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Inhibitory effects of polyphenols isolated from *Rhus verniciflua* on Aldo-keto reductase family 1 B10

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Aldo-keto reductase family 1 B10 (AKR1B10) is a member of the NADPH-dependent aldo-keto reductase (AKR) superfamily, and has been considered to be a potential cancer therapeutic target. Total extract from the bark of *Rhus verniciflua* (*Toxicodendron vernicifluum* (Stokes)) showed AKR1B10 inhibitory activity. To identify the active compounds from *R. verniciflua* responsible for AKR1B10 inhibition, nine compounds were isolated via bioactivity-guided isolation and tested for their effects against recombinant human AKR1B10 (rhAKR1B10). Results showed that butein, isolated from the ethyl acetate fraction, was most able to inhibit rhAKR1B10. The inhibitory rate of butein against rhAKR1B10 was 42.86% at 1 μM with an IC_{50} value of 1.47 μM, and enzyme kinetic analysis revealed its inhibition mode to be uncompetitive. [BMB reports 2010; 43(4): 268-272]

INTRODUCTION

Aldo-keto reductase family 1 B10 (AKR1B10), also identified as aldose reductase-like or small intestine aldose reductase (ARL-1), is a recently identified member of the aldo-keto reductase (AKRs) superfamily (1). AKR1B10 is a NADPH-dependent oxidoreductase and reduces various aldehydes and ketones, including endogenous substrates such as retinals, farnesal and geranylgeranial (2, 3). AKR1B10 is, however, highly expressed in several types of cancers, including hepatocellular carcinoma (4), lung squamous cell carcinoma, lung adnocarcinoma in smokers (5) and cervical cancer (6). AKR1B10 is also known to be highly active in the reduction of all-*trans*- and 9-*cis*-retinaldehyde, with efficiency comparable to retinaldehyde reductase. Thus, an increase in AKR1B10 activity seriously inhibits retinoic acid synthesis, which can be associated with subsequent loss of cell differentiation and cancer development (7). Recent work showed that down-regulation of the AKR1B10 gene using siRNA promoted cell death in colorectal cancer cells through enhanced cytotoxicity of reactive carbonyls produced from lipid peroxidation (8). In other studies, inhibition of AKR1B10 resulted in disruption of lipid synthesis, mitochondrial function and oxidative status, all of which are involved in important cell survival mechanisms (9, 10). Therefore, AKR1B10 is a potential therapeutic target in certain types of cancer, but specific AKR1B10 inhibitors are not yet clinically available.

Another AKR protein, Aldo-keto reductase family 1 B1 (AKR1B1) has to date been the most studied member of the AKR superfamily because it is involved in important biological processes. Also called aldose reductase (ALR2), AKR1B1 is up-regulated in hyperglycemia and reduces glucose to sorbitol, a hyperosmotic compound, which results in cellular accumulation of sorbitol. This hyperglycemic injury triggers development of secondary diabetic complications, including cataract formation in the lens (11), neuropathy (12), nephropathy (13) and retinopathy (14). Thus, much effort has been focused on finding inhibitors of AKR1B1 (ARIs) for therapeutic purposes against diabetic complications. It requires, however, further mechanistic study because knowing the mechanism would allow the development of clinical drugs without unwanted side effects. Interestingly, AKR1B1 and AKR1B10 show about 71% homology in their amino acid sequences (15), and also show similar substrate specificity towards several endogenous substrates, such as retinals (2), phospholipid aldehydes (16), and acrolein (8). For this reason, selective ARIs might also be inhibitors for AKR1B10, resulting in inhibition in certain types of cancer. According to our previous report, compounds isolated from a medicinal plant, *Rhus verniciflua*, showed potent inhibitory effects on AKR1B1 (17). *R. verniciflua* grows mainly in Southeast Asia, and the plant has been reported to have biological activities, such as anti-inflammation (18), anti-cancer (19), and anti-rheumatoid arthritis. Thus, we hypothesized that the ARIs from *R. verniciflua* could exert similar inhibitory effects on AKR1B10. In the present study, we attempted to isolate these compounds from *Rhus verniciflua* and evaluated their therapeutic potential as novel inhibitors of AKR1B10 using a recombinant human AKR1B10 (rhAKR1B10). We observed different degrees of AKR1B10 inhibition by the com-

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pounds isolated from *R. verniciflua*. These findings suggest that AKR1B10 inhibitors could be potential candidates for novel anticancer agents.

