Effect of CYP17A1 inhibitors orteronel & galeterone on adrenal androgen biosynthesis.

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Introduction

The CYP17A1 enzyme, localized in the endoplasmic reticulum, can catalyze both 17α-hydroxylase and 17,20-lyase reactions. In human adrenal glands CYP17A1 enzyme is found in both the ZF and ZR of the cortex and it also has a role in synthesis of cortisol and DHEA. The 17,20-lyase activity of CYP17A1 is key for androgen regulation. Understanding the mechanisms regulating 17,20-lyase activity is important for the understanding of hyperandrogenic disorders such as premature, exaggerated adrenarche and the polycystic ovary syndrome, and also for the design of selective 17,20-lyase inhibitors for use in hyperandrogenic states and in sex-steroid dependent cancers. The orteronel and galeterone are known to inhibit 17,20-lyase activity however the detail mechanism of the CYP17A1 inhibition remains unknown. These inhibitors have been developed to treat the castration resistant prostate cancer (CRPC) but little is known about its effect on adrenal androgen biosynthesis. The objective of this project is to study the effect of these inhibitors on CYP17A1 enzyme in adrenal androgen biosynthesis.

Methods

We used the NCI-H295R adenocarcinoma cell model to study the effect of orteronel and galeterone. We treated H295R cells from 0-2 µM orteronel and galeterone for 24 hours. Steroid production was labeled with [^{3}H] pregnenolone for 90 min. Steroids were extracted and resolved by thin layer chromatography. For specific analysis of the CYP17A1 activities, cells were treated with 1µM trilostane (a specific blocker of HSD3B) for 90 min before adding labeled with [^{3}H] pregnenolone. To study the effect of these inhibitors on steroidogenic gene expression we performed the relative quantification PCR (qRT-PCR). Effect of inhibitors on aromatase activity was measured using a tritiated water-release assay using ^{3}H labelled androstenedione as substrate.

Results

1. Role of CYP17A1 in steroidogenesis

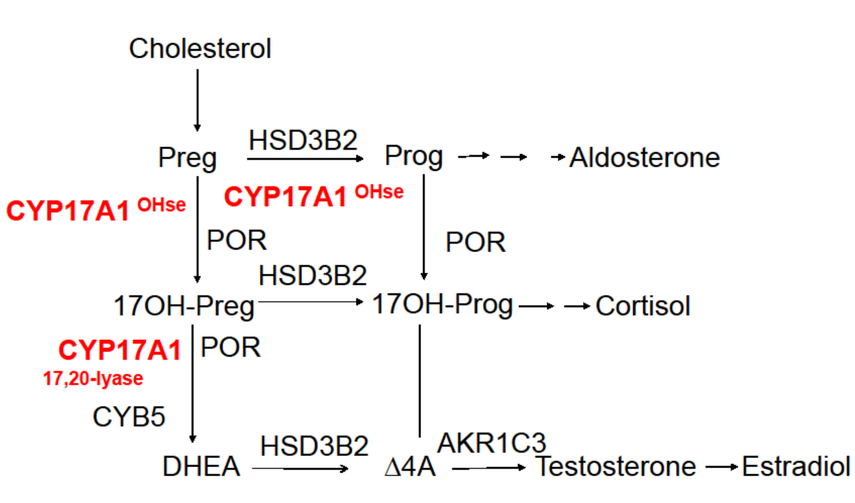

Figure 1: Short-form of human steroidogenic pathways. In the absence of CYP17A1, steroidal C_{21} 17-deoxy precursors are directed toward the production of the aldosterone. In the presence of the 17α-hydroxylase activity of CYP17A1, C_{21} 17-deoxysteroids are converted to C_{21} 17-hydroxysteroid precursors of the cortisol. In the presence of the 17,20-lyase activity of CYP17A1, C_{21} 17-hydroxysteroids are converted to C_{19} 17-hydroxysteroid precursors of sex steroids. Note that human CYP17A1 does not convert significant amounts of 17-OH progesterone to androstenedione.

