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Arginase II Inhibitory Activity from Crude Drugs†

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Abstract – Arginase competitively inhibits nitric oxide synthase (NOS) via use of the common substrate L-arginine. Arginase II has recently reported as a novel therapeutic target for the treatment of cardiovascular diseases such as atherosclerosis. In our experiment, the EtOH extracts of four-hundreds extracts drugs were investigated for the arginase inhibitory activity. Among them, four extracts exhibited over 50% inhibition of arginase II activity compared to control at a concentration of 150 μg/mL. In particular, the seed of *Arctium lappa*, gum-resin of *Boswellia carterii*, aerial part of *Artemisia apiacea* and rhizome of *Cyperus rotundus* inhibited arginase II activity, with IC_{50} values of 118.4, 135.4, 123.9 and 86.7 μg/mL, respectively. In addition, four plant extracts showed less than 20% inhibition of arginase I activity at 150 μg/mL. These plants might be the potential candidate materials in the development of the novel atherosclerosis drug.
Keywords – Arginase II, Crude drug, Atherosclerosis, *Cyperus rotundus*

Introduction

The endothelium plays a central role in overall vascular homeostasis by regulating vasoreactivity, platelet activation, leukocyte adhesion, and smooth muscle cell proliferation and migration. Endothelial nitric oxide (NO), an important vasoprotective molecule, is a major modulator of these effects, and impaired NO signaling associated with endothelial function is considered an early marker of the atherogenic process (Woo *et al*., 2010). Arginase competitively inhibits nitric oxide synthase (NOS) via use of the common substrate L-arginine (Berkowitz *et al.,* 2003; Simon *et al.,* 2003; Holowatz *et al.,* 2006). Arginase is present in 2 isoforms: arginase I, the hepatic isoform; and arginase II, the extrahepatic isoform; each of which is encoded by a distinct gene. The expression of arginase I in macrophages, hepatocytes, and vascular smooth muscle cells, is stimulated by lipopolysaccharide (LPS) and tumor necrosis factor-α, IL-13, altered oxygen tension, and balloon dilatation of coronary arteries (Klasen *et al*., 2001; Chicoine *et al*., 2004; Morris *et al*., 2004). The activation and expression of endothelial arginase II can also be induced by a variety of vascular stimulants, including OxLDL, LPS, TNF-α, IFN-γ, 8-bromo-cGMP, and hypoxia (Chicoine *et al*., 2004; Morris *et al*., 2004; Ryoo *et al*., 2006). Arginase activation/upregulation results in arginase/NOS imbalance, decreases NO production, and has been demonstrated to contribute to endothelial dysfunction, in a number of diseases/pathophysiologic processes such as aging (Berkowitz *et al*., 2003), diabetes (Bivalacqua *et al*., 2001), hypertension (Demougeot *et al*., 2005; Johnson *et al*., 2005), and atherosclerosis (Ryoo *et al*., 2006; Ryoo *et al*., 2008). For the purpose of finding specific inhibitors of arginase II, we screened four-hundreds ethanol extracts of medicinal crude drugs. As the results, some extracts found to be candidate for further study on the active principles.

Experimental

Materials – The EtOH extracts of crude drugs were purchased from Plant Extract Bank of Korea Research Institute of Biosciences and Biotechnology, Daejeon, Korea (see the list of crude drugs of Plant Extract Bank; http://extract.pdrc.re.kr/extract/). The crude drugs were identified by Dr. Hyeong-Kyu Lee, Korea Research Institute of Biosciences and Biotechnology, Korea, where

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the voucher specimens were deposited.

Arginase – Arginase I and II solutions were prepared from livers and kidneys lysates of anesthetized C57BL/6 mice.

Arginase activity – Tissue lysates of livers and kidneys were prepared using lysis buffer (50 mM Tris-HCl, pH7.5, 0.1 mM EDTA and protease inhibitors) by homogenization at 4 °C followed by centrifugation for 20 min at 14,000 × g at 4 °C. Briefly, aortic lysates were added to Tris-HCl. The hydrolysis reaction of L-arginine by Arg was performed by incubating the mixture containing activated Arg and was stopped by adding acid solution. For calorimetric determination of urea, α-isonitrosopropiophenone was added, and the mixture was heated at 100 °C for 45 minutes. After placing the sample in the dark for 10 minutes at room temperature, the urea concentration was determined spectrophotometrically by the absorbance at 550 nm (White *et al*., 2006).

