Misdiagnosis of CTX due to propofol: The interference of total intravenous propofol anaesthesia with bile acid profiling

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Abstract

Background: Cerebrotendinous xanthomatosis (CTX) is a rare genetic disorder, characterised by chronic diarrhoea, xanthomas, cataracts, and neurological deterioration. CTX is caused by CYP27A1 deficiency, which leads to abnormal cholesterol and bile acid metabolism. Urinary bile acid profiling (increased m/z 627: glucuronide-5β-cholestane-pentol) serves as diagnostic screening for CTX. However, this led to a false positive CTX diagnosis in two patients, who had received total intravenous anaesthesia (TIVA) with propofol.

Methods: To determine the influence of propofol on bile acid profiling, 10 urinary samples and 2 blood samples were collected after TIVA with propofol Fresenius 7 to 10 mg/kg/h from 12 subjects undergoing scoliosis correction. Urinary bile acids were analysed using flow injection negative electrospray mass spectrometry. Propofol binding to recombinant CYP27A1, the effects of propofol on recombinant CYP27A1 activity, and CYP27A1 expression in liver organoids were investigated using spectral binding, enzyme activity assays, and qPCR, respectively. Accurate masses were determined with high-resolution mass spectrometry.

Results: Abnormal urinary profiles were identified in all subjects after TIVA, with a trend correlating propofol dose per kilogramme and m/z 627 peak intensity. Propofol only induced a weak CYP27A1 response in the spectral binding assay, minimally affected CYP27A1 activity and did not affect CYP27A1 expression. The accurate mass of m/z 627 induced by propofol differed >10 PPM from m/z 627 observed in CTX.
Conclusions: TIVA with propofol invariably led to a urinary profile misleadingly suggestive of CTX, but not through CYP27A1 inhibition. To avoid further misdiagnoses, propofol administration should be considered when interpreting urinary bile acid profiles.

KEYWORDS
2,6-diisopropylphenol, bile acid profiling, cerebrotendinous xanthomatosis, CTX, CYP27A1, cytochrome P450 27A1, propofol

1 | INTRODUCTION

Cerebrotendinous xanthomatosis (CTX, OMIM #213700) is a rare autosomal recessive disorder, characterised by chronic diarrhoea, xanthomas, cataracts, and neurological deterioration.1,2 The estimated prevalence is <5/100 000 worldwide.1 CTX is caused by mutations in the CYP27A1 gene, which encodes the enzyme cytochrome P450 27A1 (CYP27A1, EC 1.14.15.15).3,4 CYP27A1 catalyses the hydroxylation of cholesterol and vitamin D3 and the conversion of bile acid intermediates.5,6 In CTX patients, CYP27A1 is deficient,
impairing cholesterol conversion into the bile acid chenodeoxycholic acid (CDCA) and cholic acid. This leads to increased concentrations of some cholesterol intermediates and cholestanol (Figure 1). Bile alcohol glucuronides are excreted in bile, faeces, and urine. Urinary electrospary ionisation mass spectrometry (ESI-MS) analysis of the bile alcohol glucuronides (m/z 611, glucuronide-5β-cholestanetetrol; m/z 627, glucuronide-5β-cholestanepentol; m/z 643, glucuronide-5β-cholestanhexol) serves as an easy diagnostic tool for CTX, although profiles may differ among patients. 7-11

The diagnosis of CTX is further supported by elevated cholestanol concentrations in blood and confirmed by DNA sequencing of the CYP27A1 gene. Early treatment with synthetic CDCA can prevent onset or reduce progression of symptoms in a subset of CTX patients.12,13 However, the onset and severity of CTX is highly variable, and this may lead to delayed or missed diagnoses.14,15 Recently, we identified a urinary profile suggestive of CTX (m/z 627 and 643) in a boy undergoing surgery for an epidural hematoma (index patient 1). Despite the absence of clear CTX symptoms, therapy with CDCA was initiated to prevent onset of symptoms. However, the profile was normal in a second premedication sample and no pathogenic CYP27A1 mutations were found. Similarly, a urinary profile suggestive of CTX was identified in a boy undergoing endoscopy for failure to thrive and diarrhoea (index patient 2), but plasma concentrations of cholestanol were normal. Aware of index patient 1 with an incidental abnormal urinary profile, prior urine and blood samples of index patient 2 were examined for bile acid metabolites and cholestanol, respectively. These samples all showed no abnormalities.

