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REVIEW

Assessment of the drug–drug interaction potential for therapeutic proteins with pro-inflammatory activities

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Abstract

It is well-recognized that therapeutic proteins (TPs) with pro-inflammatory activities elevate the pro-inflammatory cytokines and result in cytokine-drug interactions. In the current review, several pro-inflammatory cytokines, including IL-2, IL-6, IFN-γ, and TNF-α, as well as an anti-inflammatory cytokine IL-10, were summarized for their respective effect on major cytochrome P450 enzymes and efflux transporter PgP. Pro-inflammatory cytokines are generally associated with suppression of CYP enzymes across assay systems but have varied effect on Pgp expression levels and activities depending on the individual cytokines and assay systems, whereas IL-10 had no significant impact on CYP enzymes and P-gp. A cocktail drug-drug interaction (DDI) study design could be an ideal approach for simultaneously assess the impact of TPs with pro-inflammatory activities on multiple CYP enzymes. Clinical DDI studies using the cocktail approach have been conducted for several TPs with pro-inflammatory activities and for those TPs with pro-inflammatory activities which had no clinical DDI study conducted, languages for potential DDI risk due to cytokine-drug interaction were included in the label. Up to date drug cocktails, including clinically validated and unvalidated for DDI assessment, were summarized in this review. Most clinically validated cocktails focused either on CYP enzymes or transporters. Additional effort was needed to validate a cocktail to include both the major CYP enzymes and key transporters. In silico methods for assessment of the DDI for TPs with pro-inflammatory activities were also discussed.

INTRODUCTION

The importance of drug–drug interactions (DDI) in drug development and clinical practice is clear: DDIs may increase the risk of treatment failures and the incidence and severity of adverse events. The successful evaluation and prediction of DDIs during different phases of drug development and regulatory evaluation is critical to mitigate this risk.

Cytokines are a class of secreted small proteins (~5–25 kDa) that regulates the cell survival, growth, differentiation, and effector function.[1] Some cytokines, especially pro-inflammatory cytokines, are elevated during host immune responses to inflammation, infection, trauma, or cancer, and play an important role by controlling immune cell maturation, growth, and responsiveness. There is long-standing evidence for cytokine-drug interaction with early clinical evidence in the 1970s, which demonstrated

that acute inflammatory responses (associated with cytokine release) to infection or tissue injury could lead to altered drug pharmacokinetics.[2–4] Quinine exposure was increased during induced malaria and induced fever, and theophylline had a longer half-life and therefore higher exposure during acute respiratory viral illness.[3,4] With the advancement in the knowledge of cytokines and drug metabolism, direct link of cytokines and drug metabolizing enzymes were identified. For instance, interleukin 6 (IL-6) was found to be critical in reduction of cytochromes P450 (CYP) enzymes CYP1A2, CYP2A5, and CYP3A11 mRNA levels during turpentine-induced inflammation but not during LPS-mediated inflammation in mice.[5] In general, cytokines downregulate CYP enzyme levels, reduce CYP enzyme substrates metabolism, and increase drug exposures.

Therapeutic proteins (TPs) with pro-inflammatory activities are a class of TPs with the properties either as pro-inflammatory cytokines, such as peginterferon, or as pro-inflammatory cytokine modulators, such as blinatumomab. By definition, TPs with pro-inflammatory activities could result in persistent or transient elevation of cytokine levels in the body, therefore, administration of TPs with pro-inflammatory activities could lead to DDIs with co-administered drugs owing to the cytokine-drug interaction.[6,7] Recent US Food and Drug Administration (FDA) draft guidance on "Drug-drug interaction assessment for therapeutic proteins" laid out recommendations regarding assessing DDI risks for TPs.[8] For TPs as pro-inflammatory cytokines, a clinical DDI study is recommended, and, for TPs as pro-inflammatory cytokine modulators, a clinical DDI study may not be conducted but label language to include potential CYP/transporter mediated DDIs.

The objectives of the current review are to provide a summary of key pro-inflammatory cytokines (IL-2, IL-6, TNF-α, and IFN-γ) and their effect on major CYP enzymes and efflux transporter P-glycoprotein (P-gp); clinically observed DDIs for TPs with pro-inflammatory activities; possible drug cocktails for clinical DDI study to evaluate such DDIs; and in silico methods for assessment of such DDIs.

Cytokines effect on CYP enzymes and transporters

IL-2

IL-2 is a 15.5-16-kDa four-α-helix-bundle cytokine, secreted primarily by antigen-stimulated CD4+ T cells, and also can be secreted by CD8+ T cells, NK cells, and activated dendritic cells.[9] IL-2 plays an important role in immune homeostasis and activation via stimulation both regulatory T cells and cytotoxic effector T cells.