RESULTS AND DISCUSSION

The ethyl acetate fraction shows a strong inhibitory effect on AKR1B10

The present study was carried out in search of new potential AKR1B10 inhibitors from *R. verniciflua* that would be useful for the treatment of cancer. We have previously reported the inhibitory effects of *R. verniciflua* extract on AKR1B10 (20). In this study, we further isolated active compounds after bioactivity-guided fractionation to identify those responsible for AKR1B10 inhibitory activity in the extract. We found that, of 5 different fractions isolated, the ethyl acetate fraction showed the most potent inhibitory activity for AKR1B10, with an IC_{50} value of 1.23 μg/ml (Table 1). This result prompted us to further isolate active components from the ethyl acetate fraction and to analyze their inhibitory effects.

Butein is a potent inhibitor of AKR1B10

We isolated 9 phenolic compounds from the ethyl acetate fraction and performed spectral analysis for structural elucidation as described in our previous report (17). All 9 compounds were tested for inhibitory effects on rhAKR1B10 and their results are shown in Table 2. Among the 9 compounds, the polyphenol butein (Fig. 1A) showed the most potent inhibition against rhAKR1B10, with an IC_{50} value of 1.47 μM. At equal concentrations of 1 μM, sulfuretin (Fig. 1B) showed about half the potency of butein, and its IC_{50} value was 3.80 μM. These inhibition patterns are similar to those observed against AKR1B1 in our previous report (17) although the potency of AKR1B1 inhibition was slightly higher. These results support our hypothesis that ARIs can also inhibit AKR1B10 due to their structural similarity. In other words, compounds such as butein may be usable as both anti-diabetic and cancer therapeutic agents, although conventional *in vivo* and clinical studies should be done before developing them as drugs. Interestingly, AKR1B10 inhibition by other plant polyphenols such as magnolol, honokiol, resveratrol, and dicaffeoyl quinic acid has also been reported recently (21, 22).

To date, another ARI, tolrestat, is known to be the most potent inhibitor for AKR1B10, with an IC_{50} value in the low nM range (2, 15). Recently, numerous studies have reported that butein exerted anti-cancer effects in human bladder cancer cells and hepatoma cells by inhibition of cellular invasion and by induction of G2-M arrest and apoptosis, respectively (23, 24). Therefore, butein may exert its anti-cancer effects synergistically through the inhibition of AKR1B10 and the induction of several anti-carcinogenic cellular pathways. Another appli-

Table 1. Inhibitory effects of fractions from total extract of the bark of *R. verniciflua* on rhAKR1B10

Samples	Concentration (μg/ml)	Inhibition (%)[a]	IC_{50} (μg/ml)[a]
n-Hexane fraction	10	49.75	>10
Methylene chloride fraction	10	48.85	>10
Ethyl acetate fraction	10	96.18	1.23
n-Butanol fraction	10	36.51	>10
Water fraction	10	17.84	>10

Inhibition rate was calculated as percentage with respect to the control value. [a]Results are means of three separate experiments

Table 2. Inhibitory effects of the compounds isolated from ethyl acetate fraction of the bark of *R. verniciflua* on rhAKR1B10

Compounds	Concentration (μM)	Inhibition (%)[a]	IC_{50} (μM)[a]
Epalrestat	1	44.33	1.15
Protocatechuic acid	1	3.01	>1
Ethyl gallate	1	6.24	>1
Fustin	1	4.71	>1
Morin hydrate	1	7.49	>1
Fisetin	1	7.21	>1
Sulfuretin	1	21.74	3.80
Quercetin	1	8.39	>1
Butein	1	42.86	1.47
Pentagalloyl glucose	1	11.53	>1

Inhibition rate was calculated as percentage with respect to the control value. [a]Results are means of three separate experiments

