HIV Drug Efavirenz Inhibits CYP21A2 Activity with Possible Clinical Implications

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ABSTRACT

Background: HIV drugs lopinavir and ritonavir have been recently reported to cause transient adrenal insufficiency in preterm newborns. We, therefore, considered HIV drugs as a cause of transient elevation of 17-hydroxyprogesterone (17OHP) in a neonatal screening test for congenital adrenal hyperplasia (CAH) in a preterm girl exposed to zidovudine, efavirenz, tenofovir, and emtricitabine.

Objective: So far HIV drugs have not been tested for their effect on steroidogenesis and steroidogenic enzyme CYP21A2 specifically in an *in vitro* system.

Methods: We tested the effect of efavirenz, tenofovir, emtricitabine, and zidovudine on steroidogenesis of human adrenal H295R cells. Cells were treated with drugs at different concentrations including concentrations in therapeutic use. The effect on CYP21A2 activity was assessed by testing the conversion of radiolabeled 17OHP to 11-deoxycortisol. Cell viability was tested by an MTT assay. In addition, recombinant produced and purified human CYP21A2 protein was used to assess the direct drug effect on CYP21A2 activity.

Results: We observed significantly decreased CYP21A2 activity in both *in vitro* testing systems after treatment with efavirenz at therapeutic concentrations. Moreover, efavirenz affected cell viability. By contrast, the other test drugs did not affect steroidogenesis. Follow-up of our patient revealed elevated 17OHP and androgens during the first weeks of life, but values normalized spontaneously. Genetic testing for *CYP21A2* mutations was negative. Thus whether transient elevation of 17OHP in this baby was due to a drug effect remains unsettled.

Conclusion: The HIV drug efavirenz inhibits CYP21A2 activity *in vitro* through direct interaction with enzyme catalysis at therapeutic concentrations. This may have clinical implications for HIV treatment in children and adults. However, so far clinical data are scarce, and further studies are needed to draw clinical conclusions.

1. Introduction

Adrenal insufficiency (AI) may be a life-threatening condition due to inadequate production of glucocorticoids and mineralocorticoids. It is classified as primary AI (PAI), with the underlying cause in the adrenals and as secondary AI, with the underlying defect in the hypothalamic-pituitary control of the adrenals. In newborns, congenital adrenal hyperplasia (CAH) due to genetic mutations in the *CYP21A2* gene is the leading cause of PAI and affects about 1 in 10'000 - 15'000 newborns in Europe (1-3). CYP21A2 is essential for both mineralocorticoid and glucocorticoid production because it mediates the 21-hydroxylation of progesterone to deoxycorticosterone and 17-hydroxyprogesterone (17OHP) to 11-deoxycortisol (2). The severe, classic, salt-wasting form of CYP21A2 deficiency is a potentially life-threatening condition characterized by low or absent production of cortisol and aldosterone as well as overproduction of adrenal androgens and manifests early in life (1-4). The milder, non-classic form of CAH may manifests later in life predominantly with consequences of adrenal androgen excess. While girls with classic CAH often manifest with ambiguous genitalia at birth due to intrauterine overproduction of androgens (5), boys do not show physical signs suggestive of CAH (4,6). Therefore many countries have introduced a neonatal screening program for early diagnosis of CAH based on blood 17OHP levels reflecting CYP21A2 enzyme deficiency (3,6).

However, 17OHP blood screening by heel-stick may reveal both false positive and false negative results. Especially challenging is the interpretation of elevated results in preterm (7) or stressed newborns (8). Also, 17OHP may be elevated in other rarer forms of CAH such as HSD3B2, CYP11B2 or P450 oxidoreductase deficiencies, which also require prompt corticosteroid replacement therapy (9,10). Furthermore, the activities of adrenal enzymes can be influenced by drugs and toxins, and thereby cause PAI in rare cases. Drugs such as ketoconazole, metyrapone, etomidate, mitotane, and abiraterone are known inhibitors of steroid enzymes including CYP11A1, CYP17A1, CYP21A2 and CYP11B1/2 (11,12).

In recent clinical studies, it has been suggested that some HIV drugs may also affect steroidogenesis. Elevated serum 17OHP and dehydroepiandrosterone (DHEA) levels were found in neonates treated perinatally with HIV-1 protease inhibitors lopinavir and ritonavir (13,14). Three premature babies presented with clinical and biochemical signs of adrenal dysfunction after postnatal treatment with these drugs (13), while a full-term neonate was noted to have high serum 17OHP and potassium levels after pre- and postnatal treatment with lopinavir and ritonavir (14). In our clinic, we also followed a preterm baby of an HIV infected mother for repeatedly high levels of 17OHP in neonatal screening. Drugs efavirenz, emtricitabine, and tenofovir (Atripla®) were prescribed to the mother during pregnancy to reduce the mother to child transmission of HIV, and the neonate was treated with zidovudine for prevention postnatally. According to current literature (these) HIV drugs have not been tested for a possible effect on steroidogenesis.

