Food and Chemical Toxicology 95 (2016) 203–210

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

A risk assessment-driven quantitative comparison of gene expression profiles in PBMCs and white adipose tissue of humans and rats after isoflavone supplementation

Vera van der Velpen [a, *], Pieter van 't Veer [a], M. Ariful Islam [b, 1], C.J.F. ter Braak [c], F.X. Rolaf van Leeuwen [b], Lydia A. Afman [a], Peter C. Hollman [a, d], Evert G. Schouten [a], Anouk Geelen [a]

[a] Division of Human Nutrition, Wageningen University, Wageningen, The Netherlands
[b] Sub-Department of Toxicology, Wageningen University, Wageningen, The Netherlands
[c] Biometris, Wageningen University, Wageningen, The Netherlands
[d] RIKILT Wageningen UR, Wageningen, The Netherlands

ARTICLE INFO

Article history:
Received 4 March 2016
Received in revised form
8 July 2016
Accepted 12 July 2016
Available online 14 July 2016

Keywords:
Risk assessment
Gene expression
Species and tissue differences
Quantitative evaluation
Isoflavones
Multivariate model

ABSTRACT

Quantitative insight into species differences in risk assessment is expected to reduce uncertainty and variability related to extrapolation from animals to humans. This paper explores quantification and comparison of gene expression data between tissues and species from intervention studies with isoflavones.

Gene expression data from peripheral blood mononuclear cells (PBMCs) and white adipose tissue (WAT) after 8wk isoflavone interventions in postmenopausal women and ovariectomized F344 rats were used. A multivariate model was applied to quantify gene expression effects, which showed 3–5-fold larger effect sizes in rats compared to humans. For estrogen responsive genes, a 5-fold greater effect size was found in rats than in humans. For these genes, intertissue correlations ($r = 0.23$ in humans, $r = 0.22$ in rats) and interspecies correlation in WAT ($r = 0.31$) were statistically significant. Effect sizes, intertissue and interspecies correlations for some groups of genes within energy metabolism, inflammation and cell cycle processes were significant, but weak.

Quantification of gene expression data reveals differences between rats and women in effect magnitude after isoflavone supplementation. For risk assessment, quantification of gene expression data and subsequent calculation of intertissue and interspecies correlations within biological pathways will further strengthen knowledge on comparability between tissues and species.

1. Introduction

Isoflavones are phytoestrogens present in soy (products) and the main isoflavones are daidzein, genistein and glycitein. Epidemiological studies in Asian countries suggest that isoflavones are beneficial for health, because soy consumption was associated with lower incidence of several types of cancer, osteoporosis and cardiovascular disease (Messina, 2010). However, results from *in vitro* and animal studies still raise doubts about their safety (Andres et al., 2011; Assessment, 2007; Wuttke et al., 2007), especially because of the putative activation of estrogen receptors (ER). Recently, we showed that the ER might not be the main transcription factor responsible for the induced gene expression effects of isoflavones in human peripheral blood mononuclear cells (PBMCs) and adipose tissue (van der Velpen et al., 2013; van der Velpen et al., 2014). Also, it has been shown that the proportions of the more active free isoflavones in the circulation were markedly

List of abbreviations: ER, estrogen receptor; ERGDB, estrogen responsive gene database; GSEA, gene set enrichment analysis; OVX, ovariectomized; PBMC, peripheral blood mononuclear cells; PCA, principal component analysis; RDA, redundancy analysis; RMSE, root mean square effect; SES, summary effect size; WAT, white adipose tissue.

* Corresponding author. Present address: Department of Nutrition and Preventive Medicine, Norwich Medical School, University of East Anglia, United Kingdom.
E-mail address: v.van-der-velpen@uea.ac.uk (V. van der Velpen).
[1] Present address: Department of Agricultural Chemistry, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

http://dx.doi.org/10.1016/j.fct.2016.07.013
0278-6915/

higher (20–150 times) in rodents than in humans (Setchell et al., 2011). Furthermore, over the past few years human studies have not confirmed the adverse effects found in *in vitro* and in animal studies, except for a disputable adverse effect related to endometrial thickness (Unfer et al., 2004), which was not confirmed in more recent studies (Alekel et al., 2015; D'Anna et al., 2007; Palacios et al., 2007, 2010). Altogether, this further raises the question of the applicability of animal models to predict effects of isoflavones in humans.

In risk assessment, potential adverse health effects in humans are generally extrapolated from animal experiments, which causes one of several sources of variability and uncertainty in risk assessment (Abt et al., 2010). Application of new techniques, like quantitative structure-activity relationship modelling, physiologically based biokinetic modelling and transcriptomics, might enable better quantification of interspecies differences (Pettit et al., 2010). Especially the use of transcriptomics is promising, as this data can be derived in a similar manner from animal experiments and human studies, enabling direct comparison. Furthermore, transcriptomics can be considered a powerful tool for detection of early effect markers, especially when changes in gene expression are considered within biological pathways (Elliott et al., 2007) with e.g. gene set enrichment analysis (GSEA (Subramanian et al., 2005)). Several studies have demonstrated the use of gene expression in the risk assessment of e.g. dibutyl phtalate (Euling et al., 2013a, 2013b; Makris et al., 2013), coumarin (Kienhuis et al., 2009a), acetaminophen (Kienhuis et al., 2009c) and benzo(a)pyrene (Moffat et al., 2015). These often regard qualitative and semi-quantitative approaches (Bourdon-Lacombe et al., 2015), while quantitative approaches could enable better comparison between tissues and species and advance the use of gene expression in risk assessment (Burgess-Herbert and Euling, 2013; Chepelev et al., 2015).

