Short Communication

Agonistic effect of selected isoflavones on arylhydrocarbon receptor in a novel AZ-AhR transgenic gene reporter human cell line

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Abstract. The aryl hydrocarbon receptor (AhR) is a ligand-activated transcription factor that controls the expression of a diverse set of genes. Structurally diverse compounds bind to AhR and act as AhR agonists. Well characterised family of natural AhR ligands are isoflavones, which are compounds found predominantly in soy beans or red clover. In this study we have examined agonistic effect of selected isoflavones (genistein, daidzein, biochanin A, formononetin and equol) on AhR in the novel transgenic gene reporter human cell line AZ-AhR, a stably transfected AhR-responsive cell line allowing rapid and sensitive assessment of AhR transcriptional activity. We demonstrated that biochanin A, formononetin and genistein at concentration 10^{-4} mol/l exerted agonistic effects on AhR with fold activation of 309-fold, 108-fold and 27-fold, which is about 84.8%, 29.6% and 7.4%, respectively, of the value attained by 2,3,7,8-tetrachlorodibenzo-p-dioxin. Daidzein and equol did not show any significant effects on AhR.

Key words: Isoflavones — Arylhydrocarbon receptor — AZ-AhR cells

Aryl hydrocarbon receptor (AhR) is a member of the basic helix-loop-helix (bHLH) Per-ARNT-Sim (Pas) family and it also shares some elementary features linked to mechanism of action of nuclear receptors (Medjakovic et al. 2010). It is the only basic helix-loop-helix protein that is ligand activated; upon ligand binding, AhR translocates from cytoplasm to the nucleus, where it forms heterodimer with aryl hydrocarbon receptor nuclear translocator (ARNT) (Lee and Safe 2000). The complex AhR/ARNT binds to its cognate DNA sequence – xenobiotic response element (XRE), and subsequently, it activates the expression of AhR-target genes (Matthews and Gustafsson 2006). AhR encodes xenobiotic metabolizing enzymes, such as cytochromes P450 – CYP1A1, CYP1A2, CYP1B1 and UDP glucuronosyltransferase 1 family polypeptide A6 (UGT1A6) (Stevens et al. 2009). AhR also plays a variety of important functions in the body, such as regulation of drug metabolism, cell cycle regulation and proliferation, circadian rhythm, immune response and tumour promotion (Novotna et al. 2011). Structurally diverse chemicals bind to AhR, including highly toxic halogenated aromatics 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and chemoprotective phytochemicals such as indole-3-carbinol (13C), 3,3’-diindolylmethane (DIM) and bioflavonoids (Lee and Safe 2000). Tryptophanand UV photoproducts of tryptophan, metabolites of arachidonic acid, cAMP, heme metabolites such as bilirubin, biliverdin, indirubin are endogenous ligands for AhR (Medjakovic et al. 2010; Novotna et al. 2011). A well characterised family of natural AhR ligands are also isoflavones, which are organic compounds found in various species of the legume family, such as soy beans and in red clover (Wall et al. 2012; Bialesova et al. 2013). Isoflavones belong to the class of phytoestrogens because they have estrogenic and/or anti-estrogenic activity (Mittal et al. 2014). Isoflavone biochanin A is considered to be relatively strong AhR agonist. On the other hand, isoflavones genistein and daidzein have been also shown to be weak agonists or weak antagonists in mouse Hepa1 cells or yeast cells (Wall et al. 2012). Entireties of AhR functions that are modulated by isoflavones through agonistic or antagonistic effects are remaining still unclear. Nevertheless, isoflavones can be regarded as selective AhR modulators (sAhRMs) (Medjakovic et al. 2010).
The aim of this study was to evaluate agonistic effect of selected isoflavones (genistein, daidzein, biochanin A, formononetin, and equol) on aryl hydrocarbon receptor in a novel AZ-AhR transgenic gene reporter human cell line, which is stably transfected HepG2 cell line, allowing rapid and sensitive assessment of AhR transcriptional activity.