Both patients had received total intravenous anaesthesia (TIVA) with propofol from Fresenius Medical Care for at least 2 hours during surgery for idiopathic scoliosis. For concentration-effect estimation, urine was sampled repetitively in the 2 to 6 hours of TIVA in two subjects. Blood samples of two subjects were collected directly before and directly after TIVA with propofol. All samples were stored at −20°C until further analysis. Exclusion criteria were known disorders in cholesterol or bile acid metabolism. To evaluate the effect of direct excretion of propofol or other constituents of the propofol solution on bile acid profiling, urine from anonymous healthy controls was spiked with 0.025 mg propofol per millilitre urine.

2 | MATERIALS AND METHODS

2.1 | Urine and blood collection

Urinary samples of 10 subjects were prospectively collected directly after TIVA with 7 to 10 mg/kg/h propofol (Fresenius Medical Care) for at least 2 hours during surgery for idiopathic scoliosis. For concentration-effect estimation, urine was sampled repetitively in the 2 to 6 hours of TIVA in two subjects. Blood samples of two subjects were collected directly before and directly after TIVA with propofol. All samples were stored at −20°C until further analysis. Exclusion criteria were known disorders in cholesterol or bile acid metabolism. To evaluate the effect of direct excretion of propofol or other constituents of the propofol solution on bile acid profiling, urine from anonymous healthy controls was spiked with 0.025 mg propofol per millilitre urine.

2.2 | Urinary bile acid analysis

The diluted urine samples (30 μL urine and 470 μL MilliQ) were subjected to solid-phase extraction (SPE) using Oasis Prime HLB (Waters). After loading of the samples, the column was washed with 500 μL methanol (5%). Bile acids were recovered from the cartridge by elution with 500 μL acetonitrile/methanol (90%). A volume of 2 μL was injected and analysed by flow injection analysis (flow rate = 0.15 mL/min) using 90% acetonitrile. ESI-MS measurements in negative mode were carried out using an Acquity UPLC system, Xevo TQ-S micro MS (Waters Co). In each run, a urine sample from a CTX patient (before treatment) was measured as a positive control. Subsequently, semiquantitative analysis of m/z 627 (corresponding to the major bile alcohol glucuronide excreted by CTX patients or isomers thereof) was performed in a single run using a calibration curve with taurocholic acid (Sigma-Aldrich Co.) and taurocholic acid-D4 (Cambridge Isotope Laboratories) as internal standard.

2.3 | Plasma bile acid analysis

Bile acids were detected in plasma as described by Boot- 
sma et al.21
2.4 | Plasma cholestanol analysis

Cholestanol was measured in plasma as described before. 

2.5 | Spectral binding assay

Human recombinant CYP27A1 was expressed and purified as described by Mast et al. Propofol (Toronto Research Chemicals, Inc) and cholesterol (Steraloids, Inc) binding to CYP27A1 was analysed in vitro according to the method described by Lam et al, using propofol concentrations of 20, 40, and 100 μM and cholesterol concentrations up to 3.0 μM.

2.6 | CYP27A1 enzyme activity assay

The effect of propofol (Toronto Research Chemicals) on cholesterol hydroxylase was tested as described by Mast et al using 46 μM propofol and 2.3 μM cholesterol, the concentrations used to identify CYP27A1 inhibitors. To evaluate the effect of propofol on 5α-cholestane-3α,7α,12α-triol conversion to 5α-cholestane-3α,7α,12α,27-tetrol, the same experimental set-up was used, using 0.01 μM human recombinant CYP27A1, 3.0 μM adrenodoxin, 0.05 μM adrenodoxin reductase (EC 1.18.1.6), and 2.3 μM 5β-cholestane-3α,7α,12α-triol (Steraloids). Human recombinant CYP27A1, bovine adrenodoxin, and bovine adrenodoxin reductase were expressed and purified as described. 