Results and Discussion

Reciprocal regulation of NOS by arginase has been demonstrated in cells and organs in which NO is an important signaling molecule including the endothelium, cardiac myocytes, penis, airway, skin, and inflammatory cells (Woo *et al.,* 2010). It was demonstrated that arginase II activity is upregulated in atherosclerosis-prone mice and is associated with impaired endothelial NO production, endothelial dysfunction, vascular stiffness, and ultimately, aortic plaque development. Conversely, inhibition of endothelial arginase or deletion of the arginase II gene enhances NO production, restores endothelial function and aortic compliance, and reduces plaque burden. Therefore, arginase II representes a novel target for the prevention and treatment of atherosclerotic vascular disease (Ryoo *et al*., 2008). In the present study, we here screened anti-arginase II activity of 400 medicinal crude drugs (see the list of crude drugs of Plant Extract Bank; http://extract.pdrc.re.kr/extract/). Enzyme solutions for arginase I and II were prepared from lysates of liver and kidney of C57BL/6 mice, respectively. The predominant expression of arginase isoforms was previously confirmed by western blot analysis (Fig. 1). Among four-hundreds crude drugs assayed, four crude drugs demonstrated arginase II inhibitory activity with IC_{50} values no higher than 150 μg/mL (Table 1). The rhizome of *Cyperus rotundus* possessed the most potent inhibitory activity of arginase II with 72%, 54%, and 39% in the concentration of 50, 100, and 150 μg/mL, respectively (IC_{50} value as 86.7 μg/mL; Fig. 2). Furthermore, the seed of *Arctium lappa* showed inhibition of arginase II activity with IC_{50} value as 118.4 μg/mL followed by aerial part of *Artemisia apiacea* with IC_{50} value as 123.9 μg/mL and gum-resin of *Boswellia carterii* with IC_{50} value as 135.4 μg/mL. In addition, four crude drugs were weak inhibitory activity of arginase I with less than 20% at 100 μg/mL, respectively. The remaining crude drugs were apparently inactive or very weak activity with IC_{50} > 150 μg/mL.

The result established that the rhizome of *C. rotundus* possessed the most potent activity with IC_{50} value as 86.7 μg/mL. Phytochemical studies revealed the presence of sesquiterpenes, norsesquiterpenes, sesquiterpene alkaloids, and essential oils in *C. rotundus* (Xu *et al*., 2009; Jeong *et al*., 2000; Sonwa & Konig, 2001). The 70% EtOH extract of *C. rotundus* and sesquiterpene derivatives (valencene

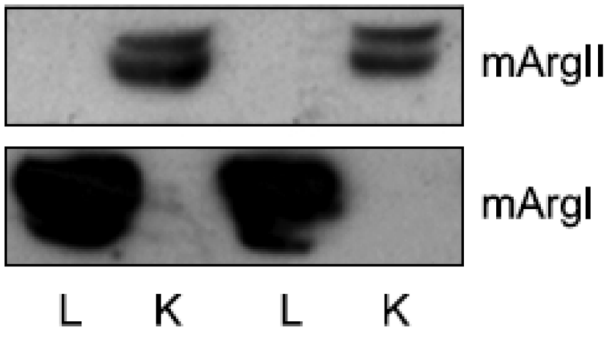

Fig. 1. Arginase solutions prepared from liver (L) and kidney (K) were confirmed to express arginse I or II by western blot analysis.

Table 1. Arginases I and II Inhibitory Activity of Crude Drugs

No	Plant species	Family	Part used	Inhibitory activity	
				Arginase I (100 μg/mL)	Arginase II (IC_{50}, μg/mL)[a]
227	*Arctium lappa*	Compositae	Seed	10 ± 0.4%	118.4 ± 1.5
234	*Boswellia carterii*	Burseraceae	Gum-resin	13 ± 0.1%	135.4 ± 1.2
274	*Artemisia apiacea*	Compositae	Aerial part	17 ± 0.7%	123.9 ± 0.7
289	*Cyperus rotundus*	Cyperaceae	Rhizome	18 ± 1.1%	86.7 ± 1.1

[a] The IC_{50} values were determined by the regression analyses and expressed as means ± SD of three replicates.

