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Rat Lens Aldose Reductase Inhibitory Activities of *Coptis japonica* Root-Derived Isoquinoline Alkaloids

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The inhibitory activity of *Coptis japonica* root-derived materials was evaluated against lens aldose reductase isolated from male Sprague−Dawley rats and compared to that of three commercially available isoquinoline alkaloids (berberine sulfate, berberine iodide, and palmatine chloride), as well as quercitrin as aldose reductase inhibitor. The biologically active constituents of *C. japonica* extract were characterized as the isoquinoline alkaloids, berberine chloride and palmatine iodide, by spectral analysis. The inhibitory effects varied with both chemical and concentration used. The IC_{50} values of berberine chloride and palmatine iodide are 13.98 and 13.45 nM, respectively. Among three berberines and two palmatines, the inhibitory activity was much greater for the choridated and sulfated analogues than for those with iodide. Quercitrin was a much more potent inhibitor than berberines and palmatines. Nonetheless, berberines and palmatines may be useful as lead compounds and new agents for aldose reductase inhibition.

KEYWORDS: Aldose reductase; *Coptis japonica*; diabetic complications; isoquinoline alkaloid; quercitrin

INTRODUCTION

Diabetes mellitus affects 300 million people worldwide and is the leading cause of blindness, kidney failure, heart attack, and amputation among adults (*1*). Achieving blood glucose levels as close to normal as possible has been considered as one of the major goals of therapy for those suffering from diabetes mellitus, as high blood glucose level is implicated in the development of macro- and microvascular complications associated with diabetes (*2*). However, in clinical practices, normalizing blood glucose levels is a formidable challenge. Even more difficult is the control of postprandial hyperglycemia. Fortunately, both dietary and pharmacological tools are now available for its management. The pharmacological agents with the greatest effect on postprandial hyperglycemia include insulin lispro, amylin analogues, and α-glucosidase inhibitors (*3*−*5*). In hyperglycemia associated with diabetes, the use of aldose reductase inhibitors has been reported for the treatment of diabetic complications (*6*−*8*).

Aldose reductase (EC 1.1.1.21) catalyzes the reduction of glucose to the corresponding sugar alcohol, sorbitol, which is subsequently metabolized to fructose by sorbitol dehydrogenase. Such conversion of glucose into fructose constitutes the polyol pathway of glucose metabolism. Under normal physiological conditions, this pathway plays a minor role in the glucose metabolism of most tissues. In hyperglycemia associated with diabetes, however, cells undergoing insulin-independent uptake of glucose produce significant quantities of sorbitol due to the poor penetration by the sorbitol through the cellular membranes and its slow metabolism by sorbitol dehydrogenase. The resulting hyperosmotic stress to cells is postulated to be the primary cause for the development of such diabetic complications as retinopathy, cataracts, neuropathy, and nephropathy (*9*). These observations suggest that the inhibition of aldose reductase can be used as a novel, potentially direct pharmacological approach toward the treatment of certain diabetic complications (*10*).

Plants constitute a rich source of bioactive chemicals (*4*−*8*). Since many of them are largely free from adverse effects and have excellent pharmacological actions, they could lead to the development of new classes of possibly safer antidiabetic agents. Additionally, some flavonoids and polyphenol as well as sugar derivatives are found to be effective on the inhibitory activities of aldose reductase (*5*−*8*, *10*). Therefore, much effort has been focused on plants for potentially useful products as commercial aldose reductase inhibitors or as lead compounds. Relatively little work has been done on aldose reductase inhibitory activity of *Coptis japonica* roots (Coptidis Rhizoma) despite its excellent pharmacological action in East Asia (*11*−*14*). Aldose reductase inhibitors isolated from *C. japonica* roots may be a good source for lead compounds as alternatives to the aldose reductase inhibitors currently used. The importance of finding effective aldose reductase inhibitors led us to further investigate natural compounds.

MATERIALS AND METHODS

Chemicals. Berberine chloride, berberine iodide, berberine sulfate, bovine serum albumin, DL-glyceraldehyde, imidazole, lithium sulfate, NADPH, palmatine sulfate, phenylmethylsulfonyl fluoride, and quer-

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citrin were purchased from Sigma Chemical Co. (St. Louis, MO). Coomassie blue reagent was purchased from Bio-Rad (Hercules, CA). Male Sprague−Dawley rats were purchased from Dong Nam Laboratory Animal Research Center Co. (Chonju, Chonbuk, South Korea), and all other chemicals were of reagent grade.