The World Health Organization (WHO) reported in 2017 that about 18 million women worldwide were infected with HIV. They also reported that HIV treatment during pregnancy was very effective and reduced the mother to child transmission of an HIV infection from 45% to 2% (15). However, this means that a significant number of neonates are exposed to HIV drugs very early in life, and possible adverse effects of drugs might be of significance.

Therefore, motivated by the clinical observations and stimulated by the fact that none of the HIV drugs in routine use have been tested for an effect on adrenal steroidogenesis, we tested some of these drugs for their effect on steroidogenesis, specifically on CYP21A2 activity *in vitro*.

2. Materials and Methods

Case Report

A 46, XX baby girl was born at 26 weeks gestation after premature rupture of membranes to an HIV infected mother. Birth weight was 760g (P 40), length 35cm (P 60). Because of premature contractions, the mother received 2 doses of betamethasone at 23 5/7 weeks gestation for induction of lung maturation. In addition, the mother was treated with HIV drugs tenofovir, efavirenz and emtricitabine (Atripla®) throughout pregnancy. The newborn was then treated with zidovudine for (post-)exposure prophylaxis during the first four weeks of life (Figure 1). The girl showed a normal physical exam at birth including normal female external genitalia without signs of virilization but received a single dose of hydrocortisone (0.1mg/kg) for low blood pressure. Initial laboratory workup in serum revealed normoglycemia, normal electrolytes and a normal 17OHP (80 nmol/l; reference value for GA and postnatal age <192nmol/l). But while glucose and electrolytes remained normal, 17OHP increased to 292nmol/l on day 15 (norm for GA and age <141nmol/l) and was 132nmol/l on day 19 (norm <104nmol/l; DELFIA Neonatal 17αOH-progesterone kit, PerkinElmer, Switzerland), and prompted further workup for possible CAH. ACTH (8.3ng/l; reference range 7.2-63.3) and cortisol (396 nmol/l; reference range 171-536nmol/l) were both normal at three weeks of age. Urine steroid profiling (GC-MS) at 3 and 6 weeks of age revealed elevated progesterone and androgen metabolites. Finally, serum 17OHP normalized after four weeks (Figure 1) and the clinical course was completely unremarkable. The baby was discharged home at the postconceptional age of 36 1/7 weeks. She is currently 3 years old and healthy.

Urinary steroid profiling

Urinary steroid profiling was performed by an established, validated *in-house* method of gas chromatography-mass spectrometry (GC-MS) (16-19). Measurements of spot urines (collected from cotton balls inserted in diapers) were normalized to creatinine (QuantiChrom Creatinine Assay; DICT-500, BioAssay Systems, Hayward, CA, USA). Accordingly, results are expressed in µg/mmol creatinine (Supplemental Table 1) as previously described for healthy neonates during the first year of life (16-18).

Genetic analysis of the CYP21A2 gene

Three fragments of the *CYP21A2* gene were amplified by PCR from genomic DNA using the CAH StripAssay® (ViennaLab Diagnostics, Vienna, Austria). In addition, all coding sequences of the *CYP21A2* gene were sequenced with the Sanger method using internal sequencing primers. For analysis, the *CYP21A2* reference sequence NM_000500.6 was used.

In vitro cell assays

Material: Antiretroviral HIV drugs zidovudine (3'-azido-3'deoxythymidine) and efavirenz were purchased from Sigma-Aldrich (Buchs, Switzerland), tenofovir and emtricitabine from Toronto research chemicals (Brisbane, Canada). Radio-labeled [^{3}H]-17-hydroxyprogesterone (17OHP; 50 Ci/mmol) was from American Radiolabel Chemicals Inc. (St. Louis, MO, USA). MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was obtained from Sigma-Aldrich (Buchs, Switzerland). The human adrenal carcinoma cell line NCI-H295R originates from American Type Culture Collection (ATCC).