In this paper, we aim to quantitatively evaluate and compare gene expression data from two tissues (PBMCs and white adipose tissue (WAT)) after isoflavone interventions in women and rats using a multivariate model.

2. Materials and methods

Data from two human intervention studies and one rat experiment were used, aligned for dose, duration and target group, and obtained with standardized gene expression methods.

2.1. Human intervention studies

The two human studies, ISO and ISO II, were conducted at the Division of Human Nutrition of Wageningen University, approved by the Medical Ethical Committee of this university and described earlier by Van der Velpen et al. (van der Velpen et al., 2013; van der Velpen et al., 2014). Both studies, were double-blind placebo-controlled crossover studies with eight week intervention periods and eight week washout periods in between.

The ISO study determined the effects on PBMC gene expression of an isoflavone supplement in 27 equol-producing postmenopausal women. In the ISO II study, the effects of the same supplement on WAT gene expression were determined in 24 postmenopausal women, both equol producers (n = 7) and non-producers (n = 17). In- and exclusion criteria for both studies and the screening procedure for equol producers have previously been described (van der Velpen et al., 2013; van der Velpen et al., 2014). In brief, exclusion criteria were use of soy products or isoflavone supplements, hormone related medication, anti-inflammatory medicines, or antibiotics in the past 3 months. In addition, women with severe heart conditions, thyroid conditions, removed ovaries or prior diagnosis of cancer were excluded, as well as known alcohol and drug abuse, smoking habits, a BMI above 35 kg/m2 and self-reported allergy to soy. These studies were registered at clinicaltrials.gov under NCT01232751 and NCT01556737.

2.2. Rat experiment

The rat experiment was performed at the Centre for Laboratory Animals (CKP, Wageningen) in compliance with the Dutch Act on animal experimentation (Stb, 1977, 67; Stb, 1996, 565, revised February 5, 1997) and was approved by the ethical committee on animal experimentation of Wageningen University. All procedures were considered to avoid and minimize animal discomfort. This experiment was a parallel study with eight-week-old female inbred ovariectomized (OVX) F344 rats, all equol producers, with an 8-week dosing period. For our analysis, data from 5 treated rats and 5 control rats were used. Further detailed experimental conditions are described by Islam et al. (Islam et al., 2016). Oral gavage stock of the supplement was daily and freshly prepared in 10 ml water containing 1% DMSO. After eight weeks, the animals were anesthetized with a mixture of isoflurane and oxygen and blood was removed from the dorsal aorta.

2.3. Supplements and doses

All 3 studies were performed with the same isoflavone supplements. In the ISO study a batch bought in October 2010 was used, which contained 60% daidzein, 13% genistein and 27% glycitein. In the ISO II study and the rat experiment a batch bought in November 2011 was used, which had a similar isoflavone profile and contained 56% daidzein, 16% genistein and 28% glycitein (Table 1).

The postmenopausal women in the two studies ingested ~100 mg isoflavones/day (aglycone equivalents), similar to daily intakes of over-the-counter isoflavone supplements. This dose equivalent to 1.34 (range 0.88–1.70) and 1.42 mg/kg bw/day (range 0.94–1.81). For the animal study, the intake was scaled to body weight and was 2.0 mg/kg bw/day.

2.4. Transcriptomics

In the human studies, gene expression after both supplement and placebo treatment was measured in PBMCs (n = 27, all equol producers) and WAT (n = 24; 7 equol producers and 17 non-producers) by Affymetrix human gene 1.1 ST arrays (van der Velpen et al., 2013; van der Velpen et al., 2014). In the rat experiment, the gene expression in PBMCs and WAT (n = 5 for the control and n = 5 for the treated group, all equol producers) was measured by Affymetrix rat gene 1.1 ST arrays.

2.5. Data analysis

Data analysis of the Affymetrix chips was described before for the human studies (van der Velpen et al., 2013; van der Velpen et al., 2014) and the animal study (Islam et al., 2016). In brief, expression values of the data were calculated using the robust multichip average (RMA) method and normalised using quantile normalisation in MADMAX (Bolstad et al., 2003; Irizarry et al., 2003; Lin et al., 2011).

To compare rat and human gene expression, the rat genes were recoded into human genes using the Homologene database (http://www.ncbi.nlm.nih.gov/homologene) and all duplicate genes were removed. After this, 80.7% of the rat genes in PBMCs and 81.5% genes in WAT that passed filtering remained as human homologs.

Due to the design differences in the human and the rat study,