Isolation and Identification. The *C. japonica* roots (5.0 kg), purchased as a commercially available product, were dried in an oven at 50 °C for 2 days, finely powdered, extracted twice with methanol (25 L) at room temperature, and filtered (Toyo filter paper no. 2). The combined filtrate was concentrated in vacuo at 40 °C to give a yield of approximately 19.1% (955 g). The extract was sequentially partitioned into hexane (71.4 g), chloroform (40.1 g), ethyl acetate (16.7 g), butanol (172.6 g), and water-soluble portions (654.2 g) for subsequent bioassay with aldose reductase. The organic solvent portions were concentrated to dryness by rotary evaporation at 40 °C, and the water portion was freeze-dried.

The chloroform fraction (15 g) was chromatographed on a silica gel column (Merck 70−230 mesh, 600 g, 5.5 mm i.d. × 70 cm) and successively eluted with a stepwise gradient of chloroform/methanol (0, 5, 10, 20, 30, 50, and 100%). The active fraction (3.6 g) was chromatographed on a silica gel column and eluted with chloroform/methanol (10:1). Column fractions were analyzed by TLC (chloroform/methanol, 10:1), and fractions with similar TLC patterns were combined. For further separation of the biologically active substances, a preparative HPLC (Waters Delta Prep 4000) was used. The column was a Bondapak C_{18} (2.9 mm i.d. × 300 mm, Waters) using methanol/water (6:4) at a flow rate of 8 mL/min and detection at 254 nm. The chemical substances in the active peak (38 mg) among three peaks showed two main yellow spots on TLC, which were developed by benzene/ethyl acetate/*n*-propanol/methanol/ethylamine (8:4:2:1:1). The two spots were colored orange-red when reacted with the Dragendorff reagent, suggesting that the chemical substances were alkaloids. The active fraction was chromatographed on a silica gel column using the previous solvent system to give fractions 1 and 2. The active compounds were isolated according to the method of Chae et al. (*14*). Fractions 1 (150 mg) and 2 (200 mg) were dissolved in water, and precipitates were obtained from the aqueous solution by adding 1 N HCl to pH 5.0 and 7.5% HI solution to pH 4.0, respectively. The precipitates were collected by filtration, washed with water, and dried under reduced pressure over P_2O_5. The yields from fractions 1 and 2 were 81.4 (compound I) and 92.5 mg (compound II), respectively. R_f values of compounds I and II were 0.67 and 0.43 in benzene/ethyl acetate/*n*-propanol/methanol/ethylamine (8:4:2:1:1), respectively. Structural determination of the active isolates was made by spectral analysis. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AM-500 spectrometer. UV spectra were obtained on a Waters 490 spectrometer, IR spectra on a Bio-Rad FT-80 spectrophotometer, and mass spectra on a JEOL JMS-DX 30 spectrometer.

Isolation of Aldose Reductase from Sprague−Dawley Rats. Crude aldose reductase was prepared from rat lenses. Lenses were removed from the eyes of 8-week-old male Sprague−Dawley rats each weighing 100−150 g and were homogenized in 12 volumes of 135 mM Na,K-phosphate buffer (pH 7.0) containing 0.5 mM phenylmethylsulfonyl fluoride and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 100000*g* for 30 min, and the resulting supernatant was retained as an enzyme preparation. All procedures were carried out at 4 °C. The activity of this preparation was determined by measuring the amount of NADP converted from NADPH per unit time at 37 °C and pH 7.0. One unit (U) of activity is defined as the amount of the enzyme catalyzing the oxidation of 1 μmol of NADPH per minute under our experimental conditions.