NCI-H295R cells were cultured under standard conditions (12,20). Test drugs were dissolved in dimethyl sulfoxide (DMSO); final concentrations used for treatment were in and above reported mean

effective serum concentrations in use for HIV therapy. Cells were grown in twelve well plates. Drugs were added to normal growth medium for 3 and 24 h. Control cells were treated with 0.1% (v/v) DMSO. Radio-labeled [^{3}H]-17-hydroxyprogesterone (50,000 cpm/well) was added to the culture medium for the last 90 min of incubation. Steroids were then extracted from cell supernatants and separated by thin layer chromatography (TLC) as described (21). Steroids were visualized on a Fuji PhosphoImager FLA-7000 (Fujifilm, Dielsdorf, Germany) and densitometrically quantified using Multi Gauge software (Fujifilm). The % conversion of 17OHP to 11-deoxycortisol (11DOC) was taken as a measure of CYP21A2 activity.

The MTT cell proliferation assay was used to determine cell viability and proliferation of NCI-H295R cells treated with HIV drugs. In brief, cells were cultured on 96-well plates at a density of 15'000 cells/well in 200 µl medium. After 48 hours cells were treated with the HIV drugs for 3 and 24 hours. Then cell proliferation was assessed by adding 20 µl of MTT to the culture medium for 3 hours before reading the absorbance at 540 nm on a Spectramax M2e microplate reader (Molecular Devices, CA, USA). In this assay, the absorbance at 540 nm correlates directly to the number of viable cells.

In vitro studies with recombinant CYP21A2 protein

Human CYP21A2 protein was expressed in *E. coli* strain C43(DE3) (Lucigen, Middleton, MI, USA) and purified via metal chelate (IMAC) and ion exchange chromatography as described (22). Carbon monoxide difference spectroscopy was carried out for a quantitative enzyme characterization following the typical absorption maximum at 450 nm with an extinction coefficient of 91 $mM^{-1}cm^{-1}$. Human NADPH-cytochrome P450 reductase (POR) was expressed in *E. coli* C43 (DE3) and purified by IMAC as described (23).

Inhibition studies were performed in reconstituted *in vitro* assays in 50 mM HEPES buffer (pH 7.4) containing 20% glycerol and 100 µM 1,2-dilauroyl-sn-glycerol-3-phosphocholine. Before use, the buffer was sonicated in a water bath for 5 minutes for the reconstitution of 1,2-dilauroyl-sn-glycero-3-phosphocholine vesicles. The final concentration of human CYP21A2 was 0.1 µM, and equal amounts of human POR were added. Additionally, the reaction contained an NADPH regeneration system consisting of 5 mM glucose-6-phosphate, 1 mM $MgCl_2$ and glucose-6-phosphate dehydrogenase. The steroid substrate 17OHP was added at a concentration of 5 µM and the drugs were tested at 5 or 50 µM concentration. The substrate concentrations were kept below saturation, but in excess over the K_m for CYP21A2. The final DMSO concentration was kept below 2%. The reaction was started with 5 mM NADPH and incubated in a shaking water bath for 4 - 7 min at 37°C. The reaction was quenched with chloroform. Steroids were extracted twice with chloroform, dried and stored at -20°C for HPLC analysis, specifically the measurement of 17OHP conversion to 11DOC.

Steroid analysis was finally carried out by RP-HPLC using a Jasco reversed phase HPLC system of the LC900 series (Jasco Inc, Easton, MD, USA) and a 4.6 mm × 125 mm NucleoDur C18 Isis Reversed Phase column (Macherey-Nagel, Düren, Germany). Samples were measured within 30 min at 240 nm and a flow rate of 0.8 mL/min with the gradient: 80% solvent A (10% acetonitrile in water) for 13 min, 60% solvent A for 7 min, 80% solvent B (100% acetonitrile) for 2 min and 80% solvent A for 8 min.

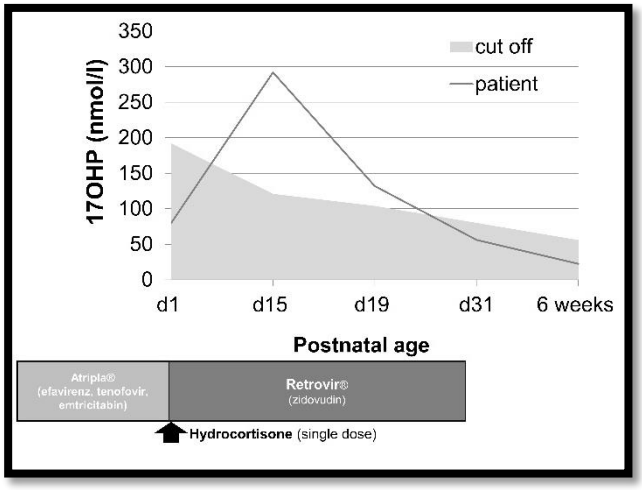

Figure 1: Transiently elevated serum 17-hydroxyprogesterone (17OHP) levels in a newborn under the influence of Atripla® (efavirenz, tenofovir, emtricitabine) prenatally and Retrovir® (zidovudine) during 4 weeks postnatally. Note that the newborn received one dose of hydrocortisone in the first 24 hours after birth. Peak 17OHP levels were seen at d15 and normalized by d28 according to normative values of the Swiss 17OHP neonatal screening test.

