Abstract:

Background:

4β-hydroxycholesterol (4β-OHC) is a metabolite resulting from CYP3A4 (and CYP3A5 to a lesser extent) metabolism of cholesterol. The ratio of 4β-OHC/cholesterol has been proposed as an endogenous biomarker of CYP3A4 and CYP3A5 activity. A single small volume blood sample can be used to measure 4β-OHC. Potential applications as a biomarker of CYP3A enzyme activity include: as a prediction of the magnitude of inhibition or induction - thus replacing the use of probe drugs for CYP3A activity; and evaluating multi-directional drug-drug interactions. Direct measurement of the metabolite could provide appropriate and accurate means of assessing CYP3A4 activity.

Methods:

The extraction process involved a liquid-liquid extraction, followed by chemical derivatization using picolinic acid. High-performance liquid chromatography (HPLC) coupled to mass spectrometry was used for chromatographic separation and MS/MS detection. The method provides sufficient accuracy and precision over a calibration range of 2-500 ng/mL based on analyte/ISTD peak area ratios with a quadratic calibration curve (weighted by $1/x^2$), and was validated according to FDA and EMA guidelines. The methodology was applied to 2 studies (Darifi and Virtual) involving HIV infected patients with viral suppression and established on protease inhibitor-based regimens to evaluate adjusted dosing approaches of darunavir/ritonavir and atazanavir/ritonavir, respectively, when administered with rifampicin. A multi-level mixed-effects approach was used to create model to investigating 4β-OHC as an endogenous biomarker to describe treatment effects on CYP3A induction and inhibition.

Results:

Within the Darifi study, a baseline ratio (4β-OHC/cholesterol) of 3.25×10^{-5} on standard darunavir /ritonavir doses of 800/100 mg daily, increased by 56(95% CI: 40, 75) % after 7 days of rifampicin. With adjusted darunavir/ritonavir of 1600/200 mg daily or 800/100 mg twice daily the ratio returned towards baseline values (32 [CI: 19, 61]% and 18 [CI: 0.4, 44]%, respectively). A limitation of the ratio, however, is that only 4 individuals completed the study which was terminated prematurely due to hepatoxicity. Within the Virtual study a baseline ratio of 1.36×10^{-5} CYP3A4 activity, increased on high dose rifampicin (600 mg daily) by 76.4(95% CI: 56.3, 99)% after 14 days, and by (+46.8%, CI:30.1,65.6)% and (+64%, CI:45.4,85.1)% with atazanavir/ritonavir 300/100 twice daily and high dose rifampicin of 1200mg. Interestingly in both studies, the ratio 4β-OHC/cholesterol decreased by 0.2% per year of age (p=0.052 and p=0.011 respectively).

Conclusion:

While we had limited sample sizes, our exploratory data suggest that the ratio of 4β-OHC-to-cholesterol may contribute to prediction and understanding of drug-drug interactions. Further studies are needed to understand the differences between populations and other factors affecting the 4β-OHC/cholesterol ratio.

This ratio is potentially useful in a non-study setting as baseline 4β-OHC/CHO ratio could influence drug-drug interactions. Finally, the ratio of 4β-OHC/CHO better correlated with CYP3A activity than when compared to 4β-OHC alone, as the CYP3A pathway is a minor pathway of Cholesterol metabolism.