Enzyme Inhibitory Assay. Aldose reductase activity was assayed according to the method described by Lee and Kim (*5*). The incubation mixture contained 135 mM Na,K-phosphate buffer (pH 7.0), 100 mM lithium sulfate, 0.03 mM NADPH, 0.04 mM DL-glyceraldehyde, and 50 μL of an enzyme preparation, with or without a plant extract, at a total volume of 1.0 mL. Each plant extract was dissolved in dimethyl sulfoxide, which was found to have no effect on the enzyme activity at less than 1%. Appropriate blanks contained all of the above-mentioned compounds except DL-glyceraldehyde. The reaction was initiated by adding NADPH at 37 °C and stopped by adding 0.5 N hydrochloric acid (0.3 mL). Subsequently, 6 N NaOH (1 mL) containing 10 mM imidazole was added, and the mixture was incubated at 60 °C for 10 min to convert NADP into a fluorescent product. The fluorescence was measured at room temperature with a spectrofluorophotometer (Aminco Bowman series 2, Spectronic Instruments, Rochester, NY) with an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Standards of NADP (0.1−5 μM) were treated in the same manner. All determinations were performed in triplicate. The concentration of each test sample giving 50% inhibition of the enzyme activity (IC_{50}) was estimated from the least-squares regression line of the logarithmic concentration plotted against the remaining activity. The protein content of the enzyme preparation was 0.021 g/L, and aldose reductase activity in the preparation was 7.38 U/L or 370.1 U/g of protein at 37 °C. The protein content of the enzyme preparation was determined using Coomassie blue reagent (Bio-Rad) according to the manufacturer's instructions, with bovine serum albumin as a standard.

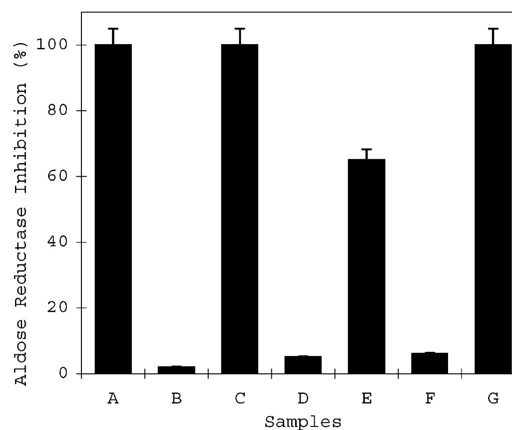

Figure 1. Aldose reductase inhibitory activity of *C. japonica* root-derived materials at a concentration of 0.1 mg/mL: (A) methanol extract, (B) hexane fraction, (C) chloroform fraction, (D) ethyl acetate fraction, (E) butanol fraction, (F) water fraction, and (G) quercitrin.

RESULTS AND DISCUSSION

Fractions obtained from methanol extracts of *C. japonica* roots were determined for the inhibitory activity against lens aldose reductase isolated from male Sprague−Dawley rats (**Figure 1**). The inhibition at a final concentration of 0.10 mg/mL was determined for each of the fractions prepared. At 0.10 mg/mL, chloroform and butanol fractions showed 100 and 65% inhibition against aldose reductase, respectively, whereas other fractions exhibited weak or no inhibition. Due to the strong activity of the chloroform fraction, purification of the biologically active compounds was done by silica gel column chromatography and HPLC, and the isolates were bioassayed. Two active principles were isolated from the chloroform fraction. The isolates were identified as isoquinoline alkaloids by color reaction with the Dragendorff reagent. Structural determination of the isolates was made by spectroscopic methods including MS and NMR and by direct comparison with authentic reference compounds, and they were characterized as the isoquinoline alkaloids berberine chloride and palmatine iodide (**Figure 2**). The ^{13}C and ^{1}H NMR spectra of berberine chloride and palmatine iodide were found to be the same as those for berberine chloride and palmatine iodide isolated from *C. japonica* roots (*14*).

The inhibitory activities of berberine chloride, berberine iodide, berberine sulfate, palmatine sulfate, and palmatine chloride against aldose reductase were compared to that of quercitrin, a naturally occurring flavonoid which has been demonstrated to be a potent aldose reductase inhibitor in vitro

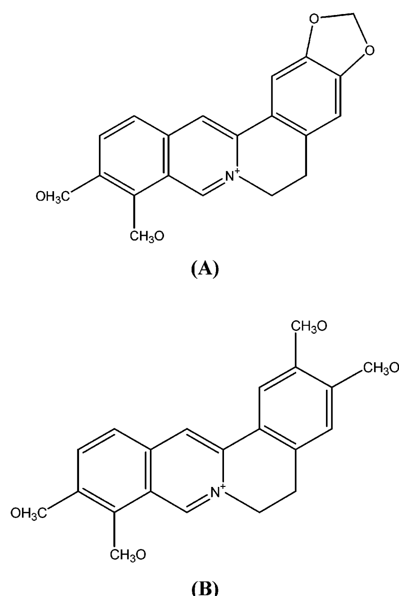

Figure 2. Structure of berberine (A) and palmatine (B).