In silico protein structure analysis

A 3D crystal structure of human CYP21A2 was obtained from the PDB database (www.rcsb.org) for docking analysis of efavirenz binding (24). However, the structure (PDB # 4Y8W) has multiple residues missing that creates gaps in the peptide backbone making it unstable during molecular dynamics analysis. A model building using sequence conservation information and secondary structure analysis was needed to fill the gaps and create a complete structure suitable for docking and molecular dynamics calculations. To get the secondary structure information of missing residues we performed sequence alignments with multiple CYP21A2 protein sequences from different organisms (Supplementary Figure 1) and made *in-silico* calculations with the programs YASARA (25) and WHATIF (26). Missing hydrogen atoms were added with YASARA (25) which was also used for all subsequent computations unless stated otherwise. Afterward, the system was subjected to 500 ps explicit solvent MD simulations at 310 K, preceded by 500 steps of steepest descent and simulated annealing minimization with the AMBER15 force field and the TIP3P water model (27,28). All subsequent MD simulations retained these settings. The resulting minimum energy structure was used with

AutoDock Vina (29) for docking experiments with efavirenz. Orthorhombic docking was grid established around the central heme. The final poses were selected based on their docking scores and resemblance to the co-crystallized progesterone in the template structure (PDB: 4Y8W). Structure models were depicted with Pymol (www.pymol.org) and rendered as ray-traced images with POVRAY (www.povray.org). Ligand interactions were analyzed.and depicted with LIGPLOT+ (http://www.ebi.ac.uk/thornton-srv/software/LigPlus/).

Statistical analysis

Statistical analysis was performed with Microsoft Excel and software Prism 6 (Graph Pad Software, Inc. San Diego, CA, USA). Student's t-test was used to evaluate the significance of differences between values. Quantitative data represent the mean of two or three independent experiments; error bars indicate the mean ± SEM. Significance was set at *$p < 0.05$ and **$p < 0.01$, ***$p < 0.001$.

