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Assessing anti-estrogenic effects of AHR ligands in primary human and rat endometrial epithelial cells

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ABSTRACT

Unopposed estrogenic action in the uterus can lead to the development of endometrial cancer in both humans and rats. Aryl hydrocarbon receptor (AHR) activation gives rise to anti-estrogenic actions and may consequently reduce the development of endometrial cancer. In this study, the anti-estrogenic potential of the AHR ligands 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and DELAQ, a metabolite of the pharmaceutical laquinimod, was assessed in in primary human and rat endometrial epithelial cells (EECs) with and without co-exposure to endogenous hormones.

In human EECs, estradiol and progesterone did not affect *AHR* gene expression, but in rat EECs, progesterone decreased *Ahr*e xpression (1.4-fold). In accordance, AHR-mediated induction of *Cyp1a1/1b1* expression by DELAQ and TCDD decreased in hormone-treated rat EECs. DELAQ was 22-fold more potent than TCDD in human EECs in inducing *CYP1A1/1B1* gene expression, while DELAQ was approximately 16 – 33-fold less potent than TCDD in rat EECs. In human EECs, 10 nM DELAQ decreased estradiol-induced expression of growth-regulated estrogen receptor binding 1 (*GREB1*) by 1.8-fold. In rat EECs, both DELAQ and TCDD did not affect the expression of estradiol-induced genes.

This study shows that AHR ligand DELAQ, but not TCDD, causes anti-estrogenic effects in primary human EECs. Furthermore, although AHR-mediated *CYP1A1/1B1/Cyp1a1/1b1* induction by DELAQ and TCDD was stronger in rat EECs than human EECs, this did not result in apparent anti-estrogenic effects in the rat cells. This study shows that primary human and rat endometrial cells respond differently towards hormones and AHR ligands. This should be considered in human risk assessment based on rodent studies.

1. Introduction

Endometrial cancer is the most common gynecologic malignancy in women in developed countries that most often affects postmenopausal women [1]. A possible mechanism by which endometrial cancer may arise is *via* a disrupted estrogen (E2)/progesterone (P4) ratio in the uterus [2,3]. These steroids are produced in the ovaria and the levels are tightly regulated throughout the menstrual and estrous cycle. E2 stimulates the epithelial endometrial cells to proliferate in order to prepare for implantation of a blastocyst. An aberrant hormonal (E2/P4) environment can therefore cause a variety of adverse effects in the uterus. Indeed, increased circulating E2 levels have been linked to a higher incidence of uterine carcinomas in animal experiments as well as in human epidemiological studies [4,5]. While spontaneous endometrial cancer is rare in young naïve rodents, endometrial adenocarcinomas are a common phenomenon in aged rats and in rat toxicity studies with environmental contaminants and pharmaceuticals [2,6]. As opposed to rat toxicity studies, epidemiological studies and clinical trials typically do not reveal a clear association between these compounds and endometrial tumors in humans. One mechanistic explanation for the endometrial cancer risk could be that estrogen signaling can be modulated through cross-talk with the aryl hydrocarbon receptor (AHR) [7,8]. Also, activation of the AHR can display species-specific responses depending on the AHR ligand [9]. AHR activation by both endogenous and exogenous compounds can result in the induction of cytochrome P450 (CYP) enzymes. In the human endometrium, E2 is metabolized by cytochrome P450 1A1 (CYP1A1) and 1B1 (CYP1B1) and AHR activation can therefore result in local metabolism of E2. AHR activation has also been shown to result in other anti-estrogenic effects by interfering with estrogen receptor (ER)-DNA binding to specific

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estrogen responsive elements that contain an overlapping dioxin response element sequence. This can lead to stimulation of ER proteasomal degradation, inhibition of selected E2-target genes and other as yet unidentified mechanisms [10]. The AHR agonist 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) has been shown to exert anti-estrogenic effects by reducing the incidence of E2-dependent tumors in rat xenograft models *in vivo*, including uterine tumors, and the E2-induced proliferation of endometrial Ishikawa and breast tumor MCF-7 cell lines *in vitro* [11–13].

A previous study by our group showed that TCDD and DELAQ are potent inducers of *CYP1A1* gene expression in primary cultures of human and rat endometrial cells, albeit with apparent species differences [14]. Considering this potency to activate the AHR, an inhibitory cross-talk with estrogen signaling is expected in human and rat endometrial cells. The present study was designed to assess the potential anti-estrogenic effects of TCDD and DELAQ in primary human and rat endometrial epithelial cells (EECs) and compare the effecs between both species.

2. Methods

2.1. Primary tissue and isolation

Endometrial tissue of eight individual female donors was obtained from the St Antonius hospital in Nieuwegein, the Netherlands. The study was approved by the hospital's Medical Ethical Committee (Study number Z15.038) and written informed consent for the use of endometrial tissue was obtained from all patients. The age of the donors varied between 43 and 56 years. None of the donors were using hormonal medication prior to or at the time of hysterectomy. No information on the time of the menstrual cycle or any other personal information was disclored due to ethical restrictions.