2. CYP17A1 inhibitors

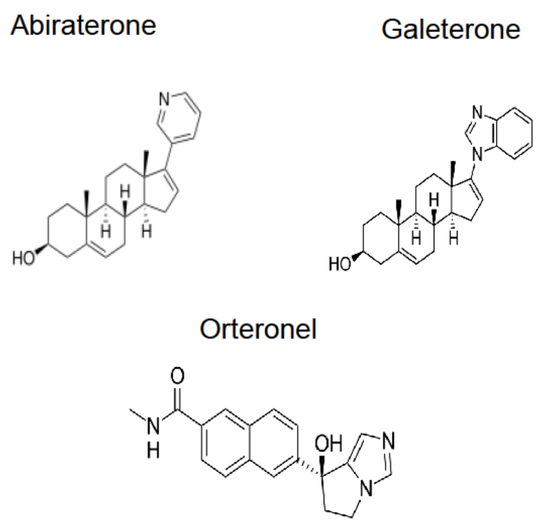

Figure 2: Structures of CYP17A1 inhibitors. Abiraterone and galeterone are steroidal inhibitors with AR antagonist activities. Orteronel is a non-steroidal CYP17A1 inhibitor.

3. Effect of inhibitors on adrenal steroid profile

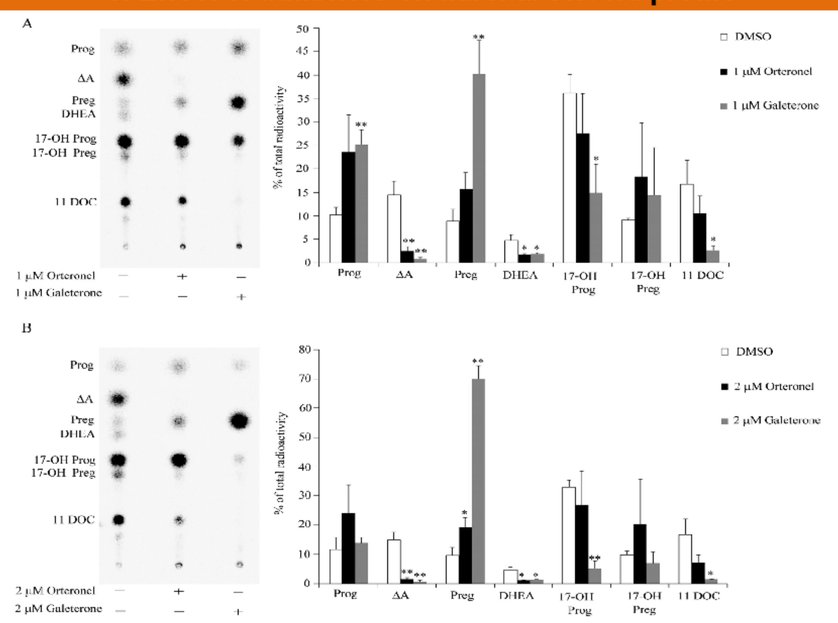

Figure 3: Steroid profiles of H295R cells treated with 1 or 2 µm orteronel and galeterone for 24 h were assessed and compared to absence of treatment. Steroid production was labeled with [^{3}H] pregnenolone for 90 min. Steroids were extracted and resolved by TLC (A-B) Steroid profiling of H295R cells treated with 1 or 2 µm of orteronel and galeterone. Prog, progesterone; Δ4A, androstenedione; Preg, pregnenolone; DHEA, dehydroepiandrostenedione; 17-OH Preg, 17α-hydroxypregnenolone; 17-OH Prog, 17α-hydroxyprogesterone; 11DOC, 11-deoxycortisol.

