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ScienceDirect
Phytomedicine 16 (2009) 258–261
Phytomedicine
www.elsevier.de/phymed

SHORT COMMUNICATION

Inhibitory effect of rhetsinine isolated from *Evodia rutaecarpa* on aldose reductase activity

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Abstract

Aldose reductase inhibitors have considerable potential for the treatment of diabetic complications, without increased risk of hypoglycemia. Search for components inhibiting aldose reductase led to the discovery of active compounds contained in *Evodia rutaecarpa* Bentham (Rutaceae), which is the one of the component of Kampo-herbal medicine. The hot water extract from the *E. rutaecarpa* was subjected to distribution or gel filtration chromatography to give an active compound, N2-(2-methylaminobenzoyl)tetrahydro-1H-pyrido[3,4-b]indol-1-one (rhetsinine). It inhibited aldose reductase with IC_{50} values of 24.1 μM. Furthermore, rhetsinine inhibited sorbitol accumulation by 79.3% at 100 μM. These results suggested that the *E. rutaecarpa* derived component, rhetsinine, would be potentially useful in the treatment of diabetic complications.

Keywords: *Evodia rutaecarpa*; Aldose reductase; Rhetsinine; Diabetic complications

Introduction

The enzymes aldose reductase and sorbitol dehydrogenase play key roles in the polyol pathway (Winegrad, 1987). These enzymes catalyze the reduction of various sugars to sugar alcohols, including glucose to sorbitol. In a diabetic condition, sufficient glucose can enter the tissues, and the pathway operates to produce both sorbitol and fructose. These abnormal metabolic results have been reported to be factors responsible for diabetic complications such as cataracts (Robinson et al., 1983; Frank et al., 1983), retinopathy (Robinson et al., 1989; Engerman, 1989) neuropathy (Young et al., 1983), and nephropathy (Dunlop, 2000). Therefore, aldose reductase inhibitors have considerable potential for the treatment of these diseases, without increased risk of hypoglycemia.

Medicinal herbal and edible plants might be expected to yield less toxic inhibitors of diabetic complications. Many kinds of aldose reductase inhibitors have been found from natural sources (Kawanishi et al., 2003). As demonstrated in the previous study (Matsuda et al., 2002), several flavonoids such as quercitrin, guaijaverin, and desmanthin-1 showed good inhibitory activity against aldose reductase. Furthermore, the structure–activity relationships revealed a catechol moiety on the B ring of flavones and flavonols play an important role against this enzyme (Okuda et al., 1982). However, flavonoids were well known to show broad inhibitory activities against various disrelated enzymes such as α-glucosidase (McDougall and Stewart, 2005) and glycogen phosphorylase (Jakobs et al., 2006).

In our search for specific aldose reductase inhibitors, we found that a hot water extract of *Evodia rutaecarpa*

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0944-7113/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.phymed.2007.04.008

Bentham exhibited significant inhibitory activity. The fruits of *Evodia rutaecarpa* Bentham (Rutaceae) have been used to aid digestion and treat stomach upsets, as a painkiller, and diuretic. Furthermore, this plant is one of the principle components of Kampo-herbal medicine, such as Goshuyu-to, Unkei-to, and Toki-shigyaku-kagoshuyu-shokyo-to. Recently, there has been undertaken scientific research to test the validity of the medicinal claims of Kampo-herbal medicine. With respect to the anti-inflammatory effects, evodiamine and rutaecarpine were reported to inhibit prostaglandin E2 synthesis and evodiamine inhibits the cyclooxygenase-2 induction and NF-kappa B activation (Choi et al., 2006).

In this paper, we report inhibition of aldose reductase by water extracts of *Evodia rutaecarpa* and the isolation of the compound responsible for the activity. In addition, we investigated the ability of this compound to suppress the accumulation of sorbitol in man.

Materials and methods

General experimental procedures

The purity of samples was checked by HPTLC on silica gel $60F_{254}$ (E. Merck) using the solvent system PrOH/AcOH/H_2O (4:1:1), and was detected by iodine vapor and Dragendorff regent. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker DRX500. Chemical shifts were expressed in ppm downfield from tetramethylsilane in $CDCl_3$ as an internal standard. Dry fruits of *Evodia rutaecarpa* Bentham were purchased from Tochimoto Tenkaido Co., (Osaka, Japan). Evodiamine, rutaecarpine, and limonin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). 1,1-Cyclopentanediacetic acid (CDA) was purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan). All other standard samples were purchased from Tokyo Kasei Kogyo Co. Ltd. (Tokyo, Japan).

Extraction and isolation

Evodia rutaecarpa (600 g) was extracted with hot water for 40 min. After cooling, an equal vol. of MeOH was added to this extract. After centrifugation, the supernatant was filtered through Celite and evaporated. This brown syrup was dissolved in 10% HCl and extracted three times with ethyl acetate. After neutralization, the water layer was extracted three times with chloroform. The chloroform layer was evaporated to give a brown oil. This oil was dissolved in 50% MeOH and chromatographed on a Toyopearl HW-40S (20–40 μm, Tosho Corporation, Tokyo, Japan) column (3.0 × 60 cm) with 50% aqueous MeOH and Sephadex LH-20 (25–100 nm, Amersham Biosciences Corp, Piscataway, USA) column (1.9 × 56 cm) with 50% aqueous MeOH as eluant and gave an active fraction (169 mg; N2-(2-methylaminobenzoyl)tetrahydro-1H-pyrido[3,4-b]indol-1-one ; rhetsinine).

Assay of enzyme activity

Recombinant aldose reductase, which retains the same properties exhibited by human muscle and retina, was purchased from Wako Pure Chemical Industries (Osaka, Japan). Aldose reductase activity was spectrophotometrically measured at 37 °C by using 100 mM D, L-glyceraldehyde as the substrate (Cappiello et al., 1994).

Determination of sorbitol in human erythrocytes

Human blood was obtained from a healthy female volunteer, who was fully informed to this study and gave written consent. Erythrocytes from heparinized blood were separated from the plasma and buffy coat by centrifuging at 3000*g* for 30 min. The cells were routinely washed three times with isotonic saline at 4 °C. During the final washing, the cells were centrifuged at 2000*g* for 5 min to obtain a consistently packed cell preparation. The packed cells (1 mL) were then incubated in a Krebs-Ringer bicarbonate buffer (pH 7.4) (4 mL) containing 28 mM glucose in the presence or absence of 40 mM samples at 37 °C in 5% CO_2 for 60 min. The erythrocytes were washed with cold saline by centrifuging at 2000*g* for 5 min, precipitated by adding 6% of cold perchloric acid (3 mL), and centrifuged again at 2000*g* for 10 min. The supernatant was neutralized with 2.5 M K_2CO_3 at 4 °C and used for sorbitol determination (Malone et al., 1980; Haraguchi et al., 1997). The reaction mixture contained the appropriate protein-free supernatant, 50 mM glycine buffer (pH 9.4), 0.2 mM NAD^+, and 1.28 units of sorbitol dehydrogenase. The incubations were performed at 37 °C for 30 min, and the relative fluorescence due to NADH was measured by a fluorescence spectrometer at an excitation wavelength of 366 nm and emission wavelength of 452 nm (Clements et al., 1969).

Results and discussion

Hot water extracts of *Evodia rutaecarpa* were filtered through Celite and evaporated (hot water Fr.). In order to remove the non-specific inhibition factor, 1 mg/mL BSA (final dose) was add to the reaction system. This hot water extract fraction at a concentration of 100 μg/mL inhibited aldose reductase activity by 90.0%. Active