2.7 | Propofol exposure to human liver organoid cultures

Human Lgr5+ cells were isolated from biopsies from livers of two healthy donors as previously described. Use for our studies was approved by the Medical Ethical Committee of our hospital (STEM study). Liver stem cell organoids were passaged to fresh matrigel and cultured in expansion medium (EM) for 3 days, after which differentiation towards hepatocyte-like cell was started by replacement of EM for differentiation medium (DM). DM was refreshed every 2 to 3 days and after 5 days, propofol (Fresenius) was added in a concentration of 0, 100, 300, or 900 μM. After 24 hours of incubation with propofol, organoids were harvested for messenger RNA (mRNA) analysis.

2.8 | RNA isolation and qPCR

RNA was isolated from liver organoid cells using Trizol LS reagent (Invitrogen), and stored at −80°C. Total RNA yield and purity were assessed using a Qubit RNA BR assay kit. cDNA was synthesised from 1 μg of RNA by performing reverse-transcription with the iScript kit (Bio-Rad Laboratories, Inc). mRNA abundance was determined by real-time PCR using validated primer pairs with the SYBR Green master mix (Bio-Rad; Appendix S1, S2, and Figure S1). Quantification cycle (Cq) values for CYP27A1 (Fw: GTGCTGCCTTTCTGGAAGCGAT, Rv: TAGCCAGACACCTGGATGCCAT) were normalised against reference gene HP1BP3 (Fw: CCCACGTCC CAAGATGGGAT, Rv: CTGATGCACACTCTCTGAGGAA) and fold change was calculated using the 2^(-ΔΔCq) method.

2.9 | High resolution measurements

SPE procedure was performed as described for bile acid analysis. A volume of 2 μL was injected in a direct flow of 0.15 mL/min (90% vol/vol acetonitrile) with a total runtime of 1 minute. Analyses were performed on an Ultimate 3000 UHPLC coupled to a Q-exactive HRMS (both Thermo Scientific, Bremen, Germany). Optimal scan parameters using negative ESI at a resolution of 240,000 were established by infusing a mixture of cholic acid, taurodeoxycholic acid, and glycocholic acid. Scan parameters were an AGC target value of Se^6 and maximum injection time of 200 (ms). Spray voltage was 3 kV, Capillary temperature was 325°C and a factor of 40 was used for the S-lens RF level. Full MS scans (negative mode) were performed in the mass range of 350 to 700 m/z. To achieve best mass accuracy, a customised mass calibration was performed using a mixture of glycine (2 mg mL^-1) and ammonium formate (1 mg mL^-1) in methanol/MilliQ water (70/30 vol/vol) and 0.1% formic acid. For this purpose, m/z of 74.02475 (C2H3NO2(-H)), 171.03872 (C4H6N2O2Na(-H)), 268.05270 (C6H12N2O2Na2(-H)), 365.06667 (C8H17N4O2Na3(-H)), 462.08064 (C10H21N5O10Na4(-H)), 559.09462 (C12H25N6O12Na5(-H)), and 753.12256 (C16H33N8O16Na7(-H)) were analysed in negative mode.

