GINSENOSIDES ARE NOVEL AH RECEPTOR LIGANDS

Hu Q[1], He G[2], Zhao J[2], Soshilov A[2], Denison MS[2], Yin H[3], Zhang A[1], Xie Q[1], Zhao B[1*]

[1]Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China; [2]Department of Environmental Toxicology, Meyer Hall, University of California, Davis, CA, USA; [3]Xiyuan Hospital, China Academy of Chinese Medical Sciences, Beijing, 100091, China

Introduction

The aryl hydrocarbon receptor (AhR) is conserved in vertebrates and invertebrates, suggesting its important biological function through evolution[1], although its endogenous function remains unclear. AhR is basic helix–loop–helix PAS-containing transcription factor, which activates gene expression in a ligand-dependent manner[2,3]. Exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD, dioxin), the prototypical and most potent AhR ligand, results in a wide variety of species- and tissue-specific toxic and biological responses, the majority of which are AhR dependent[4]. Following ligand binding, the cytosolic AhR protein complex translocates into the nucleus, and the ligand-bound AhR is released from its associated protein subunits upon dimerization with the Arnt (Ah receptor nuclear translocator) protein and is converted into its high-affinity DNA binding form. The heteromeric ligand:AhR:Arnt complex then binds to its specific DNA recognition site, the dioxin response element (DRE), upstream of cytochrome P4501A1 (CYP1A1), and other AhR-responsive genes to manipulate the transcription[5,6]. The best characterized high-affinity ligands for the AhR include a variety of halogenated aromatic hydrocarbons (HAHs), such as the polychlorinated dibenzo-p-dioxin, dibenzofurans, and biphenyls, as well as numerous polycyclic aromatic hydrocarbons (PAHs), such as benzo(a)pyrene, 3-methylcholanthrene, and others[7]. Scientists have identified and characterized a relatively large number of natural and synthetic AhR ligands (agonist and antagonists) whose structure and physicochemical characteristics are dramatically different from that of the prototypical HAH and PAH AhR ligands[6,7,8]. The spectrums of synthetic and natural chemicals that can bind to and activate the AhR remain to be established[9]. Accordingly, we have carried out AhR-based bioassay screening analyses of natural compounds in order to identify and characterize novel AhR ligands with the goal of better understanding the endogenous functions of the AhR and AhR signal transduction pathway.

Ginseng has been used as traditional medicine in China and other Asian countries for thousands of years. Seven major species of ginseng are distributed in East Asia, Central Asia, and North America. Most studies of ginseng have utilized constituents from three common species: *Panax ginseng* (Asian ginseng), *Panax quinquefolius* (American ginseng), and *Panax japonicus* (Japanese ginseng). Most pharmacological actions of ginseng are attributed to ginsenosides, which have been reported to have a number of biological effects[10]. The potential health effects of ginsenosides include anti-carcinogenic, immunomodulatory, anti-inflammatory, antiallergic, anti-atherosclerotic, anti-hypertensive, and anti-diabetic effects as well as anti-stress activity and effects on the central nervous system[10]. In general, these ginseng saponins, with structural similarity, can be divided into three groups according to the structure of the non-sugar (aglycon) part of the molecule. To date, more than 30 ginsenosides have been found in the roots and other parts of *Panax ginseng*, and a total of over 60 ginsenosides were isolated from members of the Panax genus. Many of these compounds are responsible for the wide range of medicinal effects of ginseng[10].

There is only one study showed that CYP1A1 mRNA expression was significantly increased in a concentration- and time- dependent manner in human hepatoma (HepG2) cells by two common clinically used ginsenosides Rg1 and Rb1[11]. However, whether ginsenosides can actually bind to and activate the AhR and AhR-dependent signal pathway remains unknown. Given that the structures of ginsenosides are generally considered to be too large to fit into the AhR ligand binding pocket, it remains to be determined whether the observed CYP1A1 activation is due to their direct binding to and activation of the AhR or through an alternative mechanism of AhR activation. Here we have examined the mechanism by which ginsenosides can activate the AhR signal transduction pathway.

Materials and methods

Chemicals. Ginsenosides Rc and Rh1 were provided by Dr. Huijun Yin and each compound was more than 98% pure. TCDD was purchased from Wellington Laboratories Inc. (Ontario, Canada) and dimethyl sulfoxide (DMSO) was from Sigma-Aldrich (St. Louis, MO). Cell culture media was purchased from Invitrogen (Carlsbad, CA), fetal serum was purchased from Lonza (Allendale, NJ) and G418 was from Gemini Bio-Products (Woodland, CA). All other chemicals were of analytic purity.

Cell Culture, Chemical Treatment, and AhR-Dependent Luciferase Reporter Gene Expression. Recombinant rat (H4L1.1c4) and mouse (H1L1.1c2) hepatoma cells were kindly provided by Dr. Michael S. Denison (University of California, Davis, USA) and were grown and maintained as previously described[12]. H4L1.1c4and H1L1.1c2 cells contain the stably integrated DRE-driven firefly luciferase reporter plasmid pGudLuc1.1[12]. Cells were plated into white, clear-bottomed 96-well tissue culture dishes at 75,000 cells per well and allowed to attach for 24 h. Cells were incubated with carrier solvent DMSO (1% final solvent concentration), TCDD (1 nM) or the indicated compound (for measurement of agonist activity) for 4 h at 37◦C. For luciferase measurement, sample wells were washed twice with phosphate-buffered saline, followed by addition of cell lysis buffer (Promega) and shaking of the plates for 20 min at room temperature to allow cell lysis. Measurement of luciferase activity in each well was carried out using an microplate luminometer (TECAN Infinite F200 pro, Switzerland) with automatic injection of Promega stabilized luciferase reagent. Luciferase activity in each well was expressed relative to that induced by 1 nM TCDD.