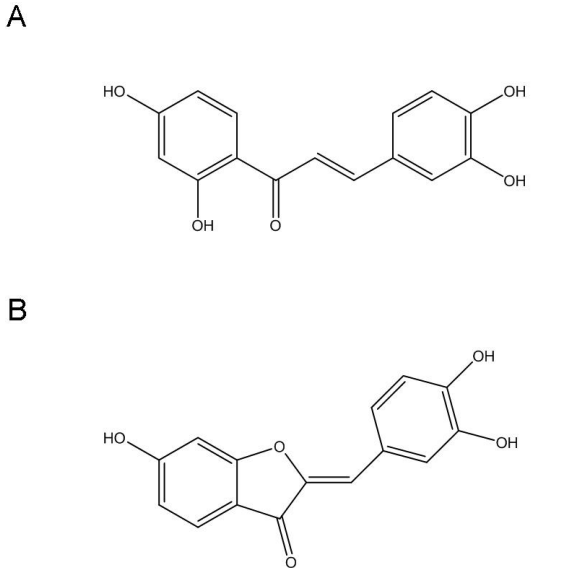

Fig. 1. Chemical structures of the polyphenolic compounds butein (A) and sulfuretin (B) isolated from the ethyl acetate fraction of the bark extract of *R. verniciflua*.

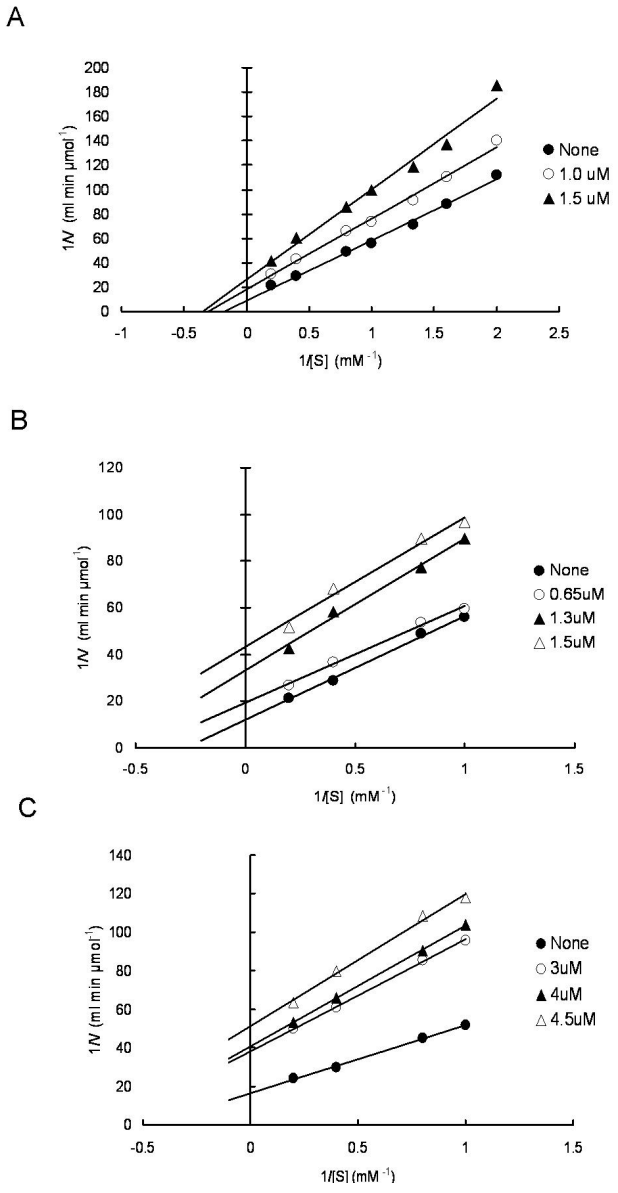

Fig. 2. Lineweaver-Burk plots of the kinetics of rhAKR1B10 in the presence of epalrestat as a positive control (A), butein (B), and sulfuretin (C). Results are representatives of three independent experiments.

cation of AKR1B10 inhibitors is that they can be used in combination with anti-cancer drugs such as daunorubicin, which has been used in the treatment of lung cancer. Since AKR1B10 inactivates daunorubicin by reducing the carbonyl group of daunorubicin in cancer cells, combinatorial treatment with butein or another AKR1B10 inhibitor could be a good strategy for blocking inactivation of anti-cancer drugs.