Rat endometrial cells were isolated from ~7-week old female Sprague-Dawley rats under approval of the Dutch law for animal welfare and protocols were approved by the Ethical Committee on Animal Research and the Animal Care Committee of Utrecht University (AVD108002015135; WP 800−15-135−01-002). This study complied with the ARRIVE guidelines.

The human and rat endometrial epithelial cells were isolated as described earlier [14]. The purity of the cellular fractions was visually assessed by the distinctly different morphological features of the cell types, i.e. the typical cobblestone-like morphology of the epithelial cells, and the elongated wave-like morphology of the stromal cells. Both human and rat EECs were cultured in RMPI-1640 medium + 1 % penicillin/streptomycin (p/s) + 10 % fetal bovine serum (FBS; Hyclone, GE Healthcare) and kept in an incubator at 37 °C in 5 % CO2. Medium was refreshed every 3 days. Both human and rat EECs were cultured with the standard medium as described before, and approximately 7 days prior to the experiment to "reset" the steroid receptor status in the cells. After that, the cells were cultured with different hormone media (containing no hormones, 100 nM E2 or 100 nM P4) for three days (see section 2.2), to "prime" the steroid receptor status of the cells. This was done to synchronize the hormonal state of the cells and to limit the effect of the differences in time of the reproductive cycle at the time of endometrial cell isolation.

2.2. Experimental conditions

It has been documented that human EECs cultured on a layer of extracellular matrix (ECM) grow in a polarized, more *in vivo* like fashion [15]. Therefore, both human and rat EECs were seeded on pre-coated ECM 24-well plates. The coating comprised of a thin layer of 1:1 medium and ECM (Sigma, the Netherlands) that was allowed to polymerize at 37 °C. After ~7 days of culture, the epithelial cells were detached from the culture flasks using Accutase (Life Technologies) and seeded with a density of approximately 90.000 cells per well. The culture medium was replaced with RMPI-1640 medium + 1 % p/s + 10 % dextran charcoal treated FBS (Hyclone, GE Healthcare). Cells were seeded with or without hormones: 0.1 % ethanol (no hormones), 100 nM estradiol (E2; Sigma) or 100 nM progesterone (P4; Sigma). After 3 days, this medium was refreshed containing the different hormones in addition to the different concentrations of TCDD, DELAQ, or 0.1 % DMSO as a vehicle control. After 48 h of exposure, RNA Instapure (Eurogentec, Belgium) was added to the cells to preserve the mRNA. Cells were stored at −80 °C until RNA isolation.

2.3. RNA isolation and qPCR

RNA was isolated by phenol–chloroform extraction method. Purity and concentration of the isolated RNA were determined spectrophotometrically at an absorbance wavelength of 260 and 280 nm. Complementary DNA (cDNA) was synthesized using the Script cDNA Synthesis Kit (Bio-Rad, The Netherlands), according to the manufacturer's instructions. The obtained cDNA was diluted 10 times and stored at 4 °C until further analysis. Quantitative reverse transcriptase-polymerase chain reaction (RT-qPCR) was performed using the CFX96 cycler (BioRad). A PCR mastermix was made containing IQ SYBR Green Supermix, forward and reverse primer with a concentration of 10 μM and RNA-se free water. Samples were heated initially at 95 °C for 15 min and then followed by 40 cycles with denaturation at 94 °C for 30 s, annealing/extension temperature (between 55 and 65 °C) for 30 s. Oligonucleotide sequences and primer-specific annealing temperatures of the human and rat are listed in Table S1. Primer efficiencies were established as between 90 % and 110 %. Gene expression was determined using the ΔΔCq method of relative quantification where gene expression was normalized to GAPDH and β-actin (human samples) or Arbp and β-actin (rat samples).

2.4. Data analysis

Experiments were performed in triplicate, unless stated otherwise. Unpaired t-tests were used to analyze the statistical difference of the mean between the hormone treatments in the vehicle controls of human and rat EECs. Concentration-response curves for CYP1A1 and CYP1B1 induction were calculated relative to 1 nM and 0.1 nM TCDD for human and rat, respectively. Symbol meaning; * = $P \leq 0.05$, ** = $P \leq 0.01$, *** = $P \leq 0.001$. The relative potencies of DELAQ to TCDD were derived by dividing the average induction of CYP1A1 and CYP1B1 for DELAQ and TCDD at similar compound concentrations (1 nM for the human cells and 0.1 nM for the rat cells).

3. Results

3.1. Hormone responses in human and rat EECs

In humans and rats, hormonal regulation of cyclic endometrial growth is regulated through ER and progesterone receptor (PR). In a preliminary experiment, ERα gene expression was not consistently induced by E2 in the human EEC, whereas ERβ gene expression was very low in human EECs, and did not respond to E2 treatment (data not shown). Therefore, the gene expression of both isoforms of the ER was not included in the assessment of anti-estrogenic effects in this study. In contrast, gene expression of PR and growth-regulated estrogen receptor binding 1 (GREB1) showed clear E2-responsiveness in human EECs. PR gene expression was statistically significantly 1.3-fold higher in the E2-treated human EECs, compared to the vehicle-treated control cells (Fig. 1A). GREB1 gene expression was statistically significantly 7.8-fold higher in the E2-treated human ECCs, compared to the vehicle-treated control cells (Fig. 1B). The expression of E2-responsive genes PR and GREB1 was not affected by P4 in human EECs. AHR gene expression did not change in the E2-treated or P4-treated human EECs, compared to the vehicle-treated control cells (Fig. 1C).

