Effects of Monocyte Chemoattractant Protein-1, Macrophage Inflammatory Protein-1α and Interferon-α2a on P450 Enzymes in Human Hepatocytes in Vitro

Maciej Czerwiński, Krystal Gilligan, Kevin Westland, and Brian W. Ogilvie
XenoTech

aaps 360
ADVANCING PHARMACEUTICAL SCIENCES, CAREERS, AND COMMUNITY

PURPOSE

Some immunomodulatory drugs stimulate the innate immune system to release cytokines that may change expression of drug-metabolizing enzymes. In drug development, regulation of multiple pro- and anti-inflammatory cytokines by Toll-like receptors (TLR) has gained attention in parallel to targeting the therapeutic potential of these receptors (Hennessy et al., 2010; Patel et al., 2014). In a previous study, tilsotolimod, an agonist of TLR9 designed to enhance T-cell responses to tumor antigens, stimulated release of cytokines in human blood (Tarantino et al., 2018). A lower dose of tilsotolimod (10 µg/mL) elevated levels of interferon gamma-induced protein-10 (IP-10) and macrophage chemoattractant protein-1 (MCP-1), while a higher dose of the drug (100 µg/mL) increased macrophage inflammatory protein-1α (MIP-1α), tumor necrosis factor-α (TNF-α), and interferon-α2a (INF-α2a) (Table 1). This plurality of cytokines contained in the tilsotolimod-stimulated plasma increased CYP1A2 mRNA by up to 50% and reduced CYP1A2 enzyme activity to 47% of control, in human hepatocytes. The tilsotolimod-stimulated plasma decreased CYP2B6 mRNA by up to 50% and decreased CYP2B6 enzyme activity to 13% of control.

OBJECTIVE

The aim of this study was to establish whether MCP-1, MIP-1α or IFN-α2a were responsible for the effects of tilsotolimod-stimulated plasma on CYP1A2 and CYP2B6 mRNA and enzyme activity levels.

METHODS

Cell Culture and Treatments, mRNA and Enzyme Activity Analysis:

The cell culture procedures, treatments and analysis of mRNA and enzyme activities were conducted as described (Czerwinski et al., 2015). The modified Chee's medium was supplemented with 10% normal human plasma in order to replicate conditions of the in vitro evaluation of tilsotolimod. Hepatocytes were treated with MCP-1 (2, 10 or 50 ng/mL), MIP-1α (0.4, 2 or 10 ng/mL) and INF α2a (0.1, 0.5 or 2.5 ng/mL) daily for three days. These concentrations were 0.21- or 5-fold the levels in plasma stimulated with tilsotolimod (100 µg/mL)(Tarantino et al., 2018).

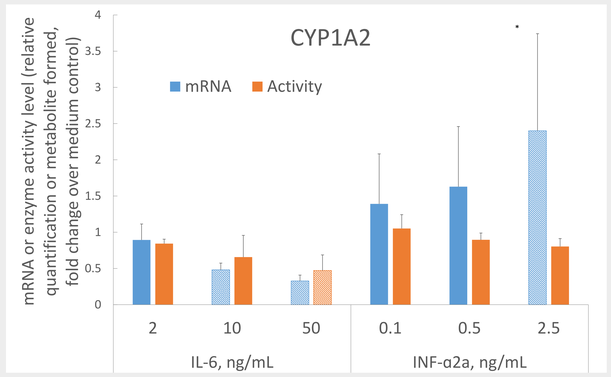
Table 1. Effects of incubation, saline, LPS and Tilsotolimod on the release of cytokines in human blood