3 | RESULTS

3.1 | Subject and propofol exposure characteristics

Characteristics of included subjects (n = 12) and propofol exposure are presented in Table 1. Mean exposure to propofol was 9 mg/kg/h for a mean duration of 5 hours.
3.2 Urinary bile acid analysis

In our two index patients, a urinary profile similar to that of a CTX patient (Figure 2A) was not seen under normal circumstances (Figure 2B), but was seen after administration of propofol (Figure 2C). Abnormal profiles were identified in all urinary samples collected after TIVA with propofol (Figure 2D), similar to the urinary profile of a CTX patient (Figure 2A). No evidence was found for direct excretion of propofol or other lipophilic constituents causing the abnormal m/z peaks as spiking of urine with propofol did not lead to an abnormal profile (data not shown). A trend could be observed correlating propofol dose per kilogramme and m/z 627 peak intensity (Figure 2D). Interestingly, these intensities were up to 60 times higher than those found in untreated CTX patients. Repetitive urinary samples taken from two subjects during TIVA with propofol showed a clear increase of the m/z 627 peak intensity with increasing duration of TIVA with propofol (Figure 2E).

TABLE 1 Characteristics of included subjects and propofol exposure

| Study subjects | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | Average (±SD) |
|----------------|---|---|---|---|---|---|---|---|---|----|----|----|--------------|
| Sex (male/female) | F | F | M | M | F | F | F | F | F | M | M | - |
| Age (years) | 14 | 14 | 16 | 9 | 10 | 9 | 16 | 18 | 6 | 13 | 15 | 4 | 12 (4) |
| Weight (kg) | 35 | 60 | 52 | 33 | 33 | 23 | 70 | 55 | 16 | 35 | 45 | 16 | 40 (16) |
| Total propofol (mg*10³) | 2.3 | 2.6 | 1.7 | 1.3 | 1.3 | 0.6 | 2.5 | 1.6 | 0.5 | 1.3 | 1.5 | 1.1 | 1.5 (0.7) |
| Propofol per body mass (mg/kg) | 66 | 43 | 32 | 40 | 39 | 24 | 35 | 29 | 30 | 39 | 33 | 68 | 42 (14) |
| Propofol per body mass per hour (mg/kg/h) | 8 | 9 | 10 | 9 | 9 | 8 | 8 | 7 | 9 | 9 | 8 | 9 | 9 (1) |
| Time from propofol start to last urine catch (min) | 475 | 300 | 225 | 265 | 270 | 190 | 365 | 250 | 210 | 230 | 255 | 435 | 297 (93) |

Note: Urinary samples were taken directly after TIVA with propofol. In subjects 7 and 8, characteristics are presented of the last of three and two sequential urinary samples, respectively.

FIGURE 2 Propofol causes a CTX-like urinary profile. Urinary profiles of a Cerebrotendinous xanthomatosis (CTX) patient (A), our second index patient under normal circumstances (B) and after receiving total intravenous anaesthesia with propofol (C). All study subjects showed urinary profiles suggestive of CTX, with relative intensity of m/z 627 comparable to or higher than an untreated CTX patient (D) and increasing after propofol administration (E).
3.3 | Plasma cholestanol and bile acid analysis

Cholestanol concentrations in plasma samples of two subjects were 3.9 and 2.9 μmol/L before and 2.9 and 1.0 μmol/L after TIVA with propofol, respectively. Similarly, bile acid profiles in these plasma samples remained normal after TIVA with propofol (data not shown).

3.4 | CYP27A1 spectral binding

We observed only a weak spectral response after addition of propofol to human recombinant CYP27A1 (Figure 3A), 20 times lower than the large spectrum response seen after addition of a known substrate (cholesterol, Figure 3B) and 10 times lower than the spectrum response seen after addition of a strong inhibitor (anastrozole, Figure 3C). This indicates that propofol binding to the haem group of CYP27A1 was absent or weak.