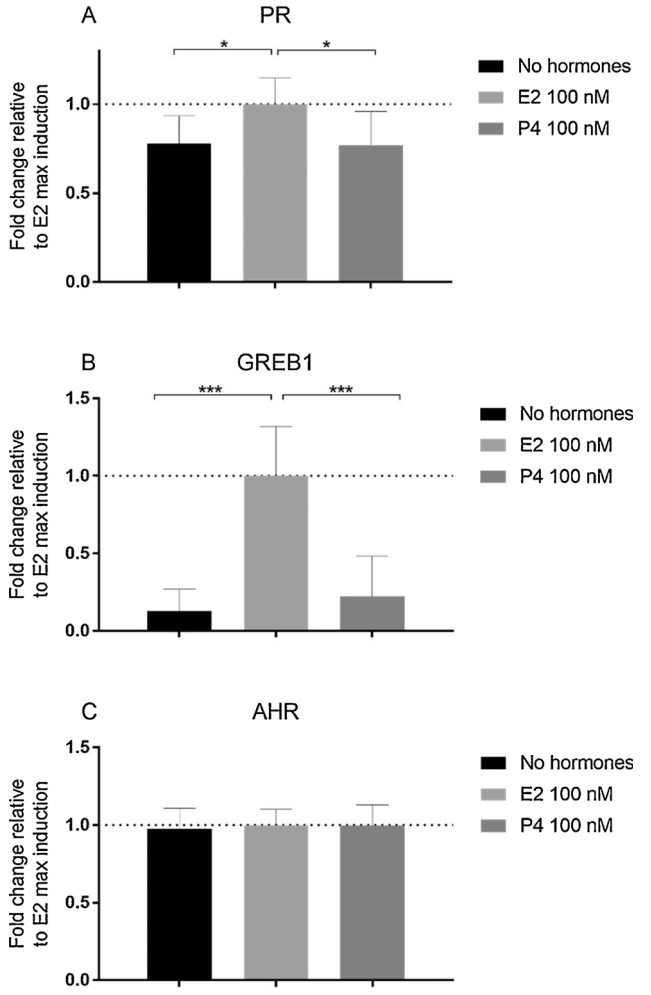

Fig. 1. Effect of hormones (no hormones (0.1 % v/v ethanol), 100 nM estradiol (E2) or 100 nM progesterone (P4)) on the expression of A) PR, B) GREB1 and C) AHR mRNA in human endometrial epithelical cells (EECs). Values are expressed as mean values and standard deviations relative to the expression in E2-treated human EECs with N = 8. Statistically significantly different with * $P \leq 0.05$ and *** $P \leq 0.001$.

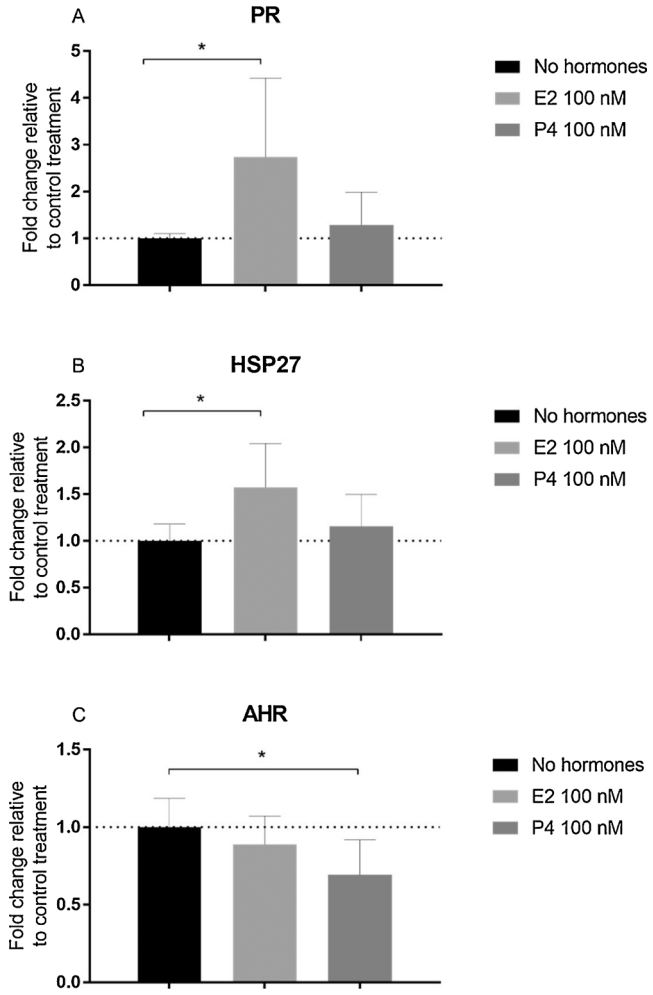

Fig. 2. Effect of hormone treatment (no hormones, 100 nM estradiol (E2) or 100 nM progesterone (P4)) on the expression of A) PR, B) HSP27 and C) AHR mRNA in rat EECs. Values are expressed as mean values and standard deviations relative to the expression in vehicle treated (0.1 % ethanol) control cells with N = 5. Statistically significantly different from no-hormone treated EECs with * $P \leq 0.05$.