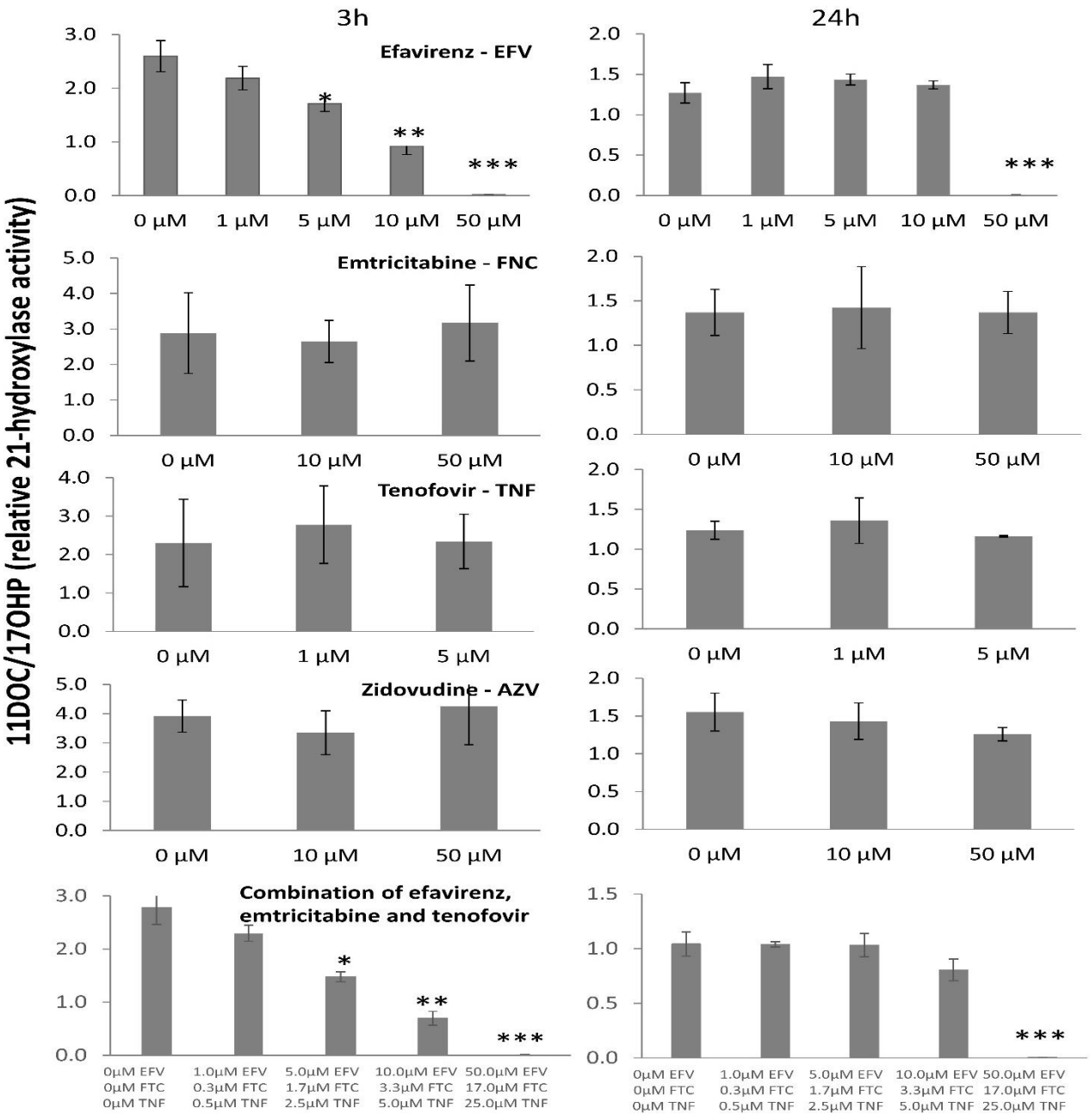

Figure 2 –Efavirenz inhibits CYP21A2 activity at concentrations in therapeutic use, while no effect was seen for the other tested HIV drug compounds. Human adrenal NCI-H295R were treated with efavirenz, emtricitabine, tenofovir, and zidovudine in various concentrations for 3 and 24 hours to test for their effect on steroidogenesis. Steroids were extracted from cell supernatants and separated by thin layer chromatography (TLC). Relative CYP21A2 activity was calculated by assessing the conversion of 17-hydroxyprogesterone (17OHP) to 11-deoxycortisol (11DOC). Data are the mean ± SEM of two to four independent experiments. *$p < 0.05$ and **$p < 0.01$, ***$p < 0.001$

3. Results

Biochemical data of neonates exposed to HIV drugs lopinavir and ritonavir suggested an inhibitory effect on adrenal steroidogenesis (13,14). Similarly, we observed elevated serum 17OHP levels and urinary steroid profile abnormalities consistent with diminished CYP21A2 and maybe diminished CYP17A1 activities in a preterm girl after exposure to tenofovir, efavirenz and emtricitabine *in utero*, and zidovudine postnatally (see Case Report; Figure 1). Genetic testing excluded mutations in the *CYP21A2* gene.

Efavirenz inhibits CYP21A2 activity

The effect on steroidogenesis of antiviral HIV drugs zidovudine, emtricitabine, tenofovir, and efavirenz was tested in the adrenal H295R cell line. Efavirenz is a non-nucleoside reverse transcriptase inhibitor. Its reported mean effective serum concentration is 1.6-9.1µM (https://www.medicines.org.uk/emc/product/8659/smpc). The drug was tested at four concentrations (1µM, 5µM, 10µM, 50µM) after 3h and 24h incubation. A significant decrease of CYP21A2 activity was observed after 3h incubation starting at 5µM ($p < 0.05$), with a clear additional dose effect at higher concentrations (10µM, 50µM; $p < 0.01$, $p < 0.001$) (Figure 2, left panel). By contrast, after 24h incubation CYP21A2 inhibition was only observed at the highest (50µM) efavirenz concentration ($p < 0.001$) suggesting that longer incubation may allow the cells to compensate for the drug effect to some degree (Figure 2, right panel).