3.5 | CYP27A1 enzyme activity

Inhibition of CYP27A1 activity by propofol was also very limited. Propofol induced a nonstatistically significant reduction of cholesterol hydroxylation to 92% (±3) and of 5β-cholestan-3α,7α,12α-triol conversion to 91% (±1), whereas addition of anastrozole almost completely inhibited cholesterol hydroxylation activity under the

![Figure 3](https://example.com/figure3.png)

**Figure 3** Propofol is not a strong direct inhibitor of CYP27A1. Propofol induced only a weak spectral response in CYP27A1 (A) when compared with the spectral response induced by binding to CYP27A1 by a known substrate (cholesterol, B) and a strong inhibitor (anastrozole, C). Propofol had barely any effect on cholesterol hydroxylation (D) and triol conversion (E) activity, compared to the strong inhibition by anastrozole (F). Propofol did not influence CYP27A1 expression in human liver organoids (G).
experimental conditions used, even in lower concentrations (Figure 3D,E,F). When trying to determine the IC\textsubscript{50} concentration of propofol for cholesterol hydroxylation and 5β-cholestan-3α,7α,12α-triol conversion, inhibition remained <15% (Figure 3D,E) despite propofol concentrations up to 1 mM, possibly due to limited drug solubility at these concentrations. Collectively, the spectral binding and enzyme activity results suggest that under our experimental conditions, CYP27A1 inhibition by propofol is virtually absent.

3.6 | CYP27A1 expression

Exposure to propofol for 48 hours did not affect CYP27A1 expression in liver organoids derived from two individual donors (Figure 3G).

3.7 | High resolution measurements

The full MS scan of a urine sample extract of a patient with CTX showed a m/z 627.37532 and m/z 643.37012 for glucuronide-5β-cholestane-pentol and glucuronide-5β-cholestane-hexol, respectively. The differences between observed and theoretical mass for these species are 1.3 and 0.34 ppm, respectively. These accurate masses differed from m/z 627.24816 and m/z 643.24307 observed in a urine sample extract of a patient exposed to propofol. Mass differences observed between the two samples were approximately 200 ppm for both compounds. For identification with high resolution MS in metabolomics studies a mass error of 5 to 10 ppm is used.

4 | DISCUSSION

We have shown that TIVA with propofol invariably caused abnormal urinary profiles suggestive of CTX. Because CTX symptoms are variable and may occur later in life, this might lead to misdiagnoses and unnecessary treatment of patients.

Because the urinary profiles induced by propofol and genetic CYP27A1 deficiency showed comparable m/z peaks when measured with ESI/MS, we hypothesised that propofol would inhibit CYP27A1 activity. The mechanism of numerous pharmacological examples of CYP inhibition generally involves direct binding of the drug to the haem group of the CYP enzyme, resulting in two types of characteristic spectral changes in the Soret region of the visible spectrum.\textsuperscript{20,26} However, we did not see evidence of binding to the haem group as propofol only induced a minimal spectral response in CYP27A1. Concurrently, propofol only weakly inhibited human recombinant CYP27A1 activity in vitro at high concentrations and we did not find evidence that propofol decreased CYP27A1 expression in human liver organoid cells. In addition, we did not find abnormalities in cholestanol concentrations and bile acid profiles in plasma of the subjects receiving TIVA with propofol. We therefore reconsidered our hypothesis and performed high resolution mass spectrometry. These accurate mass measurements revealed that the m/z 627 and 643 peaks in the urinary profiles of our study subjects were not glucuronide-5β-cholestane-pentol and glucuronide-5β-cholestane-hexol, the substances that accumulate in CTX patients.

The m/z peaks induced by propofol must be the result of an in vivo mechanism, since direct excretion of propofol or other lipophilic constituents of propofol Fresenius did not cause the abnormal urinary m/z peaks, as evidenced by analysis of control urine spiked with propofol Fresenius. For intravenous use, propofol is dissolved in the lipid emulsion intralipid, consisting of refined soya-bean oil, purified egg phosphatides, glycerol, oleic acid, sodium hydroxide, and water. Hypothetically, one of these additional substances could create the abnormal urinary profile. However, Intralipid is commonly used in parenteral feeding and although widely studied, there is no description in literature of (or personal experience with) Intralipid-induced abnormal urinary profiles similar to those seen in our subjects. These findings suggest that the observed m/z peaks in our study subjects were induced by propofol through a yet undefined in vivo mechanism.