The development and properties of this transgenic gene reporter human cell line were described elsewhere (Novotna et al. 2011). Isoflavones: genistein (Gen), daidzein (DAI), biochanin A (BiA) formononetin (Fo), and equol (EQ), and other compounds were purchased from Sigma Aldrich (Schnelldorf, Germany). For MTT test, AZ-AhR were seeded on 96-well plates at density 20 000 cells per well. Following 24 h incubation, cells were incubated with tested isoflavones at concentrations ranging from $10^{-8}$ to $10^{-4}$ mol/l (for genistein (Gen), daidzein (DAI), biochanin A (BiA) and formononetin (Fo)), and at the concentrations from $10^{-8}$ to $10^{-5}$ mol/l (for equol (EQ)). AhR, aryl hydrocarbon receptor.

For a gene reporter assay, AZ-AhR cells were seeded on 96-well plates at density 20 000 cells per well. Following 24 h incubation, cells were incubated with tested isoflavones at concentrations ranging from $10^{-4}$ to $10^{-8}$ mol/l. In parallel, the cells were treated with vehicle (DMSO; 0.1%, v/v) and Triton X-100 (1%, v/v) to assess the minimal (positive control) and maximal (negative control) cell response, respectively. MTT test, a colorimetric assay for assessing cell viability was employed in experiments. After 24 h treatment with tested compounds, samples were measured spectrophotometrically at 540 nm (TECAN, Schoeller Instruments LLC). The treatments were performed in quadruplicates in three independent cell passages.

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In this work, we have evaluated agonistic effects of selected isoflavones (Gen, DAI, BiA, Fo, EQ) on aryl hydrocarbon receptor in gene reporter human AZ-AhR cells. Firstly, we have tested effects of isoflavones on viability of AZ-AhR cells. Gen, DAI and Fo were not cytotoxic up to $10^{-4}$ mol/l, while BiA was found to be slightly toxic ($IC_{50} = 8.25 \times 10^{-5}$ mol/l) (Fig. 1). Gene reporter assays revealed the fold inductions of AhR-dependent luciferase activity in AZ-AHR cells induced by BiA (309-fold), Fo (108-fold), Gen (27-fold) and TCDD (364-fold), whereas daidzen (DAI) and equol (EQ) did not exert any agonistic effects on aryl hydrocarbon receptor (Fig. 2). TCDD was used at the concentration of $5.0 \times 10^{-9}$ mol/l, which is not toxic against cell line AZ-AhR (Novotna et al. 2011).

In the current paper, we performed dose-response analyses with selected isoflavones and methylisoflavones against AhR transcriptional activity in transgenic AZ-AhR cells. We used broad spectrum of concentrations, since in vivo levels of tested compounds and their metabolites may vary significantly. Relatively low plasma concentrations of polyphenolic compounds originating from oral intake are in submicromolar range. On the other hand, intestinal concentrations of polyphenolics may be several orders of magnitude higher as compared to plasmatic ones, taking in account enormously high content of polyphenolic compounds in some food supplements.