Similar to human EECs, ERα gene expression was not consistently and strongly induced by E2 treatment in rat EECs in this study (data not shown). PR gene expression also showed clear E2-responsivess in rat EECs and was statistically significantly 2.7-fold higher in the 100 nM E2-treated cells, compared to the vehicle-treated control cells (Fig. 2A). As gene expression of GREB1 in rat uterine tissue has not been reported in literature, heat shock protein 27 (HSP27) gene expression was also investigated as E2-responsive gene in rat. In rat EECs, HSP27 gene expression was statistically significantly 1.6-fold higher in the E2-treated cells compared to the vehicle control treated cells (Fig. 2B). mRNA expression of E2-responsive genes, PR and HSP27, was not affected by P4 in rat EECs. In contrast to human EECs, AHR gene expression was affected by homones in rat EECs. AHR gene expression slightly decreased 1.1-fold upon treatment with E2, and statistically significantly decreased 1.4-fold upon treatment with P4 (Fig. 2C).

3.2. AHR gene battery in human and rat EECs

To determine the effects of TCDD and DELAQ on AHR-dependent genes, gene expression of *CYP1A1/Cyp1a1* and *CYP1B1/Cyp1b1* were studied in human and rat EECs. In human EECs, *AHR* gene expression did not statistically significantly change upon 1 nM TCDD or 0.01 − 10 nM DELAQ treatment alone or in combination with E2 or P4 (Supplemental Fig. 1).

Normalized *CYP1A1* gene expression induced by TCDD varied from 1.2-fold to 17.8-fold between donors (Supplemental Fig. 2). As a result of these interindividual differences, the variation in the grouped data was relatively large. To correct for these interindividual differences in *CYP1A1* inducibility, changes in *CYP1A1* gene expression are displayed relative to *CYP1A1* gene expression as induced by 1 nM TCDD for every individual experiment. This concentration TCDD significantly induced CYP1A1 expression. *CYP1A1* gene expression induced by TCDD was not affected by E2 or P4 in human EECs (Fig. 4). *CYP1A1* gene expression was statistically significantly induced by DELAQ in a concentration-dependent manner compared to the vehicle control, and was not affected by either E2 or P4 (Supplemental Fig. 3). In the human EECs, the induction of *CYP1A1* gene expression by 1 nM TCDD was equipotent to 0.03 nM DELAQ (Figs. 3,4A, intersection of dotted line and fitted curve). Therefore, the mean relative effect potency of DELAQ to TCDD for *CYP1A1* expression in human EECs is ~33.

CYP1B1 gene expression induced by 1 nM TCDD was less variable between donors than *CYP1A1* gene expression, with an interindividual ~4-fold variability (Supplemental Fig. 4). The CYP1B1 gene expression induced by 1 nM TCDD varied from 1.5-fold to 6.5-fold between donors. The induction of *CYP1B1* gene expression by 1 nM TCDD in human EECs was on average 1.5-fold lower than the induction of *CYP1A1*

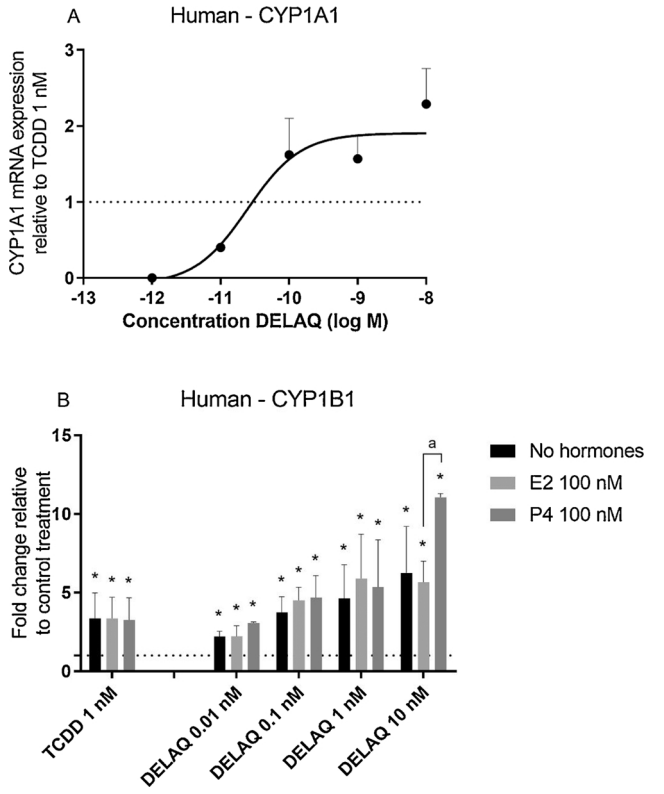

Fig. 3. mRNA expression of CYP1A1 (A) and CYP1B1 (B) in human EECs. Data is expressed as mean induction (+standard deviation) relative to 1 nM TCDD (CYP1A1) or relative to the vehicle (0.1 % DMSO) treated control cells (CYP1B1) with total N = 7. *Statistically significantly different from the vehicle-treated control cells with P ≤ 0.05. [a]Statistically significantly different between the E2 and P4 co-treatments with P ≤ 0.05.