Table 1. Aldose Reductase Inhibitory Activities of *C. japonica* Root-Derived Isoquinoline Alkaloids and Various Isoquinoline Alkaloids

compound	final concn (mg/mL)	aldose reductase inhibition (%)	IC_{50} (nM)
berberine chloride	0.1	100	13.98
	0.05	88	
	0.01	68	
	0.005	45	
	0.001	29	
berberine sulfate	0.1	100	13.45
	0.05	92	
	0.01	78	
	0.005	51	
	0.001	38	
berberine iodide	0.1	100	32.84
	0.05	78	
	0.01	44	
	0.005	15	
palmatine sulfate	0.1	98	51.78
	0.05	72	
	0.01	43	
	0.005	10	
palmatine iodide	0.1	88	68.00
	0.05	59	
	0.01	28	
	0.005	0	
quercitrin	0.1	100	11.15
	0.05	95	
	0.01	89	
	0.005	83	
	0.001	68	
	0.0005	49	
	0.0001	32	

(**Table 1**). Inhibitory responses varied with the chemicals tested. At 0.1 mg/mL, all five compounds produced significant inhibition (88−100%) against aldose reductase. On the basis of their effective inhibitory activity, the inhibitory effect was determined as the concentration of compound was decreased step by step. The IC_{50} values of berberine chloride, berberine sulfate, and berberine iodide are 13.98, 13.45, and 32.84 nM, respectively. Furthermore, the IC_{50} values of palmatine sulfate and palmatine iodide are 51.78 and 68.0 nM, respectively. These results indicate that the berberines are somewhat more active than the palmatines. Among three berberines and two palmatines, the IC_{50} values of berberine chloride and berberine sulfate are approximately 2.5 times higher than that of berberine iodide, and the IC_{50} value of palmatine sulfate is approximately 1.3 times higher than that of palmatine iodide. These results indicate that inhibitory activity against aldose reductase was much more pronounced in chloridated and sulfated analogues than in the iodides. However, quercitrin (IC_{50} = 11.15 nM) was much more potent than berberines and palmatines.

It has been well acknowledged that plant-derived extracts and phytochemicals are potential alternatives to synthetic inhibitors against aldose reductase (*5*−*8*, *15*). Currently, the compounds isolated from plants as aldose reductase inhibitors are classified as flavonoids and flavonoid-related compounds. These include 5,7,4′-trihydroxy-3,6-dimethoxyflavone isolated from *Acanthospermum australe* (*15*), myricetin 3-*O*-(4′′-acetyl)fucoside from *Anthocepharus chinensis* (*16*), and dihydroflavonol rhamnosides and quercitrin 3-rhamnoside from *Engelhardtia chrysolepis* (*17*). In this study, the components isolated from *C. japonica* roots active against aldose reductase were identified as the isoquinoline alkaloids berberine and palmatine, although the inhibitory responses varied with chemical and concentration tested. It has been reported that the *Coptis* root-derived materials including isoquinoline alkaloids have antibacterial (*14*), antitumor (*18*), antidiarrhea (*19*), and antiinflammatory effects (*20*). It might be expected, then, that the active components isolated from *C. japonica* roots have a range of pharmacological actions for antidiabetic principles.

Aldose reductase inhibitors including quercitrin are currently the most commonly used oral agents for good penetration of sorbitol through cellular membranes and fast metabolism of sorbitol by sorbitol dehydrogenase. More importantly, they are considered as prospective therapeutics for treatment of diabetic complications such as retinopathy, cataracts, neuropathy, and nephropathy (*9*). Berberines and palmatines may be used as lead compounds for the development of antidiabetic therapeutics, although the inhibitory activities of berberines and palmatines were lower than that of quercitrin.

In conclusion, these results indicate that *C. japonica* root-derived materials have inhibitory effects in vitro against rat lens aldose reductase. Based upon our limited data and some earlier findings, the inhibitory action of *C. japonica* root-derived isoquinoline alkaloids confirms their potential utility as antidiabetic agents, although their use in vivo and the clinical efficacies remain to be evaluated.

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