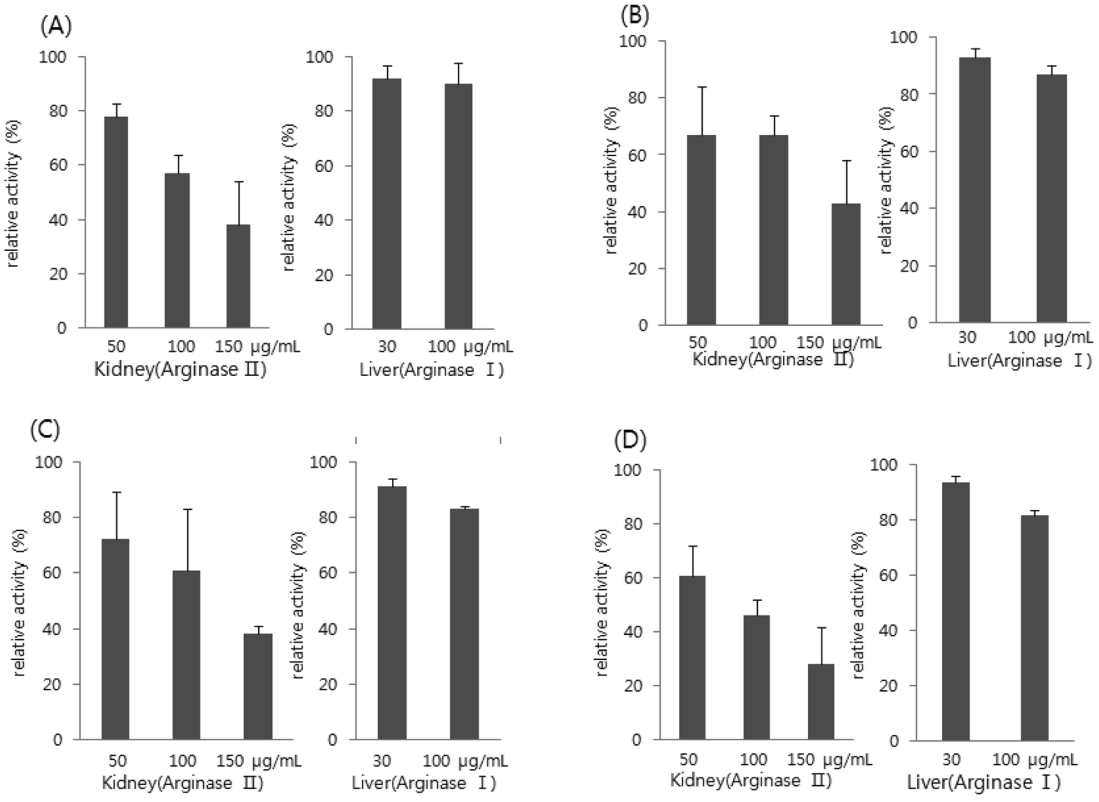

Fig. 2. Arginases I and II Inhibitory Activity of the EtOH extracts of Crude Drugs (A) The seed of *Arctium lappa*; (B) The gum-resin of *Boswellia carterii*; (C) the aerial part of *Artemisia apiacea*; (D) the rhizome of *Cyperus rotundus*.

and nootkatone) showed anti-allergic activity in rat basophilic leukemia-1 cells and inhibited the delayed-type hypersensitivity reaction in mice (Jin *et al*., 2011). In addition, the extract of this plant exhibited high reduction capability and powerful free radical scavenging (DPPH and superoxide anion) as well as a moderate effect on NO (Yazdanparast & Ardestani, 2007). Lee *et al*. reported the neuroprotective effects of a water extract of *C. rotundus* against 6-hydroxydopamine-induced neuronal damage (Lee et al., 2010). Previous studies indicated the effects of EtOH extract and its isolated sesquiterpene (nootkatone) on collagen-, thrombin-, and AA-induced platelet aggregation (Seo *et al*., 2011). Nevertheless, no report has been described the inhibitory effect on arginase II which were used in this study.

The other plants presented interesting activity against arginase II enzyme such as *Arctium lappa, Boswellia carterii*, and *Artemisia apiacea*. The seed of *A. lappa* has been used in patients with diabetes traditional medicine. In alloxan-induced diabetic rats, its lignin-fraction showed significant reductions in plasma glucose, triglycerides, and total cholesterol (Xu *et al*., 2008). Recently, arctigenein from *A. lappa* showed anti-inflmmatory effect in the in vitro assay through inhibition on iNOS pathway and interleukin-2 (Zhao *et al*., 2009; Tsai *et al*., 2011). In addition, phytochemical studies revealed the presence of oleanane- and ursane-type triterpenes in the gum-resin of *B. carterii* (Morikawa *et al*., 2010). The triterpenes from *B. carterii* have significant anti-arthritic, anti-inflammatory, and apoptosis activities (Fan *et al*., 2005; Buchele *et al*., 2006). *A. apiacea* is an important medicinal plant which has been used for the treatment of eczema and jaundice (Lee *et al*., 2003). Its n-hexane-soluble fraction showed anti-oxidant activity (Kim *et al*., 2003). Three plants, *A. lappa, B. carterii*, and *A. apiacea*, have not yet been assessed for arginase II inhibitory activity and atherosclerosis studies.