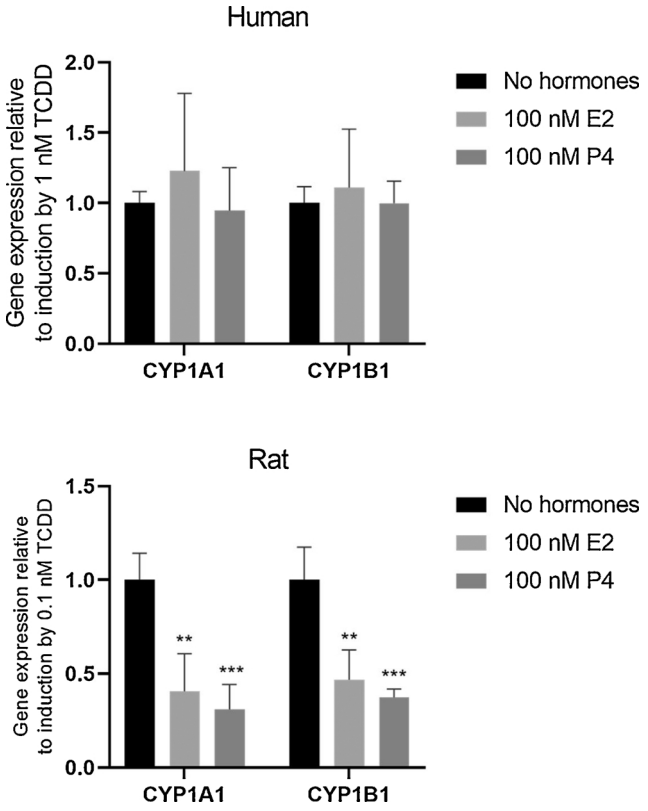

Fig. 4. mRNA expression of CYP1A1, CYP1B1, *Cyp1a1* and *Cyp1b1* in human and rat endometrial epithelial cells. Data is expressed as mean induction (+standard deviation) relative to induction by 1 nM TCDD (human) or 0.1 nM TCDD (rat) in the no hormone (0.1 % ethanol) treatment with N = 8 (human) and N = 4 (rat). Statistically significantly different from no-hormone treated EECs with ** P ≤ 0.01; *** P ≤ 0.001.

regardless of the hormonal co-treatment (Supplemental Fig. 2 and 4). Similarly to *CYP1A1*, *CYP1B1* gene expression was not affected by E2 or P4. However, the gene expression of the endometrial cells treated with 10 nM DELAQ in combination with P4 deviated from the other treatment groups (Fig. 3B). Upon P4 treatment, gene expression was 1.8-fold higher compared to the no hormone treatment, and statistically significantly 2.0-fold higher compared to the E2 treatment. *CYP1B1* gene expression was statistically significantly induced by 0.01 − 10 nM DELAQ compared with vehicle-treated control cells in a concentration-dependent manner. Similar to *CYP1A1* induction, the mean relative effect potency of DELAQ to TCDD for *CYP1B1* gene expression in human EECs was ~33.

In rat EECs, basal *Cyp1a1* gene expression was extremely low in the vehicle-treated control EECs. As a result, normalized *Cyp1a1* gene expression induced by 0.1 nM TCDD varied from 98-fold to 333-fold in the no hormone exposed cells, 13-fold to 2003-fold in the E2 exposed cells and 10-fold to 740-fold in the P4 exposed cells, when calculated relative to the vehicle-treated control EECs. To correct for this large variation as a result of low basal gene expression, changes in *Cyp1a1* gene expression were calculated relative to *Cyp1a1* gene expression induced by 0.1 nM TCDD. This concentration TCDD significantly, but not maximally induced *Cyp1a1* mRNA (Fig. 5A). As rats are generally more sensitive to AHR activation than humans, this concentration was 10-fold lower than the concentration used to express the CYP1A1 expression in human EECs.

Cyp1a1 gene expression induced by 0.1 nM TCDD statistically significantly decreased 2.5-fold after co-exposure with E2 and 3.1-fold after co-exposure with P4 compared with no-hormone treated EECs (Fig. 4). *Cyp1a1* gene expression was induced by both TCDD and DELAQ in a concentration-dependent manner in rat EECs (Fig. 5). In rat EECs, the induction of *Cyp1a1* gene expression by 0.1 nM TCDD was equipotent to 2.1 nM DELAQ in the vehicle-treated control cells, and 1.6 nM in the E2 and P4 treated cells (Fig. 5A intersection dotted line and fitted curves). The mean relative effect potency of DELAQ to TCDD for induction of *Cyp1a1* expression in rat EECs compared to TCDD is ~0.05 in the no hormone treatment and ~0.06 in the E2 and P4 treatments.

