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\textsubscript{50} values of 24.1 \textmu M. Furthermore, rhetsinine inhibited sorbitol accumulation by 79.3\% at 100 \textmu M. These results suggested that the *E. rutaecarpa* derived component, rhetsinine, would be potentially useful in the treatment of diabetic complications.

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**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 \textbeta-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*...
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 60F254 (E. Merck) using the solvent system PrOH/AcOH/H2O (4:1:1), and was detected by iodine vapor and Dragendorff regent. 1H NMR (500 MHz) and 13C NMR (125 MHz) spectra were recorded on a Bruker DRX500. Chemical shifts were expressed in ppm downfield from tetramethylsilane in CDCl3 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-Cyclopentanedicarboxylic 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 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 anduffy coat by centrifuging at 3000g 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 2000g 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% CO2 for 60 min. The erythrocytes were washed with cold saline by centrifuging at 2000g for 5 min, precipitated by adding 6% of cold perchloric acid (3 mL), and centrifuged again at 2000g for 10 min. The supernatant was neutralized with 2.5 M K2CO3 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 relativefluorescence 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...
The aqueous layer was neutralized with Na2CO3 and (EtOAc) to give the EtOAc fraction and aqueous layer. The active compound was then dissolved in 10% HCl and extracted with ethyl acetate. Compounds were isolated by bioassay-guided fractionation method. The hot water extract fraction was ineffective. These results suggested that the active compound has an alkaloidal moiety. The chloroform fraction was further chromatographed with Toyopearl HW-40S and Sephadex LH-20 to give an active compound (169 mg). The active compounds were isolated as a yellow powder with optical rotation [α]D 320.1400 [M+H]+; C 19H18O2N3 requires 320.1399). The 13C NMR (CDCl3, 125 MHz) spectroscopic data revealed the presence of two amide groups at C-14 (δ 163.1) and C-5 (δ 167.4). The 1H NMR spectral data was in accord with those of N2-(2-methylaminobenzoyl)tetrahydro-1H-pyrido[3,4-b]indol-1-one (rhetsinine, Fig. 1) (Joshi et al., 1991).

Major alkaloidal components, evodiamine, rutaecarpine, and the bitter principle limonin, have been reported with various biological activities. Evodiamine induces human melanoma cell death through an IL-1 mediated pathway (Wang et al., 2005a) and rutaecarpine gives protection from gastric mucosa injury (Wang et al., 2005b). Limonin has been known to inhibit HIV-1 replication on infected human mononuclear cells (Battinelli et al., 2003). On the other hand, the isolation of rhetsinine has previously been reported (Yuan et al., 1997) but the biological activity has not been reported. Interestingly, the major alkaloidal components exhibited less than 50% inhibition, even at concentrations of 5 mg/mL. On the other hand, rhetsinine showed good inhibitory activity with IC50 values of 24.1 μM. It has already been reported that the activity of erythrocyte aldose reductase increases in diabetic patients (Malone et al., 1984) and erythrocyte sorbitol levels in rats are positively correlated with the levels in the lens, sciatic nerve, and retina (Hotta et al., 1985; Kinoshita, 1974). Thus, we investigated the effect of rhetsinine on sorbitol in human erythrocytes. The effects of rhetsinine and a positive control CDA (Iimura et al., 1989) with IC50 values of 11.2 μM against aldose reductase on sorbitol accumulation in human erythrocyte are shown in Fig. 2. Sorbitol accumulation was 15-fold greater when cells were incubated in a high glucose medium, compared to that in a glucose-free incubation. Rhetsinine and CDA inhibited sorbitol accumulation by almost 79.3% and 82.8% at 100 μM, respectively. These results suggested that rhetsinine could prevent the accumulation of sorbitol in human erythrocyte.

The most serious problem in diabetes is that complications develop slowly and cause significant tissue damage before clinical signs appear. In recent years, the possibility of preventing the onset of diabetes using dietary supplements and/or herbal medicines has attracted increasing attention. In this study, hot water extracts of Evodia rutaecarpa and their component, rhetsinine showed inhibitory activity against aldose reductase. Furthermore, rhetsinine significantly suppressed sorbitol accumulation in human erythrocytes. This compound could be potentially useful in the treatment of diabetic complications.

Fig. 2. Effects of rhetsinine on sorbitol accumulation in human erythrocyte. Erythrocyte was incubated for 60 min in a Krebs-Ringer bicarbonate buffer containing 28 mM glucose and in the presence or absence of 100 μM rhetsinine or 1,1-cyclopentane diacetic acid (CDA). Each value represents the mean ± SEM (n = 3). ** Significant difference (p < 0.01) compared with the control.

Fig. 1. Structure of rhetsinine.

References


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