In order to determine the mode of inhibition by butein and sulfuretin against rhAKR1B10, a kinetic analysis of rhAKR1B10 activity in the presence and absence of the inhibitors was performed using Lineweaver-Burk plots. Epalrestat was used as a positive control because it is a well-known, available ARI (25). As shown in Fig. 2, both butein and sulfuretin showed uncompetitive inhibition against rhAKR1B10, as did epalrestat, indicating that they do not bind the substrate or the nucleotide-binding region of rhAKR1B10.

Overall, our findings suggest that the ethyl acetate fraction from *R. verniciflua* has an AKR1B10 inhibitory effect. In particular, butein isolated from the ethyl acetate fraction of this plant was the most potent component responsible for the observed inhibitory effects. Thus, *R. verniciflua* contains an active component, butein, that could be developed as a potential cancer therapeutic agent for targeted inhibition of AKR1B10.

MATERIALS AND METHODS

Plant materials

The bark of *R. verniciflua* was purchased from the Gyungdong market (Seoul, Korea), and it was deposited as a voucher specimen (DGR-1001) at the herbarium of KIST Gangneung Institute, Korea.

Isolation and identification of compounds from *R. verniciflua*

Extraction, fractionation and isolation procedures were described in the previous report (17). Briefly, the residue (130.0 g) of ethanol extract with reflux four times was partitioned sequentially with *n*-hexane, methylene chloride, ethyl acetate, and *n*-butanol. The ethyl acetate fraction (21.9 g) was purified by chromatography on silica gel eluted with methylene chloride and increasing proportions of methanol (from 10 : 1 to 5 : 5), resulting in 6 fractions (fraction 1-6). Further purification of fraction 2 by preparative RP-HPLC (YMC J'sphere-H80, 4 μm, 250x20 mm) and recycling preparative RP-HPLC (LC-9104, Japan Analytical Industry) yielded 8 compounds: protocatechuic acid, ethyl gallate, fustin, morin hydrate, fisetin, sulfuretin, quercetin, and butein. Pentagalloyl glucose was purified from fraction 3 by RP-HPLC (YMC J'sphere-H80, 4 μm, 250x20 mm). All compounds were identified by LC-MS and NMR spectra and direct comparison with authentic compounds.

Preparation of recombinant human AKR1B10 (rhAKR1B10)

To produce rhAKR1B10 in *E. coli*, the expression plasmid, pET23b-AKR1B10, described in the previous study (26) was transformed into *E. coli* BL21 (DE3). rhAKR1B10 expression and purification followed the procedure as described in reference 26. Briefly, the transformant was grown in LB media, induced by IPTG addition and harvested. Harvested cells were lysed using BugBuster master mix lysis solution (Merck, Darmstadt, Germany), and rhAKR1B10 was purified by immobilized metal affinity chromatography using Ni-NTA resin (Merck, Darmstadt, Germany). The concentration of purified

rhAKR1B10 protein was determined by means of Bradford dye (BioRad, CA, USA) and adjusted to a final concentration of 40 μM.

Measurement of rhAKR1B10 activity

To assay rhAKR1B10 activity spectrophotometrically, we measured the decrease in absorption of NADPH at 340 nm over a 5 min period with DL-glyceraldehyde as a substrate using BioTek Power Wave XS spectrophotometer (BioTek Instruments, VT, USA) (27). Reaction mixtures consisted of 1 μM of rhAKR1B10, 0.1 M sodium phosphate buffer (pH 7.0), and 0.3 mM NADPH with or without 10 mM substrate and inhibitor in a total volume of 1 ml. IC_{50} values, which refer to the inhibitor concentration that gives 50% inhibition of enzyme activity, were calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity. When determining the inhibition type of rhAKR1B10 by the compounds, the reaction mixtures consisted of 0.1 M potassium phosphate (pH 7.0), 0.16 mM NADPH, and 1 μM of rhAKR1B10 with various concentrations of DL-glyceraldehyde substrate and AKR1B10 inhibitors (epalrestat, butein or sulfuretin) in a total volume of 200 μl. Concentrations ranged from 0.5 mM to 5 mM for DL-glyceraldehyde, from 1.0 μM to 1.5 μM for epalrestat, from 0.65 μM to 1.5 μM for butein, and from 3.0 μM to 4.5 μM for sulfuretin.

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