In conclusion, we have demonstrated that TIVA with propofol leads to urinary profiles suggestive of CTX when measured with ESI/MS. The clinical impact of our findings is clearly illustrated by our cases. Propofol is one of the safest and most commonly used anaesthetic agents and urethral catheterization during anaesthesia offers an opportunity for urinary sampling for metabolic screening. Misdiagnosis with CTX has significant impact, because CTX is a severe progressive disorder that requires lifelong expensive treatment. Due to the lack of precision of standard bile acid profiling, interpretation must be performed with caution. Therefore, upon observing an abnormal urinary profile suggestive of CTX after propofol administration, it is important to repeat urinary bile acid profiling without propofol interference, conduct additional measurements in the same urine by high resolution mass spectrometry, determine cholestanol concentration or bile acid profiles in plasma, and/or perform genetic testing.
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CONFLICT OF INTEREST
All authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS
J.C. and S.F. responsible for the planning of the study, inclusion of subjects, and reporting of the work described in the article. E.K. helped with the inclusion and sample collection of subjects and was responsible for the interpretation of anaesthesia details. J.J., M.d.S.-v.V., and M.v.H. involved in metabolic analyses and interpretation of the mass spectra data. I.S. tested CYP27A1 expression in response to propofol in human liver organoids. N.M. and I.P. performed the spectral binding and CYP27A1 enzyme activity assays.

1 SENTENCE TAKE-HOME MESSAGE
Total intravenous anaesthesia with propofol leads to a urinary profile misleadingly suggestive of CTX, and necessitates additional investigation.

COMPLIANCE WITH ETHICS GUIDELINES
All procedures followed were in accordance with the ethical standards of the Medical Ethical Committee of the University Medical Center Utrecht and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

This article does not contain any studies with human or animal subjects performed by the any of the authors.

The corresponding author for this article, who serves as guarantor for the article, accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish, is Sabine Fuchs.