Zidovudine and emtricitabine are nucleoside reverse transcriptase inhibitors. Reported mean effective serum concentration for zidovudine is 4.45µM - 8.5µM (https://www.medicines.org.uk/emc/product/6811/smpc) and for emtricitabine it is 4.5µM - 10µM (https://www.medicines.org.uk/emc/product/18/smpc), respectively. H295R cells were treated with zidovudine and emtricitabine in two concentrations (10µM and 50µM) for 3h and 24h. Both drugs showed no effect on CYP21A2 activity at either timing (Figure 2). Tenofovir is also a nucleoside reverse transcriptase inhibitor, but by contrast to zidovudine and emtricitabine its mean effective serum concentration is markedly lower (0.74 - 1.13 µM) (https://www.medicines.org.uk/emc/product/771/smpc). Therefore, tenofovir was tested at lower concentrations of 1 and 5µM for 3 and 24h. No effect on CYP21A2 activity was observed (Figure 2).

As the HIV drugs efavirenz, tenofovir, and emtricitabine are often in use as triple medication Atripla®, we also tested the three compounds together in our H295R cell system. These experiments revealed the same inhibitory effect on CYP21A2 as observed with efavirenz alone (Figure 2, bottom).

Furthermore, to assess whether the tested drugs may affect H295R cell viability, we performed an MTT cell proliferation assay. Efavirenz starting at 5µM reduced cell proliferation significantly compared to control cells Efavirenz at 10 and 50µM seemed extremely cytotoxic. The same effect was also observed in H295R cells treated with the combination drug Atripla®, while no effect was seen with single treatments of zidovudine, emtricitabine, and tenofovir.

Efavirenz is a direct inhibitor of CYP21A2 activity

We also tested the direct effect of the four HIV drugs on enzyme catalysis using human recombinant CYP21A2 protein and 17OHP as a substrate. Similar to the cell culture experiments, we found that efavirenz inhibited CYP21A2 activity to a significant degree, decreasing the product formation to 30 % at a concentration of 50 µM efavirenz (Figure 3). By contrast, no effect on enzyme activity was found for the other three test drugs (Figure 3).

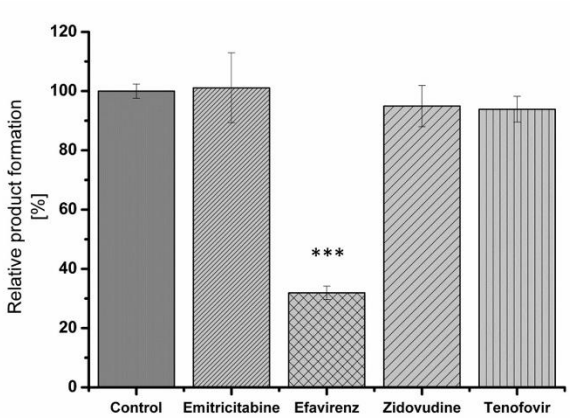

Figure 3 – Effect of HIV-drugs on CYP21A2 activity using human recombinant CYP21A2 protein produced in bacteria. Protein was produced as published. CYP21A2 activity was tested with 5 µM 17OHP as substrate and HIV-drugs at following concentrations: emtricitabine 50 µM, efavirenz 50 µM, zidovudine 50 µM, and tenofovir 5 µM. Steroids were extracted and analyzed by RP-HPLC (see Methods).

Computational docking of efavirenz into the human CYP21A2 crystal structure

Efavirenz was docked into the crystal structure of human CYP21A2 using Autodock VINA (Figure 4A). Superimposition of CYP21A2 structures with either progesterone or efavirenz docked into the active site revealed similarities in binding poses (Figure 4B and 4C). We observed a distance of 2.8 Å between efavirenz and heme iron at the active site of CYP21A2 (Figure 4A). Efavirenz is a smaller molecule than progesterone and may achieve several potential binding poses inside the CYP21A2. However, the occupation of the CYP21A2 catalytic site by efavirenz would inhibit the binding and metabolism of CYP21A2 steroid substrates. A comparison of the CYP21A2 and CYP17A1 crystal structures in complex with steroid substrates and docked efavirenz into the crystal structure of CYP21A2 revealed similarities in interacting residues and the distance of the efavirenz nitrogen to the central heme iron of CYP21A2 was in similar range (Figure 4A). Binding of efavirenz with CYP21A2 shares many similarities with natural substrates of CYP21A2 with identical active site residues such as Ser109, Gly292, and Leu364 involved in binding for both chemicals (Figure 4B and 4C).

