi Koki Co., Ltd), dialyzed at 4°C against 3 changes of phosphate-buffered saline (PBS; pH 7.35). The LDL solution was flushed with N_2, stored in the dark at 4°C, and used within 5 days from the time of the preparation. Protein was measured by the Bradford method using bovine serum albumin as standard.

2.3. Determination of Lipophilicity

R_M values, defined as a logarithmic function of the R_F values, were determined as a measure of lipophilicity. Octadecyl silica (ODS) reverse phase plates (MERCK RP-18 $WF_{254\,s}$) were used as the stationary phase. A methanol/PBS (pH 7.35) mixture (90/10, v/v) was used as the mobile phase. The plates were developed in closed chromatography tanks, saturated with the mobile phase. Spots were detected under UV light (254 nm). R_F values were determined from three individual measurements. R_M values were calculated from the corresponding R_F values, using the equation: $R_M = \log (1/ R_F - 1)$.[11]

2.4. Calculation of log*P* and log*D*

The *n*-octanol-water partition coefficient (log*P*) and distribution coefficient (log*D*, pH 7.35) of antioxidants were calculated by the program Pallas 3.0 (CompuDrug International Inc., Arizona, USA).

2.5. Assay of Free Radical Scavenging Activity

Free radical scavenging activity was determined by using DPPH at 517 nm according to the method of Blois[12] with some modifications. To 3.0 mL of 100 μM DPPH ethanol solution (60%), containing 40 mM MES (pH 5.5), was added with 3 μL of different concentrations of antioxidants tested. The mean effective concentration of antioxidants, which was required to decrease by 0.200 in the absorption in the reaction with DPPH after 30 min ($IC_{0.200}$), was calculated from the relation between the absorbance vs. the concentration.

2.6. Assay of LDL Oxidation

The effects of antioxidants on kinetics of lipid oxidation of human LDL (50 μg protein/mL) were evaluated by spectrophotometric monitoring of conjugated diene lipid hydroperoxide formation at 234 nm,[13] during copper-induced oxidation (5 μM $CuSO_4$, 10 mM PBS) using a Hitachi U-3300 spectrophotometer at 37°C with a temperature controller SPR-10. The duration of the lag phase, that is the lag time, was calculated by extrapolating the propagation phase. Lipid peroxides were measured as thiobarbituric acid reactive substances (TBARS) by the method of Yagi[14]. The concentration (IC_{50}) leading to 50% decrease of the amount of TBARS was estimated by linear regression analyses from three individual measurements.