The maximal induction of *Cyp1b1* gene expression by 0.1 nM TCDD in rat EECs was approximately 26-fold lower than the induction of *Cyp1a1* relative to the vehicle-treated control EECs, with no co-treatment of hormones. *Cyp1b1* gene expression was induced in a concentration dependent manner from 0.01–1 nM TCDD and 0.1–10 nM DELAQ (Fig. 5B). Similarly to *Cyp1a1*, *Cyp1b1* gene expression induced by 0.1 nM TCDD statistically significantly decreased 2.2-fold after co-exposure with E2 and 2.7-fold after co-exposure with P4 (Fig. 4). The induction of *Cyp1b1* gene expression by 0.1 nM TCDD was equipotent to 1.8 nM DELAQ in the E2 treatment and 3.5 nM in the P4 treatment as calculated by expressing *Cyp1b1* gene expression relative to the treatment of 0.1 nM TCDD (data not shown). No comparison could be made for the no hormone treatment, as *Cyp1b1* induction by DELAQ up to 10 nM did not reach the level of 0.1 nM TCDD-induced *Cyp1b1* gene expression in the no hormone treatment. The relative effect potency of DELAQ to TCDD for *Cyp1b1* gene expression in the rat EECs is −0.06 in the E2 treatment and −0.03 in the P4 treatment.

3.3. Human and rat EECs and anti-estrogenicity

Activation of the AHR can result in an inhibitory cross-talk with estrogen signaling pathways. To determine anti-estrogenic potential, the effects of AHR ligands TCDD and DELAQ on E2-responsive genes

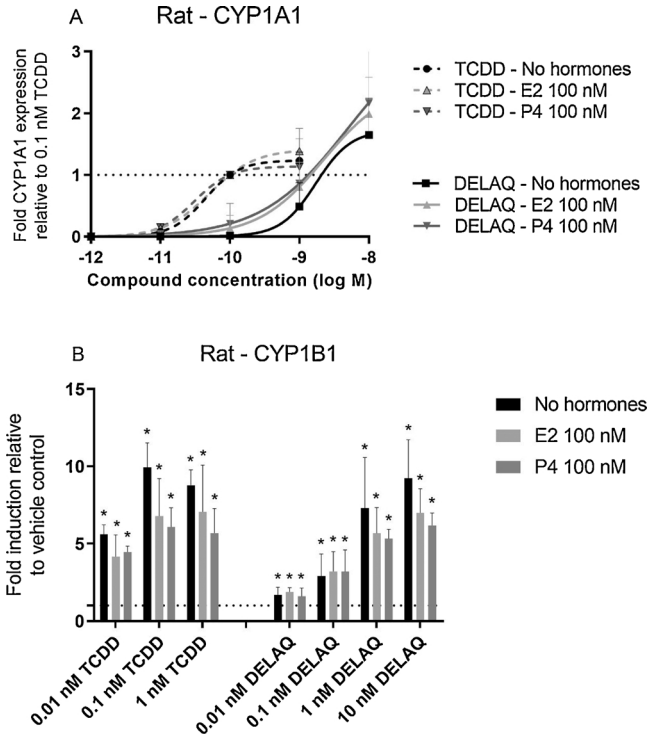

Fig. 5. mRNA expression of *Cyp1a1* (A) and *Cyp1b1* (B) in rat endometrial epithelial cells. Data is expressed relative to 0.1 nM TCDD (A) or vehicle control (B) treated cells for every hormonal treatment with total N = 4. *Statistically significantly different from the vehicle-treated control cells with P ≤ 0.05.

was assessed in human and rat EECs.

Although a slightly decreasing trend in *PR* gene expression was observed after exposure to DELAQ in E2-treated human EECs, both TCDD or DELAQ did not cause a statistically significant reduction of E2-induced *PR* gene expression nor in the other hormonal treatments (Supplemental Fig. 5). In our study, E2 alone increased *PR* gene expression 1.3-fold, but increased *GREB1* gene expression 7.8-fold in human EECs (Fig. 1). Anti-estrogenic effects on *GREB1* expression were observed in human EECs following treatment with DELAQ (Fig. 6, left panel). DELAQ reduced E2-induced gene expression of *GREB1* in a concentration-dependent manner. E2-induced *GREB1* expression decreased by 1.6-fold and 1.8-fold by 1 and 10 nM DELAQ treatment, respectively. Yet, this effect was not considered statistically significant as P-values were 0.06 for both 1 and 10 nM DELAQ. TCDD (1 nM) did not statistically significantly affect the gene expression of *GREB1* induction by E2 (Fig. 6, left panel). In contrast to the human EECs, no changes in the gene expression of E2-responsive genes *Pr* and *Hsp27* were observed in rat EECs after treatment with TCDD or DELAQ (Fig. 6, right panel).

4. Discussion

This study showed clear species-differences in AHR-mediated responses in human and rat primary EECs. Firstly, E2 and P4 reduced *AHR/Ahr* and *CYP1A1/Cyp1a1* gene expression in rat, but not in human EECs. Secondly, maximal induction of AHR-responsive genes was higher in rat EECs compared to human EECs. Thirdly, anti-estrogenic effects of DELAQ were only apparent in human EECs, and not in rat EECs; whereas exposure to the best-known AHR agonist TCDD did not show any anti-estrogenic effect on *GREB1* or *Pr/Hsp27* gene expression in primary human or rat EECs, respectively. The potency for AHR activation by DELAQ was higher than TCDD in human EECs, but lower in rat EECs. This may explain why anti-estrogenic effects of AHR activation were only apparent in human EECs upon DELAQ exposure, and not in rat EECs. These fundamental species-differences should be considered when assessing endometrial tumor development in rat studies and its relevance for the human situation.