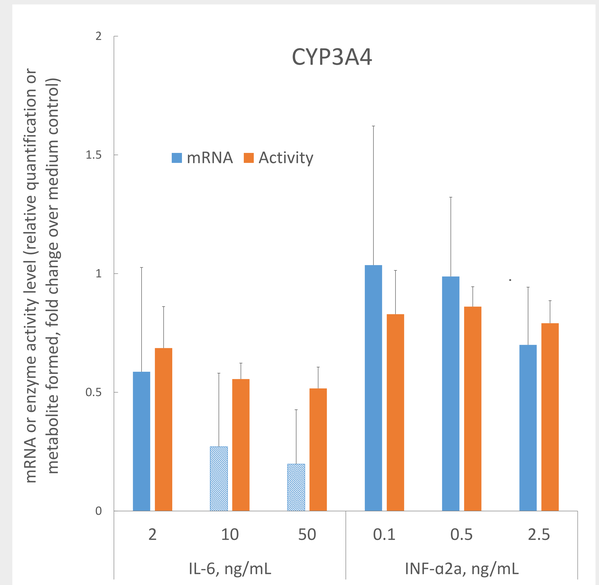
Cytokine	Treatment					
	Incubation 0 h, untreated	Incubation 24 h, untreated	Saline 1 %, v/v	LPS 10 ng/mL	Tilsotolimod 10 µg/mL	Tilsotolimod 100 µg/mL
IP-10	323 ± 211[a]	367 ± 268	353 ± 269	25400 ± 38500 (9[b])**	21600 ± 34900 (7[b])**	5650 ± 3850 (9[b])**
MCP-1	191 ± 30.6	5550 ± 1770*	6840 ± 2250	1590 ± 803**	11200 ± 3220**	11500 ± 3110**
MIP-1 α	17.5 ± 9.92 (7[c])	998 ± 1170*	732 ± 855	ALQ	991 ± 1013	2510 ± 1210**
IL-2	0.335 ± 0.167 (8[c])	0.386 ± 0.198 (9[c])	0.325 ± 0.205	1.15 ± 0.285**	0.370 ± 0.206 (9[c])	0.417 ± 0.197
IL-6	2.75 ± 3.33 (5[c])	147 ± 243	135 ± 229	8400 ± 2760**	163 ± 237	64.4 ± 33.2
IL-12p70	1.31 ± 0.704 (7[c])	3.15 ± 3.04 (8[c])	2.67 ± 2.66 (9[c])	74.0 ± 27.8**	2.72 ± 2.63	1.98 ± 0.921
TNF-α	1.98 ± 1.02	18.5 ± 17.0*	15.6 ± 17.4	3590 ± 1120**	19.7 ± 19.0	43.4 ± 25.9**
IFN-α2a	BLQ	0.325 (1[c])	BLQ	0.325 (1[c])	166 ± 145	487 ± 319

a Values are the mean ± standard deviation of duplicate determinations in blood from 10 donors in pg/mL
b (n) ALQ results not reported
c (n) BLQ results not reported

ALQ = Above the limit of quantification
BLQ = Below the limit of quantification
*Statistically different from 0 h incubation control (t test: two samples, equal variance, one-tail, P < 0.05)
**Statistically different from saline control (t test: two samples, equal variance, one-tail, P < 0.05)

RESULTS





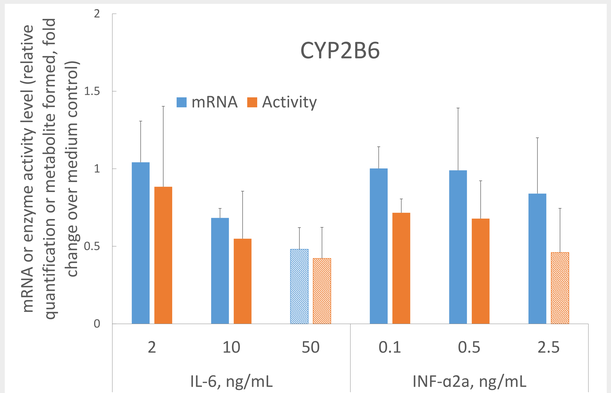


Figure 1: CYP Enzyme Activity

In three cultures of human hepatocytes, recombinant INF-α2a (2.5 ng/mL) increased CYP1A2 mRNA expression (blue bars) 2.4-fold over control ($P<0.05$) but did not change CYP2B6 or CYP3A4 mRNA more than our reporting criteria of a 2-fold increase or a 50% decrease. Neither MCP-1 nor MIP-1α had effect on the CYPs mRNAs that was greater than our reporting criteria (data not shown).

As expected, IL-6 (50 ng/mL) reduced CYP1A2, CYP2B6 and CYP3A4 mRNA by more than 50% of control. INF-α2a (2.5 ng/mL) reduced CYP2B6 enzyme activity to 46% of control but did not have an effect on CYP1A2 or CYP3A4 enzyme activities. IL-6 (50 ng/mL) reduced CYP1A2 and CYP2B6 enzyme activities to 47% and 42% of control, respectively. MCP-1 or MIP-1α did not have effect on CYP1A2, CYP2B6 or CYP3A4/5 enzyme activity.

Patterned bars indicate observations of mRNA or enzyme activity levels below 50% of the control or observations of enzyme activity above 2-fold of the control.