IL-2 treatment of cryopreserved human hepatocytes had no significant impact on either mRNA levels or enzyme activities for CYP1A2, 2C9, or 3A4, but modestly decreased CYP2B6 (mRNA only) and CYP2C19 (enzyme activity only) by less than 25% (Table 1).[10] On the contrary, the CYP2D6 mRNA expression increased ~50%, whereas CYP2D6 enzyme activity decreased ~22%.[10] In primary human hepatocyte culture, IL-2 was shown to have transient suppression of CYP3A activity, but demonstrated a sustained and concentration-dependent 50% to 70% suppression of CYP3A activity in hepatocyte/Kupffer cell coculture.[11] Indinavir, primarily eliminated by CYP3A4 metabolism, was found to have significant increase in area under the curve (AUC; 88% increase on day 5 vs. day 1) after IL-2 infusion in patients infected with the human immunodeficiency virus.[12] In addition, high-dose daily administration (9 or $12*10^6$ units/m^2) of IL-2 in patients with hepatic metastases from colon or rectum carcinomas resulted in significant reduction of total CYP enzyme protein level by 34%, CYP1A2 protein level by 37%, CYP2C protein level by 45%, CYP2E1 protein level by 60%, and CYP3A4 protein level by 39%.[13]

IL-2 significantly reduced P-gp mRNA expression in human colon carcinoma cell lines Lo VO, HT115, and SW480 cells, but had no effect on P-gp expression in LS174T cells (Table 2).[14] In another two human colon carcinoma cells, HCT15 and HCT116, a transient and reversible reduction of P-gp mRNA and protein levels when incubated with IL-2 was observed.[15] In a mouse model, chronic IL-2 treatment led to a significant decrease of 57% in P-gp protein expression but no marked differences in P-gp mRNA level in the intestines, and, as a result, IL-2 pretreatment increased orally administered P-gp substrate digoxin exposure by 2.8-fold in mice.[16] Incubation of IL-2 with human peripheral blood mononuclear cells (hPBMCs) resulted in reduced CYP2B6 and CYP3A4 mRNA and protein levels; in contrast, IL-2 increased the P-gp mRNA level to ~4.7-fold and protein level to ~2-fold, and resulted in reduced cellular accumulation of digoxin by 17% and saquinavir by 28%.[17] In another study using hPBMCs, IL-2 incubation was shown to significantly increase P-gp mRNA and protein levels in lymphocytes, and flow cytometry further confirmed that P-gp expression was significantly augmented on CD4+, CD8+, and CD19+ cells.[18]

IL-6

IL-6 is a 21-26-kDa four-α-helix-bundle cytokine with an additional short α-helix in the CD loop, secreted primarily by various cell types, including fibroblasts, endothelial cells, macrophages, T cells, and myocytes.[19] IL-6 plays an important role in host defense through the stimulation

TABLE 1 The impact of cytokines on CYP enzymes.