Natural products still remain a prime source of drugs for the treatment of atherosclerosis and can provide leads for the development of novel anti-atherosclerosis agents. Our screening of 400 crude drugs has led to the identification of the anti-arginase II activity. Four crude drugs showed specific inhibitory activity against arginase II. Thus, the search for new drugs is imperative and the results of our screening need for future isolation and characterization of active compounds by bio-assay guided fractionation.

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References

Berkowitz, D.E., White, R., Li, D., Minhas, K.M., Cernetich, A., Kim, S., Burke, S., Shoukas, A.A., Nyhan, D., Champion, H.C., and Hare, J.M., Arginase reciprocally regulates nitric oxide synthase activity and contributes to endothelial dysfunction in aging blood vessels. *Circulation* **108**, 2000-2006 (2003).

Bivalaqua, T.J., Hellstrom, W.J., Kadowitz, P.J., and Champion, H.C., Increased expression of arginase II in human diabetic corpus cavernosum: in diabetic-associated erectile dysfunction. *Biochem. Biophys. Res. Commun.* **283**, 923-927 (2001).

Buchele, B., Zugmaier, W., Estrada, A., Genze, F., Syrovets, T., Paetz, C., Schneider, B., and Simmet, T., Characerization of 3a-acetyl-11-keto-a-boswellic acid, a pentacyclic triterpenoid inducing apoptosis in vitro and in vivo. *Planta Med.* **72**, 1285-1289 (2006).

Chicoine, L.G., Paffett, M.L., Young, T.L., and Nelin, L.D., Arginase inhibition increases nitric oxide production in bovine pulmonary arterial endothelial cells. *Am. J. Physiol. Lung Cell Mol. Physiol.* **287**, L60-68 (2004).

Demougeot, C., Prigent-Tessier, A., Marie, C., and Berthelot, A., Arginase inhibition reduces endothelial dysfunction and blood pressure rising in spontaneously hypertensive rats. *J. Hypertens.* **23**, 971-978 (2005).

Fan, A.Y., Lao, L., Zhang, R.X., Zhou, A.N., Wang, L.B., Moudgil, K.D., Lee, D.Y., Ma, Z.Z., Zhang, W.Y., and Berman, B.M., Effects of acetone extract of *Boswellia carterii* Birdw. (Burseraceae) gum resin on adjuvant-induced arthritis in lewis rats. *J. Ethnopharmacol.* **101**, 104-109 (2005).

Holowatz, L.A., Thompson, C.S., and Kenney, W.L., L-Arginine supplementation or arginase inhibition augments reflex cutaneous vasodilatation in aged human skin. *J. Physiol.* 574, 573-581 (2006).

Jeong, S.J., Miyamoto, T., Inagaki, M., Kim, Y.C., and Higuchi, R., Rotundines A-C, three novel sesquiterpene alkaloids from *Cyperus rotundus*. *J. Nat. Prod.* **63**, 673-675 (2000).

Jin, J.H., Lee, D.U., Kim, Y.S., and Kim, H.P., Anti-allergic activity of sesquiterpenes from the rhizomes of *Cyperus rotundus*. *Arch. Pharm. Res.* **34**, 223-228 (2011).

Johnson, F.K., Johnson, R.A., Peyton, K.J., and Durante, W., Arginase inhibition restores arteriolar endothelial function in Dahl rats with salt-induced hypertension. *Am. J. Physiol. Regul Integr. Comp. Physiol.* **288**, R1057-1062 (2005).

Kim, K.S., Lee, S., Lee, Y.S., Jung, S.H., Park, Y., Shin, K.H., and Kim, B.K., Anti-oxidant activities of the extracts from the herbs of *Artemisia apiacea*. *J. Ethnopharmacol.* **85**, 69-72 (2003).

Klasen, S., Hammermann, R., Fuhrmann, M., Lindemann, D., Beck, K.F., Pfelischifter, J., and Racke, K., Glucocorticoids inhibit lipopolysaccharide-induced up-regulation of arginase in rat alveolar macrophages. *Br. J. Pharmacol.* **132**, 1349-1357 (2001).

Lee, C.H., Hwang, D.S., Kim, H.G., Oh, H., Park, H., Cho, J.H., Lee, J.M., Jang, J.B., Lee, K.S., and Oh, M.S., Protective effect of Cyperi rhizome against 6-hydroxydopamine-induced neuronal damage. *J. Med. Food* **13**, 564-571 (2010).