The AHR is constitutively expressed in the human endometrium. In this study, *AHR* gene expression did not change in primary human EECs *in vitro* upon E2 or P4 treatment. This is in line with several studies showing no changes in *AHR* gene expression in endometrial tissue obtained during follicular and luteal phases of the female menstrual cycle [16,17]. In contrast, in rat EECs, *Ahr* gene expression decreased upon treatment with E2 and P4. This decrease was also observed in a previous study performed by us using a co-culture of primary epithelial and stromal endometrial cells after exposure to hormones [14]. Rataj et al. also described that *Ahr* mRNA was downregulated by both E2 and P4 in uteri of ovariectomized rats *in vivo* [18,19]. The authors described a stronger effect of E2 on AHR downregulation than P4, while in the present study, P4 exerted a stronger effect on AHR downregulation in rat EECs *in vitro*. Possibly, the uteri of ovariectomized rats respond stronger to E2 which can account for the differences between the

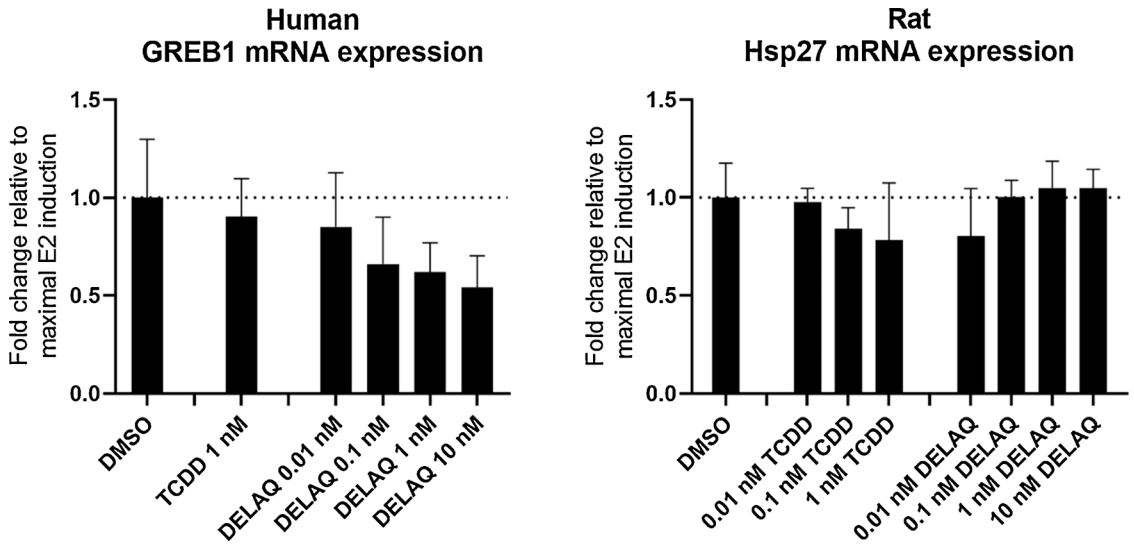

Fig. 6. mRNA expression of *GREB1* in human endometrial epithelial cells (left panel) and *Hsp27* in rat endometrial epithelial cells (right panel). Data is expressed relative to the induction in the 100 nM E2 vehicle-treaded control cells. N = 6 with N = 6 for TCDD, N = 2 for 0.01 nM and 10 nM DELAQ and N = 3 for 0.1 and 1 nM DELAQ for the human cells and N = 4 for the rat cells.

studies. Chaffin et al. reported a decrease in *Ahr* gene expression in ovarian cells in Lewis rats *in vivo* during the pro-estrus, when both E2 and P4 levels are high [20]. These data combined indicate a species-specific difference in AHR expression rather than a cell type specific effect in response to hormones. However, the modulating effect of circulating/ background hormones on AHR expression in *in vivo* studies cannot be excluded.

Besides the differences in AHR expression, also species-specific differences in AHR activation was observed in our study. Here, we demonstrate that DELAQ is more potent than TCDD in *CYP1A1/1B1* activation in human endometrial cells, while the reversed was observed in rat cells. The potencies of DELAQ compared to TCDD to induce *CYP* gene expression was on average, approximately 0.05 in rat and 33 in human EECs. These species differences in AHR ligand response may result from differences in ligand binding domains of the AHR which can result in ligand binding specificity [21,22].