Results and discussion

We first examined the AhR agonist activity of ginsenosides by testing their abilities to stimulate AhR-dependent reporter gene expression in recombinant rat (H4L1.1c4) and mouse (H1L1.1c2) hepatoma cells that contain the stably transfected DRE-luciferase reporter plasmid pGudLuc1.1. Dose-dependent induction of luciferase by ginsenosides at 4 h was observed in this cell line and Rc (Figure 1) and Rh1 (data not shown) were found to stimulate AhR-dependent reporter gene expression in both of the recombinant cell types. Rc amazingly shows 54.26±9.48% of that induced by 1 nM TCDD in H4L1.1c4 cells (Figure 1). These results combined with the lack of induction by ginsenosides at concentrations ≤1μM (Figure 1) indicate that ginsenosides are relatively weak AhR agonists when compared to TCDD and other potent HAH and PAH ligands.

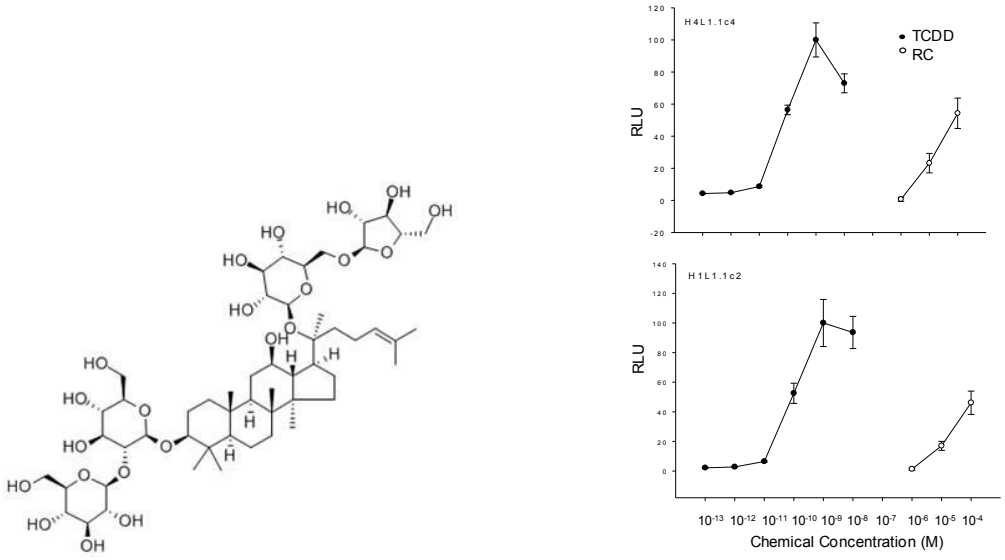

Figure 1. Dose-dependent induction of luciferase activity by TCDD and Rc in H4L1.1c4 and H1L1.1c2 cells. Cells were incubated with the indicated concentration of Rc for 4 h or 24 h and luciferase activity was determined as described in Materials and Methods section. Schematic

diagram of the chemical structure of Rc is shown in left panel. Values are expressed in the figure as the percentage of maximal TCDD induction and represented as Mean ± SEM, n=4, each with triplicate determinants. All concentrations of TCDD $\geq 10^{-11}$ M and of Rc $\geq 10^{-5}$ M were significantly greater than DMSO-treated sample at $p < 0.01$ as determined by Student's t-test.

This is the first study to demonstrate that the ginsenosides could activate AhR and AhR-dependent pathway. Our data show that Rc is a significantly weaker inducer of AhR dependent gene expression than that of TCDD. The weaker activity of Rc may be due to its relatively larger size which leads to steric hindrance in its binding within the AhR ligand binding pocket and whether the entire compound fits into the binding site is unclear. Ginseng has been used for traditional medicine in China and other Asian countries for thousands of years, however, caution has been raised in order to safeguard consumers using these herbal medicines. Exposure of cells or animals to ginsenosides is reported to cause a variety of biological and physiologic effects, and most research uses ginsenosides as therapies to enhance cholesterol biosynthesis, modulate immune system, inhibit inflammatory activity and induct cancer cell differentiation, apoptosis and angiogenesis, but few studies have focused on the side effects of ginsenosides. Chan et al. tested the embryotoxicity of Rc and Re to rat whole embryo *in vitro*, and found that there was a significant variability in embryotoxic effects of different ginsenosides[13]. Whether the AhR pathway plays a role in the ability of ginsenosides to produce their clinical, biological or side effects remains to be determined. The role of the AhR signaling pathway in the mechanisms of action of gensenosides may provide insights into how gensing can produce some of its biological and medicinal effects. Considering the currently large consumption of ginseng, further studies into the molecular mechanisms of ginsenosides action, including that of the AhR pathway, are needed in order to better understand the diverse effects of these chemicals.

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