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REFERENCES
1. Nie S, Chen G, Cao X, Zhang Y. Cerebrotendinous xanthomatosis: a comprehensive review of pathogenesis, clinical manifestations, diagnosis, and management. Orphanet J Rare Dis. 2014;9:179-190.
2. Sekijima Y, Koyama S, Inaba Y, Koinuma M, Yoshinaga T. Nationwide survey on cerebrotendinous xanthomatosis in Japan. J Hum Genet. 2018;63(3):271-280.
3. Jiao H, Olin M, Hansson M, et al. Unique case of cerebrotendinous xanthomatosis revisited: All the mutations responsible for this disease are present in the CYP27A1 gene. J Intern Med. 2018;283(6):604-606.
4. Orphanet. Cerebrotendinous xanthomatosis. https://www.orpha.net/consor/cgi-bin/OC_Exp.php?Lng=EN&Expert=909. Accessed July 1, 2018.
5. Gupta R, Patrick K, Bell N. Mutational analysis of CYP27A1: assessment of 27-hydroxylation of cholesterol and 25-hydroxylation of vitamin D. Metabolism. 2007;56(9):1248-1255.
6. TM Šarenac TM, Mikov M. Bile acid synthesis: from nature to the chemical modification and synthesis and their applications as drugs and nutrients. Front Pharmacol. 2018;9:939.
7. de Sain-van der Velden MG, Verrits A, Prinsen BH, de Barse M, Berger R, Visser G. Elevated cholesterol precursors other than cholesterol can also be a hallmark for CTX. J Inherit Metab Dis. 2008;31(2):387-393.
8. Pierre G, Setchell K, Blyth J, Preece MA, Chakrapani A, McKiernan P. Prospective treatment of cerebrotendinous xanthomatosis with cholic acid therapy. J Inherit Metab Dis. 2008;31(2):241-245.
9. Pitt JJ. High-throughput urine screening for Smith–Lemli–Opitz syndrome and cerebrotendinous xanthomatosis using negative electrospray tandem mass spectrometry. Clin Chim Acta. 2007;380(1–2):81-88.
10. Haas D, Gan-Schreier H, Langhans CD, et al. Differential diagnosis in patients with suspected bile acid synthesis defects. World J Gastroenterol. 2012;18(10):1067-1076.
11. Shimazu K, Kuwabara M, Yoshii M, et al. Bile alcohol profiles in bile, urine, and feces of a patient with Cerebrotendinous Xanthomatosis. J Biochem. 1986;99(2):477-483.
12. Amador MDM, Masingue M, Debs R, et al. Treatment with chenodeoxycholic acid in cerebrotendinous xanthomatosis: clinical, neurophysiological, and quantitative brain structural outcomes. J Inherit Metab Dis. 2018;41(5):799-807.
13. Duell PB, Salen G, Eichler FS, et al. Diagnosis, treatment, and clinical outcomes in 43 cases with cerebrotendinous xanthomatosis. J Clin Lipidol. 2018;12(5):1169-1178.
14. Verrits A, van Engelen BG, Wevers RA, et al. Presence of diarrhea and absence of tendon xanthomas in patients with cerebrotendinous xanthomatosis. Arch Neurol. 2000;57:520-524.
15. Appadurai V, DeBarber A, Chiang PW, et al. Apparent under-diagnosis of cerebrotendinous xanthomatosis revealed by analysis of ~60,000 human exomes. Mol Genet Metab. 2015;116(4):298-304.
16. Yang L, Yu WF, Cao YF, Gong B, Chang Q, Yang GS. Potential inhibition of cytochrome P450 3A4 by propofol in human primary hepatocytes. World J Gastroenterol. 2003;9(9):1959-1962.
17. Lejus C, Fautrel A, Mallédant Y, Guillouzo A. Inhibition of cytochrome P450 2E1 by propofol in human and porcine liver microsomes. Biochem Pharmacol. 2002;64(7):1151-1156.
18. Gemayel J, Géloën A, Mion F. Propofol-induced cytochrome P450 inhibition: an in vitro and in vivo study in rats. *Life Sci.* 2001;68(26):2957-2965.

19. Lam M, Mast N, Pikuleva IA. Drugs and scaffold that inhibit cytochrome P450 27A1 in vitro and in vivo. *Mol Pharmacol.* 2018;93(2):101-108.

20. Mast N, Lin JB, Pikuleva IA. Marketed drugs can inhibit cytochrome P450 27A1, a potential new target for breast cancer adjuvant therapy. *Mol Pharmacol.* 2015;88(3):428-436.

21. Bootsma AH, Overmars H, Van Rooij A, et al. Rapid analysis of conjugated bile acids in plasma using electrospray tandem mass spectrometry: application for selective screening of peroxisomal disorders. *J Inherit Metab Dis.* 1999;22(3):307-310.

22. Mast N, Murtazina D, Liu H, et al. Distinct binding of cholesterol and 5beta-cholestane-3alpha,7alpha,12alpha-triol to cytochrome P450 27A1: evidence from modeling and site-directed mutagenesis studies. *J Biochem.* 2006;45(14):4396-4404.

23. Sagara Y, Hara T, Ariyasu Y, Ando F, Tokunaga N, Horiuchi T. Direct expression in *Escherichia coli* and characterization of bovine adrenodoxins with modified amino-terminal regions. *FEBS Lett.* 1992;300:208-212.

24. Sagara Y, Wada A, Takata Y, Waterman MR, Sekimizu K, Horiuchi T. Direct expression of adrenodoxin reductase in *Escherichia coli* and the functional characterization. *Biol Pharm Bull.* 1993;16:627-630.

25. Huch M, Gehart H, van Boxtel R, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. *Cell.* 2015;160(1–2):299-312.

26. Isin E, Guengerich F. Substrate binding to cytochromes P450. *Anal Bioanal Chem.* 2008;392(6):1019-1030.

**SUPPORTING INFORMATION**

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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