CYP1A1 and CYP1B1 are considered AHR-responsive genes, but the CYP enzymes also play important roles in estrogen metabolism thus normal physiology. Furthermore, endometrial tumors are considered a consequence of disbalance between estrogen and progesterone in the uterus. Estrogen metabolism plays a crucial role herein as it affects estrogen levels. AHR activation can modulate estrogen metabolism *e.g. via* the induction of estrogen metabolizing enzymes like CYP1A1 and 1B1. Similarly to present study, a large interindividual variability in *CYP1A1* and *CYP1B1* gene expression patterns was reported in various human primary endometrial cell types [17]. In addition, Bulun et al. did not find any difference in basal *CYP1A1* and *CYP1B1* gene expression between uterine samples from the proliferative and luteal phases. This is in line with our observation that in human EECs, *CYP1A1* and *CYP1B1* gene expression was not affected by E2 or P4 exposure. Remarkably, studies have demonstrated an estrogen responsive element (ERE) in the CYP1B1 promotor, suggesting a stimulatory effect of E2 on CYP1B1 [23]. Possibly, the effect of E2/ERE on *CYP1B1* induction is tissue-specific, as this was not observed in our study [24]. However, to the best of our knowledge, no studies have investigated endometrium-specific regulation of CYP1B1 or the role of CYP1B1 in ER regulation in primary endometrium.

Despite species-, ligand-, and tissue differences in AHR-mediated effects, typically, CYP1A1 is more highly induced than CYP1B1 upon AHR activation [25–27]. The preferential induction of *CYP1A1* over *CYP1B1* was also observed in human and rat EECs in this study. We also observed a large interindividual difference in, in particular CYP1A1 induction in the human EECs. One factor that can contribute to the variability of human sensitivity to CYP induction is the expression of the AHR. This may, for example, be caused by life-style factors, such as exposure to AHR agonists, including cigarette smoke or PAHs from air pollution, which may have led to differences in AHR expression as well as background and inducible levels of CYP1A1 in humans. Other studies have previously also shown a large variability in basal and induced CYP1A1 expression [28]. The differential induction of *CYP1A1* and *CYP1B1* is especially important, when considering the role of these CYPs in estradiol metabolism [29]. CYP1A1-mediated metabolism leads to predominantly 2-hydroxyestrogen formation, which is considered the “good” metabolite. CYP1B1-mediated estrogen metabolism, in contrast, results in formation of the potentially carcinogenic 4-hydroxyestrogens [7,30,31]. Based on normalized gene expression, the *CYP1A1/1B1* ratio upon AHR activation by TCDD or DELAQ was approximately 1 in human EECs, but this was approximately 0.5 and 0.7 in rat EECs for TCDD and DELAQ, respectively. This suggests a shift in estrogen metabolism, favoring CYP1B1-mediated E2 metabolism in rat EECs, as opposed to human EECs. CYP1B1-mediated metabolism of E2 can result in the formation of DNA adducts and mutagenicity which can ultimately initiate breast and other cancers [32]. Indeed, it was reported that many tumor cells express a higher level of CYP1B1 compared to the surrounding normal tissue [33]. This is also supported by a −15 − 200-fold higher excretion of estrogen-DNA adducts in urine from women with breast cancer compared to women without breast cancer [34]. It would be extremely informative for future studies to determine the effects of changes in *CYP1A1/1B1* ratio in endometrial cells and link this to actual estrogen metabolite levels and/or cancer markers.

Taken together, AHR activation in human EECs is related to anti-estrogenic actions and is considered protective against, especially, endometrial cancer. For example, smoking is associated with lower endometrial cancer risk due to AHR activation by polycyclic aromatic hydrocarbons in cigarette smoke [35]. In the present study, anti-estrogenic effects of AHR ligands in human and rat EECs were assessed using *PR/Pr* (both species), *GREB1* (human) and *Hsp27* (rat) gene expression as markers of E2-responsiveness [36–39]. *Hsp27* gene expression was assessed in the rat EECs as, to the best of our knowledge, gene expression of *Greb1* in rat uterine tissue has not been reported in literature. No anti-estrogenic effects, based on changes in *Pr/Hsp27* gene expression were observed in rat EECs upon TCDD or DELAQ exposure. E2-induced *Pr* levels in rat mammary glands were also reported not changed by TCDD [40]. However, anti-estrogenic effects of TCDD have been reported in rats *in vivo*. Chaffin et al. 1996 showed reduced circulating E2 levels in pregnant Holtzman rats after exposure to TCDD [41]. Yet, effects on uterine wet weights, an E2-dependent endpoint, were not reported [41]. In contrast, exposure to DELAQ did attenuate E2-induced expression of *GREB1* in human EECs. No anti-estrogenic effects for TCDD were observed in human EECs, which may be explained by the higher potency of DELAQ to activate the AHR and induce *CYP1A1* and *CYP1B1* expression in human EECs compared to TCDD. As to the lack of anti-estrogenic effect in rats, this may be explained by a low basal AHR expression. Although the relative induction of *CYP* gene expression, as a proxy for AHR activation, is high in rat EECs as a result of a very low basal expression, the absolute CYP expression may not be high enough to induce the anti-estrogenic effects as observed in the human EECs.

5. Conclusion

This study cleary shows that primary rodent and human endometrial cells respond differently towards hormones and AHR ligands. This should be taken into account when performing human risk assessment based on rodent studies and warrants the development of human-relevant models to assess endometrial effects of xenobiotics.

Declaration of interest and funding

The authors have no conflicts of interest to declare. This study was performed for and financed by Teva Pharmaceutical Industries Ltd. with study number DS-2017 − 063.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.reprotox.2020.07.003.

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