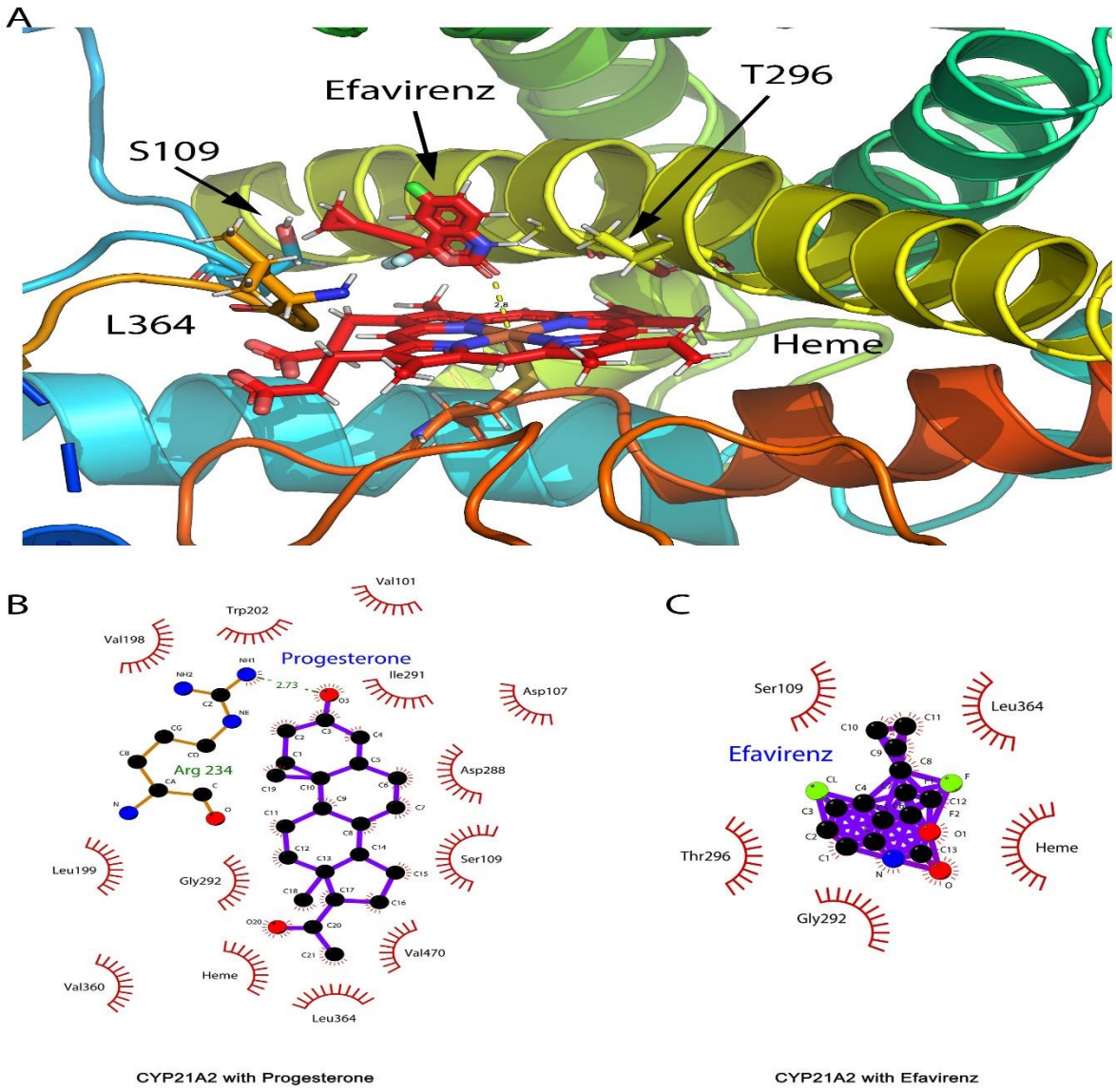

Figure 4 –Structure analysis of HIV-drug efavirenz interaction with the CYP21A2 enzyme. A. Efavirenz docked into the protein structure of CYP21A2. B. Progesterone versus C. Efavirenz binding poses revealed by the CYP21A2 structure and docking studies.

4. Discussion

Laboratory investigations of neonates exposed to HIV drugs suggest that these compounds may alter adrenal steroidogenesis (13,14). However, so far these drugs had not been tested for their possible effect on adrenal steroidogenesis. In this study, we show that the antiretroviral drug efavirenz inhibits steroidogenic enzyme CYP21A2 at therapeutic concentrations in human adrenal NCI-H295R cells and in direct kinetic protein interaction assays. Our bioinformatic studies suggest that efavirenz is a direct competitor to substrates of the enzyme. By contrast, no inhibitory effect on CYP21A2 activity was found for zidovudine, emtricitabine, tenofovir.