Lee, S., Kim, K.S., Shim, S.H., Park, Y.M., and Kim, B.K., Constituents from the non-polar fraction of *Artemisia apiacea*. *Arch. Pharm. Res.* **26**, 902-905 (2003).

Morikawa, T., Ominami, H., Matsuda, H., and Yoshikawa, M., Four new ursane-type triterpenes, olibanumols K, L, M, and N, from traditional Egyptian medicine olibanum, the gum-resin of *Boswellia carterii*. *Chem. Pharm. Bull.* **58**, 1541-1544 (2010).

Morris, C.R., Poljakovic, M., Lavrisha, L., Machado, L., Kuypers, F.A., and Morris, S.M. Jr., Decreased arginine bioavailability and increased serum arginase activity in asthma. *Am. J. Respir. Crit. Care Med.* **170**, 148-153 (2004).

Ryoo, S., Gupta, G., Benjo, A., Lim, H.K., Camara, A., Sikka, G., Lim, H.K., Sohi, J., Santhanam, L., Soucy, K., Tuday, E., Baraban, E., Ilies, M., Gerstenblith, G., Nyhan, D., Shoukas, A., Christianson, D.W., Alp, N.J., Champion, H.C., Huso, D., and Berkowitz, D.E., Endothelial arginase II: a novel target for the treatment of atherosclerosis. *Circ. Res.* **102**, 923-932 (2008).

Ryoo, S., Lemmon, C.A., Soucy, K.G., Gupta, G., White, A.R., Nyhan, D., Shoukas, A., Romer, L.H., and Berkowitz, D.E., Oxidized low-density lipoprotein-dependent endothelial arginase II activation contributes to impaired nitric oxide signaling. *Circ. Res.* **99**, 951-960 (2006).

Seo, E.J., Lee, D.U., Kwak, H.H., Lee, S.M., Kim, Y.S., and Jung, Y.S., Antiplatelet effects of *Cyperus rotundus* and its component (+)-nootkatone. *J. Ethnopharmacol.* in press (2011).

Simon, A., Plies, L., Habermeier, A., Martine, U., Reining, M., and Closs, E.I., Role of neutral amino acid transport and protein breakdown for substrate supply of nitric oxide synthase in human endothelial cells. *Circ. Res.* **93**, 813-820 (2003).

Sonwa, M.M. and Konig, W.A., Chemical study of the essential oil of *Cyperus rotundus*. *Phytochemistry* **58**, 799-810 (2001).

Tsai, W.J., Chang, C.T., Wang, G.J., Lee, T.H., Chang, S.F., Lu, S.C., and Kuo, Y.C., Arctigenin from *Arctium lappa* inhibits interleukin-2 and interferon gene expression in primary human T lymphocytes. *Chin. Med.* **25**, 12 (2011).

White, A.R., Ryoo, S., Li, D., Champion, H.C., Steppan, J., Wang, D., Nyhan, D., Shoukas, A.A., Hare, J.M., and Berkowitz, D.E., Knockdown of arginase I restores NO signaling in the vasculature of old rats. *Hypertension* **47**, 245-251 (2006).

Woo, A., Min, B.S., and Ryoo, S., Piceatannol-3'-O-β-D-glucopyranoside as an active component of rhubarb activates endothelial nitric oxide synthase through inhibition of arginase activity. *Exp. Mol. Med.* **42**(7), 524-532 (2010).

Xu, Y., Zhang, H.W., Wan, X.C., and Zou, Z.M., Complete assignments of ^{1}H and ^{13}C NMR for two new sesquiterpenes from *Cyperus rotundus* L.. *Magn. Reson. Chem.* **47**, 527-531 (2009).

Xu, Z., Wang, X., Zhou, M., Ma, L., Deng, Y., Zhang, H., Zhao, A., Zhang, Y., and Jia, W., The antidiabetic activity of total ligna from Fructus Arctii against alloxan-induced diabetes in mice and rats. *Phytother. Res.* **22**, 97-101 (2008).

Yazdanparast, R., and Ardestani, A., In vitro antioxidant and free radical scavenging activity of *Cyperus rotundus*. *J. Med. Food* **10**, 667-674 (2007).

Zhao, F., Wang, L., and Liu, K., In vitro anti-inflammatory effects of artigenin, a lignin from *Arctium lappa* L., through inhibition on iNOS pathway. *J. Ethnopharmacol.* **122**, 457-462 (2009).

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