Our results well correspond to the analyses of the Medjakovic group (Medjakovic and Jungbauer 2008), describing red clover isoflavones BiA and Fo transactivating effects on the aryl hydrocarbon receptor. They have compared their results with transactivating capacity of other flavonoids and Figure 2. Gene reporter assay. Agonistic effect of selected isoflavones Gen, DAI, BiA and Fo at the concentrations ranging from $10^{-8}$ to $10^{-4}$ mol/l, isoflavone EQ at the concentrations from $10^{-8}$ to $10^{-5}$ mol/l and natural ligand of AhR (TCDD, $5 \times 10^{-9}$ mol/l) on the induction of AhR in stable transfected AZ-AhR cell line after 24 h incubation. TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin. For more abbreviations see Fig. 1.
biologically active derivatives of indol, BiA and Fo behaved as strong AhR agonists in vitro, with EC\(_{50}\) 2.5 × 10\(^{-7}\) mol/l (BiA) and 1.3 × 10\(^{-7}\) mol/l (Fo). These compounds were 10-times more potent in activation of AhR as compared to indolderivates (indol-3-carbinol EC\(_{50}\) 5.8 × 10\(^{-6}\) mol/l and diindolylmethan EC\(_{50}\) 1.1 × 10\(^{-6}\) mol/l) (Medjakovic and Jungbauer 2008). Han and co-workers (2006) have shown the effect of BiA on the nuclear accumulation of activated AhR induced by DMBA (7,12-dimethylbenz[a]anthracene) in the nuclear extract of the MCF-7 cell line, which has been treated with BiA at concentrations ranging from 1 × 10\(^{-5}\) mol/l to 5 × 10\(^{-5}\) mol/l in the presence and in the absence of DMBA. BiA at the concentration of 5 × 10\(^{-5}\) mol/l increased activity of AhR. The other study performed by Wall’s group (Wall et al. 2012), in which they tested agonistic effects of synthetically prepared 2-amino-isoflavones (Chr), has shown that Chr-3, -15, -16 and -19, at the concentration 10\(^{-5}\) mol/l induced significant changes in the AhR-dependent luciferase reporter activity in human re-combinant HGL26.1c3 cells (derived from HepG2 cell line), while Chr-7, synthetic isoflavone with similar structure to BiA, showed almost no changes in the AhR induction (Wall et al. 2012). It seems that particular isoflavones may behave as AhR agonists, and this effect largely depends on the compound structure. In our experiments, we have confirmed Fo as the second strongest agonist of AhR, as compared to BiA, the compound lacking a hydroxyl group. Experiments in mice H1L6.1c2 cells (derived from Hepa1c1c7) showed that the strongest activators of AhR are isoflavones Chr-13 and Chr-15 (Wall et al. 2012). Other authors showed that Gen and DAI were capable to activate AhR in stable transfected mouse hepatoma Hepa-1 cells. On the other hand, in human MCF-7 and HepG2 cell lines, they do not activate AhR (Zhang et al. 2003). Wall et al. (2012) observed similar effects using synthetically prepared isoflavone Chr-18 with similar structure to DAI. In contrast to data obtained by Zhang’s group (Zhang et al. 2003), our experiments using DAI on the permanently transfected HepG2 cells showed the same result, but when using Gen, we have observed only slight agonistic effect (7.4% as compared to TCDD). What was the reason of a modest increase of AhR activation remains unclear.

In conclusion, we have described agonistic effect of selected isoflavones on arylhydrocarbon receptor in a novel AZ-AhR transgenic gene reporter human cell line. Detailed mechanism of isoflavones action is still not fully understood, and in addition to existing research of their effects mainly in terms of the nature of estrogen, it would be appropriate to also focus more on the research of their other biological properties.

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References


http://dx.doi.org/10.1615/CritRevEukarGeneExpr.v18.i3.20


http://dx.doi.org/10.4149/gpb_2013064


http://dx.doi.org/10.1007/BF02969267


http://dx.doi.org/10.1155/2014/520763


http://dx.doi.org/10.1093/toxsci/58.2.235


http://dx.doi.org/10.1621/nrs.04016


http://dx.doi.org/10.1016/j.jshmb.2007.10.001


http://dx.doi.org/10.3390/nu2030241


http://dx.doi.org/10.4103/2229-516X.125686


http://dx.doi.org/10.1095/brl.120.000716

Stevens E., Mezrich D., Bradfield A. (2009): The aryl hydrocarbon receptor: a perspective on potential roles in the immune system. Immunology 127, 299–311

http://dx.doi.org/10.1111/j.1365-2567.2009.03054.x


http://dx.doi.org/10.1016/j.tox.2012.03.011
http://dx.doi.org/10.1289/ehp.6322

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