Medications in use for HIV treatment fall in different categories concerning drug safety (https://aidsinfo.nih.gov/guidelines). Tested drugs suspected to inhibit steroidogenesis are listed in the category characterized by limited experience in pregnancy and with incomplete data on teratogenicity, toxicity and drug interactions. In literature, efavirenz has been linked to congenital anomalies in monkeys and humans in some studies (https://aidsinfo.nih.gov/guidelines, (30,31)), but these adverse effects were not confirmed in others (32,33). Currently no restrictions apply to the use of efavirenz during pregnancy, neither in British nor US HIV treatment guidelines (https://www.bhiva.org/pregnancy-guidelines and https://aidsinfo.nih.gov/guidelines**)**. Fetal exposure to tenofovir was shown to lower bone mineral content in infants compared to controls (34). By contrast, no congenital anomalies or adverse effects have been reported with the HIV drug emtricitabine (33,35,36). Concerning interactions of HIV drugs with cytochrome P450 enzymes, nothing was known for steroidogenic P450s so far. But ritonavir has been reported to inhibit

drug metabolizing CYP2D6 and CYP3A4 (37,38), while an enzyme induction of ritonavir/lopinavir on CYP1A2 and CYP2C9 and CYP2C19 activities has been observed (39). These drugs have not been tested for their effect on CYP21A2 in this studies, but are currently being tested in cell experiments with preliminary results showing an inhibitory effect of lopinavir but not ritonavir on CYP21A2 and less on CYP17A1.

Overall these results suggest that HIV drugs should be carefully tested for their possible effect on P450s including steroidogenic P450 enzymes as this may have clinical implications. Mild inhibition of adrenal steroid biosynthesis may not have consequences in healthy individuals, but can result in inability to sustain stressful situations with severe disease where within minutes there is a high demand for stress hormones. In addition, long term lowgrade inhibition of CYP21A2 or other steroid enzyme activities may lead to an imbalance between adrenal corticosteroids and androgens with all its consequences. This is best exemplified by late-onset CAH due to mild CYP21A2 deficiency. Such effect may also result from medications interfering with steroid enzyme activities as shown for antiepileptic drugs, which can cause an increase in androgen production and a polycystic ovary syndrome phenotype (40,41).

The premature baby described in our case report showed positive newborn screening for transient elevation of plasma 17OHP and increased urinary excretion of androgens, estrogens and progesterones after treatment with HIV drugs during fetal life (tenofovir, efavirenz, emtricitabine) and postnatally (zidovudine). While elevated plasma 17OHP with neonatal screening motivated us to investigate the effect of the HIV drugs in question on CYP21A2 activity, elevated urinary excretion of progesterones may not be explained by CYP21A2 inhibition but rather by CYP17A1 deficiency. On the other hand, CYP17A1 deficiency decreases androgen production. Thus the abnormal biochemical findings in our case with spontaneous resolution may or may not have been due to an adverse drug effect (of efavirenz). However, our *in vitro* and *in silico* studies show that such an effect is clearly seen for efavirenz. Its impact should be further explored in larger clinical studies. It is therefore important to bring the possible inhibitory effect of HIV drugs on adrenal steroidogenesis to the attention of the HIV community, as it may have clinical implications beyond the neonatal age.

In conclusion, HIV drug efavirenz inhibits CYP21A2 activity in human adrenal NCI-H295R cells at therapeutic concentrations apparently by a competitive mechanism. This effect has been suspected by laboratory abnormalities found in few newborns treated with HIV drugs. Whether this effect is of clinical relevance to the adrenal function in health and disease remains to be seen in larger clinical studies including children and adults under treatment with HIV drugs. So far we suggest to list some HIV drugs (such as efavirenz) as medications that may cause a positive neonatal screening test for elevated 17OHP.

5. Statements

5.1. Acknowledgment

We thank the patient and her family for providing the data for the case report.

5.2. Ethics statement

Parents have given written informed consent for the Case Report. The institutional Ethics committee approved the study.

5.3. Disclosure statement

The authors have no conflicts of interest to declare.

5.4. Funding sources

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5.5. Author contribution

Project idea: JM, TH, JMD, CEF. Clinical studies: TH, JMD, CEF. Experimental studies: JM, RF, SS, MG, SBA, RB, AVP, CEF. Bioinformatic studies: AVP. Data analysis: JM, AVP, CEF. Manuscript draft: JM, TH, CEF. Manuscript approval: all authors.

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