4. Effect of inhibitors on CYP17A1 enzyme activity

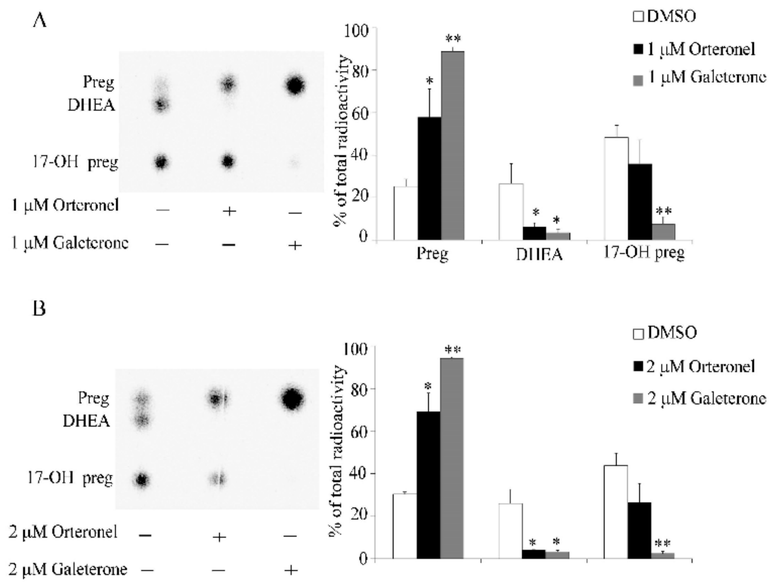

Figure 4: The enzyme activities of CYP17A1 were assed in H295R cells treated with 1 or 2 µM of orteronel and galeterone. CYP17A1 activities were assessed by looking at the conversion of [^{3}H] pregnenolone (Preg) to 17α-hydroxypregnenolone (17OH Preg) for 17α-hydroxylase and to DHEA for 17,20-lyase activity. (A-B) Representative TLCs are depicted on the left, summaries and quantifications of the results are given on the right. Data are given as mean±SEM of two independent experiments. *, P ≤ 0.05,** p<0.01.

5. Impact on androgen biosynthesis genes

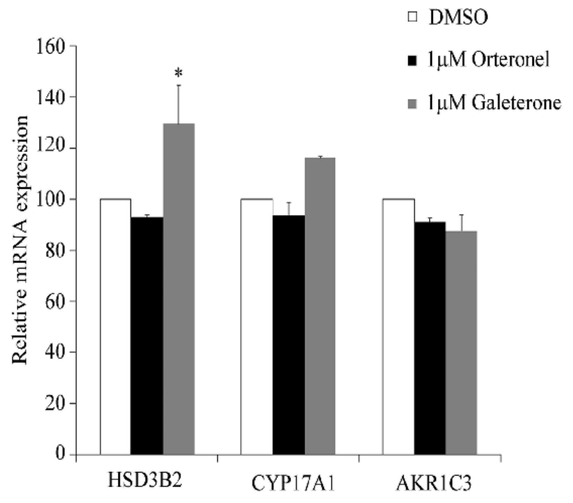

Figure 5: H295R cells were treated with 1 µM orteronel and galeterone for 72h and then RNA was isolated and transcribed to cDNA. Results of quantitative reverse transcription PCR validation of HSD3B2, CYP17A1 and AKR1C3 genes relative to the housekeeping gene cyclophilin A. Expression of the genes was analyzed by SYBR Green real-time PCR. Analysis of relative gene expression was determined by the $2^{-\Delta\Delta Ct}$ method. Results are shown as quantification of two independent qRT-PCR experiments (mean±SD). * p<0.05.

6. Effect on CYP19A1 enzyme activity

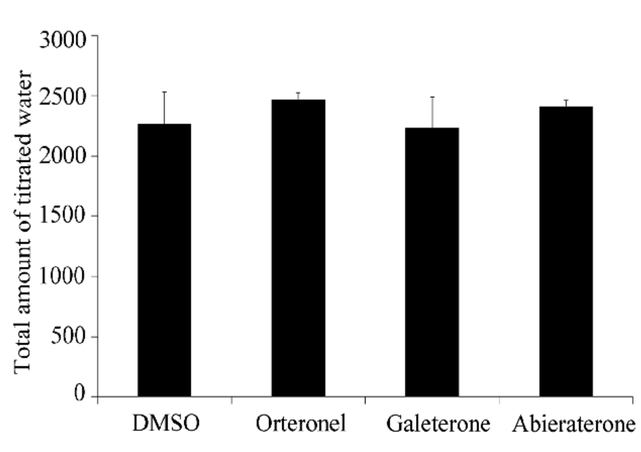

Figure 6: Effect of inhibitors was studied on aromatase activity in human placental JEG-3 cells. Cells were treated with DMSO or 1 µM of orteronel, galeterone and abieraterone for 24 h before aromatase activity was assayed. Aromatase activity was assayed with androstenedione with cold 2 µM substrate and ^{3}H labeled androstenedione ([1β-^{3}H(N)]-androstene-3,17-dione; ~100,000 cpm/well) as labelled. Androstenedione was added to the treated cells for 6 h. Aromatase activity was then assessed by the tritiated water release assay.

Conclusions

- Based on our results we can conclude that orteronel is a more potent inhibitor of 17,20-lyase activity than galeterone in H295R cells.
- Additionally, we found slight change in HSD3B2 gene expression especially due to treatment of galeterone but in presence of orteronel no significant change was observed suggesting that it does not affect steroidogenic gene expression.
- We also showed that CYP17A1 inhibitors has no effect on aromatase (CYP19A1) enzyme activity.
- Discovery of these drug actions on specific CYP17A1 17, 20-lyase activity would be of great clinical value for understanding adrenal androgen regulation.

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