Isoacteoside induces heme oxygenase-1 via activation of ERK and NF-E2 related factor 2 transcription factor

Dong Ok Ko, Kyoung Ah Kang, and Jin Won Hyun

Department of Biochemistry, Jeju National University School of Medicine,Jeju, Korea

Abstract

Isoacteoside was isolated from Clerodendron trichotomum Thunb that belongs to Verbenaceae family. We found that isoacteoside increased the heme oxygenase-1 (HO-1) expression in a time dependent manner. Furthermore, isoacteoside increased the expression of NF-E2 regulated factor 2 (Nrf2), which is a transcription factor of HO-1, in a time dependent manner. Moreover, isoacteoside increased the phospho Erk expression. Our data suggested that isoacteoside showed antioxidant effects via Erk activation, induction of Nrf2, and HO-1, in Chinese hamster lung fibroblast cells. (J Med Life Sci 2009;6:359-361)

Key Words : Isoacteoside, NF-E2 related factor 2 (Nrf2), Antioxidant effect

Introduction

Reactive oxygen species (ROS) includes the free radicals such as superoxide anion (O_2^-), hydroxyl radical (OH.) and hydrogen peroxide (H_2O_2). The generation of ROS is known to be involved in tissue damage and diseases such as aging, cancer, arteriosclerosis and diabetes.[1-4] Aerobic organisms possess antioxidant defense mechanisms that protect against these oxidative stresses.[5] Heme oxygenase-1 (HO-1), which is a antioxidant enzyme, catalyzes the rate-limiting step in heme catabolism, leading to the formation of biliveridin, freeiron and carbon monoxide. In the presence of biliveridin reductase, biliveridin is further converted to bilirubin, which is a potent antioxidant.[6, 7] HO-1 expression is induced in response to various chemically or physiologically produced oxidative stresses in cells and tissues.[8, 9] The HO-1 pathway represents a prime cellular defense system against oxidative stress.[10, 11] Recently, a new class of activator protein-1 (AP-1) related sequences has been shown to mediate the oxidative stress responsiveness of the HO-1 gene. These regions, termed stress responses elements (StRE) or antioxidant responses elements (ARE), are tightly regulated by the redox sensitive transcription factor, NF-E2 regulated factor 2 (Nrf2).[12] MAPKs pathway associates with the modulation of ARE driven gene expression via Nrf2 activation. ERK and p38 MAPK pathways involved the nucleus binding of Nrf2 to the ARE.[13] Isoacteoside was isolated from Clerodendron trichotomum Thunb that belongs to Verbenaceae family.[14, 15] It has pharmacological effects and anti-inflammatory effects.[16, 17] Many researchers have studied the effect of isoacteoside in a variety of cells. However, HO-1 induction of isoacteoside in lung cells has not been reported until now.The present study investigates the antioxidant effect via HO-1 induction of isoacteoside in V79-4 cells.

Address for correspondence : Jin Won Hyun
Department of Biochemistry, Jeju National University School of Medicine, 66 Jejudaehakno, 690-756, Jeju, Korea
E-mail : jinwonh@jejunu.ac.kr

Materials and methods

1. Reagents

Isoacteoside was provided by Dr. Sungwook Chae of Korea Institute of Oriental Medicine, Daejeon, Korea. Primary rabbit polyclonal phospho ERK, Nrf2 antibodies were purchased from Santa Cruz Biotechnology, Santa Cruz, CA, USA. Primary mouse monoclonal HO-1 antibody was purchased from Stressgen Corp., Victoria, Canada.

2. Cell culture

Chinese hamster lung fibroblasts (V79-4) cells from the American type culture collection (Rockville, MD, USA) were maintained at 37 ℃ in an incubator, with a humidified atmosphere of 5% CO_2 and cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf

serum, streptomycin (100 g/ml) and penicillin (100 units/ml).

3. Western blot analysis

The cells were harvested, washed twice with PBS, lysed on ice for 30 min in 100 $\mu\ell$ of a lysis buffer [120 mM NaCl, 40mM Tris (pH 8), 0.1% NP 40] and then centrifuged at 13,000×g for 15 min. The supernatants were collected from the lysates and the protein concentrations were determined. Aliquots of the lysates (40 μg of protein) were boiled for 5 min and electrophoresed in 10% sodium dodecylsulfate-polyacrylamidegel. The blots in the gels were transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA), which were then incubated with the primary antibody. The membranes were further incubated with the secondary immunoglobulin-G-horseradish peroxidase conjugates(Pierce, Rockford, IL, USA). Protein bands were detected using an enhanced chemiluminescenceWestern blotting detection kit (Amersham, Little Chalfont, Buckinghamshire, UK), and then exposed onto X-ray film.

Results and Discussion

Oxidative stresses are involved in the process of multi-stage carcinogenesis. Flavonoid has polyphenol groups which are found in coffee, beer, wine and fruits and vegetables. Polyphenols have reported to reduce the risk of cardiovascular disease and increase the antioxidant capacity in KB cells.[18, 19] Although many studies have reported the antioxidant effects of flavonoids, there is no report on the antioxidant effects via HO-1 induction of isoacteoside, which belongs to flavonoid group, in lung fibroblast cells. HO-1 is a defensive enzyme against oxidative stress. HO-1 degrades heme into carbon monoxide (CO), iron, and biliverdin. As shown Fig 2, treatment of isoacteoside increased the HO-1 protein expression in a time dependent manner. Moreover, the transcription factor, Nrf2, regulates the antioxidant response element (ARE) of the phase 2 detoxifying and antioxidant enzymes, resulting in induction of HO-1 expression. Isoacteoside increased Nrf2 expression up to 48h as shown in Fig 3. Furthermore, isoacteoside increased the phospho Erk expression, which is upstream of Nrf2, at 6h(Fig 4).