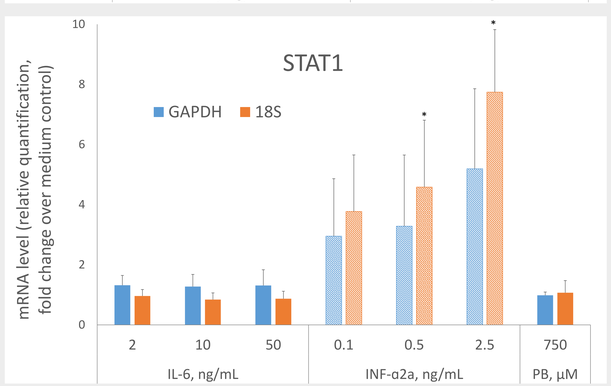


Figure 2: STAT1

In three cultures of human hepatocytes, recombinant INF-α2a (0.5 - 2.5 ng/mL) increased signal transducer and activator of transcription 1 (STAT1) mRNA expression up to eight fold. STAT1 mRNA induction, the INF-α target gene, demonstrated functionality of interferon receptor in the cultured cells in the study. IL-6 (2 – 50 ng/mL) did not change STAT1 mRNA expression.

Patterned bars indicate mRNA above 2-fold of the control.

CONCLUSIONS

INF-α2a, in a dose-dependent manner, increased CYP1A2 mRNA and reduced CYP2B6 enzyme activity in human hepatocytes in vitro.

We concluded that INF-α2a, but not chemokines MCP-1 or MIP-1α, was likely responsible for appreciable P450 changes observed in hepatocytes cultured with plasma from tilsotolimod-treated blood. It is possible that other cytokines that can be modulated by tilsotolimod, but were not evaluated in this study, also could modify CYP enzymes.

MCP-1 or MIP-1α did not affect CYP1A2, CYP2B6 nor CYP3A4 mRNA expression or enzyme activity.

The effects of MCP-1 or MIP-1α on P450 enzymes were not reported, but since these chemokines are expected to be elevated by some drugs targeting TLRs, they need to be considered from a drug safety perspective (Ozato et al., 2002). It is a limitation of this study that cytokine-stimulated differentiation of monocytes to macrophages could not be evaluated in vitro, as macrophages are a source of pro-inflammatory cytokines (Arango Duque and Descoteaux, 2014).

Cell culture conditions may change hepatocyte response to INF-α2a.

Chen and coauthors reported that INF-α2b (0.1 – 10 ng/mL) induced CYP3A4 mRNA and protein, although in our study INF-α2a did not have such effect (Chen et al., 2011). It is suspected that differences in handling of the cells contributed to the discrepant CYP3A4 results from the two studies. However, it is possible that the CYP3A4 mRNA response to INF-α2a was attenuated by the addition of normal plasma (10% v/v) to the cell culture medium in our study. The absence of appreciable effects of recombinant INF-α2a on CYP3A4 is consistent with the conclusion that interferon-α can be co-administered with drugs metabolized by CYP1A2 and CYP3A4 in patients with chronic hepatitis C without significant risks of drug interactions, although an evaluation of CYP2B6 was not provided (Pageaux et al., 1998).

REFERENCES

Arango Duque G and Descoteaux A (2014) *Front Immunol* **5:**491.
Chen C, Han YH, Yang Z, and Rodrigues AD (2011) *Xenobiotica* **41:**476-485.
Czerwinski M, Kazmi F, Parkinson A, and Buckley DB (2015) *Drug Metab Dispos* **43:**42-52.
Hennessy EJ, Parker AE, and O'Neill LA (2010) *Nat Rev Drug Discov* **9:**293-307.
Ozato K, Tsujimura H, and Tamura T (2002) *Biotechniques* **Suppl:**66-68, 70, 72 passim.
Pageaux GP, le Bricquir Y, Berthou F, Bressot N, Picot MC, Blanc F, Michel H, and Larrey D (1998) *Eur J Gastroenterol Hepatol* **10:**491-495.
Paris BL, Ogilvie BW, Scheinkoenig JA, Ndikum-Moffor F, Gibson R, and Parkinson A (2009) *Drug Metab Dispos* **37:**2045-2054.
Patel MC, Shirey KA, Pletneva LM, Boukhvalova MS, Garzino-Demo A, Vogel SN, and Blanco JC (2014) *Future Virol* **9:**811-829.
Tarantino P, Sullivan T, Ogilvie BW, and Czerwinski M (2018) *Hepatology* **68:**1444A.

XENOTECH
A BioIVT Company