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Supplemental Data

Drug Metabolism and Disposition

CYP and non-CYP drug metabolizing enzyme families exhibit differential sensitivities towards pro-inflammatory cytokine modulation

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Supplemental materials and methods: LC-MS/MS method to quantify CYP activity

Quantification of acetaminophen, 4'hydroxymephentoin, 1'hydroxymidazolam, and 4'hydroxydiclofenac in cell supernatant was done using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system consisting of a Nexera LC-40 high-performance liquid chromatography (HPLC) system equipped with a DGU-403 degassing unit, two LC-40D pumps, a SIL-40C autosampler, and a CTO-40S column oven (Shimadzu, 's-Hertogenbosch, the Netherlands). A Kinetex C18 column (1.7 μM, 50x2.1 mm) (Phenomenex, Utrecht, The Netherlands) with a SecurityGuard Ultra C18, 2.7 μm, 5 × 2.1 mm cartridge (Phenomenex, Utrecht, The Netherlands) as guard column were used to separate probe metabolites from other analytes present in the sample matrix. Mobile phases consisted of water (A) and methanol (B) both containing 0.1% formic acid. The gradient, with a flow rate of 0.4 ml/min, started at 5% B and increased to 100% B in 4 min, maintaining 100% B for 2 min, and then returned to initial conditions for another 2 min. The column was kept at 50 ˚C and the injection volume was 10 μL or 20 μL depending on the analyte. The HPLC was coupled to a Sciex QTRAP 6500+ mass spectrometer (AB Sciex Netherlands B.V., Nieuwerkerk aan den Ijssel, The Netherlands) operating in positive electrospray mode (ESI+).

The MS conditions were as follows: curtain gas 20 psi, collision gas "medium", ion source gas 1 40 psi, ion source gas 2 40 psi, ion spray voltage 5500 V and temperature 550 ˚C. The MS was operated in the multiple reaction monitoring (MRM) mode and was optimized by direct infusion of the standards individually. The optimized MRM transitions, retention time, declustering potential (DP), collision energy (CE) and cell exit potential (CXP) used are summarized in Supplemental Table 2. Analyst software version 1.4 (AB Sciex Netherlands B.V., Nieuwerkerk aan den Ijssel, The Netherlands) was used for data analysis.

Supplemental materials and methods: LC-MS/MS method to quantify FMO3 and UGT2B7 activity

Quantification of benzydamine N-oxide and morphine-3-glucuronide in cell supernatant was done using a liquid chromatography-tandem mass spectrometry (LC-MS/MS) system consisting of a Nexera-X2 ultra high-performance liquid chromatography (UHPLC) system equipped with a DGU-20A degassing unit, three LC-30 pumps, a SIL-30ACMP autosampler, and a CTO-30A column oven (Shimadzu, 's-Hertogenbosch, the Netherlands).

For benzydamine N-oxide, separation was achieved with an Acquity BEH column (1.7 μm, 2.1x50 mm) from Waters (Etten-Leur, The Netherlands). Elution of benzydamine-N-oxide was performed using a high pressure gradient, with a flow of 0.4 ml/min, from 5% to 95% acetonitrile with 0.1 % formic acid. The column was kept at 40 ˚C and the injection volume was 10 μL.

For morphine-3-glucuronide, separation as achieved with a Vision HT Basic column (3μm, 150×3 mm) (Grace, Breda, the Netherlands). An online solid-phase extraction (SPE) method was used to clean the samples, using a Hysphere GP cartridge (Spark Holland, Emmen, the Netherlands). Samples were injected into the SPE column and washed with 1 ml 10 mM ammonium acetate buffer at pH 10 for 1 minute to remove salts and other interferences, after which they were injected into the LC-column. Elution into the LC system was performed with a gradient of 3% to 97% acetonitrile with 0.1 % formic acid in 4 minutes, at a flow of 300μL/min and re-equilibrated at 3% acetonitrile. The column was kept at 40 ˚C and the injection volume was 5 μL.

The UHPLC was coupled to a TSQ Vantage mass spectrometer (Thermo Fisher, Breda, The Netherlands) operating in positive electrospray mode (ESI+). The MS conditions were as follows: curtain gas 20 psi, collision gas 0.5 atm ion source gas 5 psi, ion spray voltage 3000 V and temperature 350 ˚C. The MS was operated in MRM mode and was optimized by direct infusion of the standards individually. The optimized MRM transitions, retention time, declustering potential (DP) and collision energy (CE) used for both analytes are summarized in Supplemental Table 3. Thermo XCalibur Software LCQuan 2.7 was used for data analysis.