Taken together, we have shown that isoacteoside increase antioxidant effect via HO-1 induction in V79-4.

Figure 1. Chemical structure of isoacteoside.

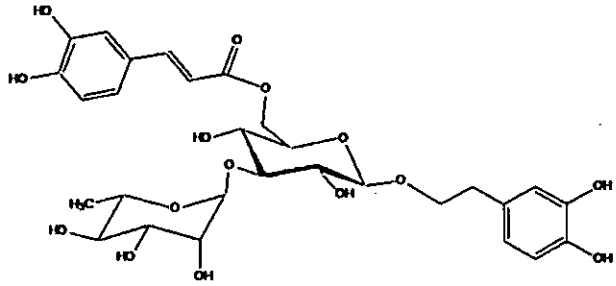


Figure 2. Effect of isoacteoside on HO-1 expression. Western blot analysis was performed using HO-1 specific antibody.

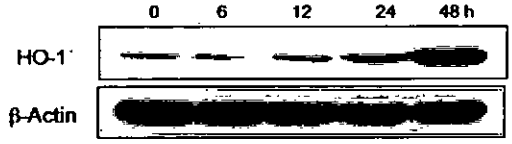


Figure 3. Effect of isoacteoside on Nrf2 expression. Western blot analysis was performed using Nrf2 specific antibody.

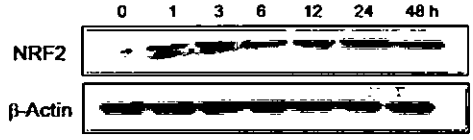
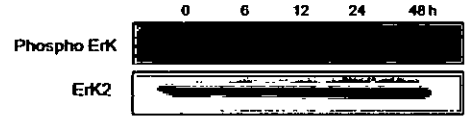


Figure 4. Effect of isoacteoside on phospho ErK expression. Western blot analysis was performed using phospho ErK specific antibody.



Reference

1) Cooke MS, Mistry N, Wood C, Herbert KE, Lunec J. Immunogenicity of DNA damaged by reactive oxygen species-Implications for anti-DNA antibodies in lupus. *Free Radical Biol. Med.* 22:1997:151-159.
2) Farinati F, Cardin R, Degan P, Rugge M, Mario FD, Bonvicini P, Naccarato R. Oxidative DNA damage accumulation in gastric carcinogenesis. *Gut* 42:1998:351-356.
3) Palinski W, Miller E, Witztum JL. Immunization of low density lipoprotein (LDL) receptor deficient rabbits with homologous malondialdehyde-modified LDL reduces

atherogenesis. *Proc. Natl.Acad. Sci. USA* 921995;821-825.

4) Parthasarathy S, Steinberg D, Witztum JL, The role of oxidized low-density lipoproteins in the pathogenesis of atherosclerosis. *Annu. Rev. Med.* 43;1992;219-225.

5) Halliwell B, Gutteridge JMC, Free radicals in biology and medicine. New York:Oxford University Press. 1998;22-35.

6) Maines MD, Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J. 2;1988;2557-2568.

7) Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, Ames BN, Bilirubin is an antioxidant of possible physiological importance. Science 235;1987;1043-1046.

8) Applegate LA, Luscher P, Tyrrell RM, Induction of heme oxygenase: a general response to oxidant stress in cultured mammalian cells. Cancer Res. 51;1991;974-978.

9) Rossi A, Santoro MG, Induction by prostaglandin A1 of haem oxygenase in myoblastic cells: an effect independent of expression of the 70 kDa heat shock protein. Biochem. J. 308;1995;455-463.

10) Yamada, N., Yamaya, M., Okinaga, S., Lie, R., Suzuki, T., Nakayama, K., Takeda, A., Yamaguchi, T., Itoyama, Y., Sekizawa, K. and Sasaki, H. (1999) Protective effects of heme oxygenase-1 against oxidant-induced injury in the cultured human tracheal epithelium. Am. J. Respir. Cell. Mol. Biol. 21, 428-435.

11) Foresti, R. and Motterlini, R. (1999) The heme oxygenase pathway and its interaction with nitric oxide in the control of cellular homeostasis. Free Radic. Res. 31, 459-475.

12) Nguyen T, Sherratt PJ, Pickett CB, Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. Annu. Rev. Pharmacol. Toxicol. 43;2003;233-260.

13) Yu R, Chen C, Mo YY, Hebbar V, Owuor ED, Tan TH, Kong AN, Activation of mitogen-activated protein kinase pathways induces antioxidant response element-mediated gene expression via a Nrf2-dependent mechanism. J. Biol. Chem. 275;20003;9907-39913.

14) Ichi T, Shimizu T, Yoshihira K, Biotechnology in agricuture and forestry. Medicinal and aromatic plants IX. 37;1996;108.

15) Lee WT, Lineamenta *Florae Koreae*, Seoul: Academic Books. 1996;934-935.

16) Ahn DK, *Illustarated book of Korean medicinal herb.* Kyo-Hak Publishing. 2003;323. Seoul:

17) Huang KC, The pharmacology of Chinese herbs, Boca Raton, FL: CRC Press, 2003;71-72.

18) Arts IC, Hollman PC, Polyphenols and disease risk in epidemiologic studies.American Journal Clinical Nutrition 2005;81;317-325.

19) Mei Y. We D, Liu J, Reversal of Multi drug Resistance in KB Cells with Tea Polyphenol Antioxidant Capacity. 2005;4;468-473.