Cytokines	Assay system	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4	References
IL-2	Cryopreserved human hepatocyte	↔ mRNA and activity	↓ mRNA (<25%) only		↔ mRNA and activity	↓ activity (<25%) only	↑ mRNA (~50%) ↓ activity (<22%)	↔ mRNA and activity	10
	Primary human hepatocyte							↓ activity (transient)	11
	Primary human hepatocyte/Kupffer coculture							↓ activity (50% - 70%)	11
	Human	↓ protein (37%)		↓ protein (45%)	↓ protein (45%)	↓ protein (45%)		↓ protein (39%)	13
	hPBMCs		↓ mRNA and protein					↓ mRNA and protein	17
IL-6	Primary human hepatocyte and hepatocyte/Kupffer coculture							↓ activity (~80% - 90%)	11
	Primary human hepatocyte		↓ mRNA (~75%) ↓ protein (~60%)	↓ mRNA (~50%)	↓ mRNA (~35%) ↓ protein (~80%)	↓ mRNA (~40%)		↓ mRNA (~90%) ↓ protein (~50%)	20
	Primary human hepatocyte	↓ mRNA (>75%) ↓ activity (NS)	↓ mRNA (>60%) ↓ activity (NS)	↓ mRNA (>60%) ↓ activity (NS)	↓ mRNA (>60%) ↓ activity (NS)	↓ mRNA (>30%) ↓ activity (NS)	↓ mRNA (~50%)	↓ mRNA (>80%) ↓ activity (NS)	21
	HepaRG cells	↓ mRNA (>80%) ↓ activity (>80%)	↓ mRNA (>60%) ↓ activity (>60%)	↓ mRNA (>60%) ↓ activity (>80%)	↓ mRNA (>75%) ↓ activity (>60%)	↓ mRNA (>75%) ↓ activity (>60%)		↓ mRNA (>80%) ↓ activity (>80%)	21
	Cryopreserved human hepatocyte	↓ mRNA (27%) ↓ activity (22%)	↓ mRNA (63%) ↓ activity (30%)		↓ mRNA (63%) ↓ activity (35%)	↓ mRNA (72%) ↓ activity (65%)	↑ mRNA (2.4-fold) ↓ activity (39%)	↓ mRNA (98%) ↓ activity (76%)	10
	Caco-2 cells							↓ mRNA (~15%)	22
	hPBMCs		↔ mRNA ↓ protein (~40%)					↔ mRNA ↓ protein (~20%)	17
IFN-γ	Primary human hepatocytes		↓ mRNA (~75%) ↓ protein (~70%)	↓ mRNA (~50%) ↓ protein (~80%)	↔ mRNA ↓ protein (~60%)	↔ mRNA ↓ protein (~60%)		↓ mRNA (~70%) ↓ protein (~50%)	20
	hPBMCs		↓ mRNA (~60%) ↓ protein (~60%)					↓ mRNA (~40%) ↓ protein (~50%)	17
	Caco-2 cells							↔ mRNA	22
TNF-α	Primary human hepatocytes		↔ mRNA ↓ protein (~80%)	↓ mRNA (~60%) ↓ protein (~80%)	↔ mRNA	↔ mRNA		↓ mRNA (~80%) ↓ protein (~60%)	20
	Cryopreserved human hepatocytes	↓ mRNA (45%) ↓ activity (~75%)	↔ mRNA ↓ activity (~35%)		↔ mRNA ↓ activity (~20%)	↔ mRNA ↓ activity (~80%)	↓ mRNA (45%) ↓ activity (~45%)	↓ mRNA (87%) ↓ activity (~70%)	10

TABLE 1 (Continued)

Cytokines	Assay system	CYP1A2	CYP2B6	CYP2C8	CYP2C9	CYP2C19	CYP2D6	CYP3A4	References
	HepaRG cells	↓ mRNA (>40%) ↓ activity (>80%)	↓ mRNA (>40%) ↓ activity (>80%)	↓ mRNA (>40%) ↓ activity (~30%)	↓ mRNA (>40%) ↓ activity (>80%)	↓ mRNA (>40%) ↓ activity (>80%)		↓ mRNA (>40%) ↓ activity (>80%)	21
	hPBMCs		↑ mRNA ↑ protein					↔ mRNA ↔ protein	17
	Caco-2 cells							↔ mRNA	22
IL-10	hPBMCs		↔ mRNA ↔ protein					↔ mRNA ↔ protein	17
	Human	↔ activity			↔ activity		↔ activity	↓ activity (~12%)	35

Abbreviations: hPBMCs, human peripheral blood mononuclear cells; NS, not significant.

of acute phase responses, hematopoiesis, and immune reactions.[19]

IL-6 was shown to have significant suppression of CYP3A activity (~80%–90%) in both human primary hepatocyte culture and hepatocyte/Kupffer cell coculture (Table 1).[11] In another study with primary human hepatocytes, IL-6 was shown to significantly reduce the mRNA levels of all the CYP isoforms tested (CYP2C8, 2C9, 2C19, 3A4, and 2B6), with the least at ~35% for CYP2C9 and the greatest reduction at ~90% for CYP3A4.[20] IL-6 also reduced CYP2B6, 2C9, and 3A4 protein levels by ~50–80%.[20] Similarly, co-incubation of primary human hepatocytes with IL-6 downregulated the major CYP enzymes mRNA by at least 40%, with CYP1A2 decreased by greater than 75%, CYP3A4 by greater than 80%, CYP2C9 by greater than 60%, and CYP2D6 by ~50%, and P-gp mRNA decreased by ~40%; similar findings were observed in HepaRG cells with significant downregulation by at least 60% for almost all P450 isoforms with co-incubation of IL-6, but only marginal reduction in the P-gp mRNA level.[21] Consistently, IL-6 treatment significantly reduced the enzymes activities of P450s 1A2, 2B6, 2C8/9/19, and 3A4 in HepaRG cells, and markedly reduced the enzymes' activities in primary human hepatocyte but not statistically significant due to high interindividual variability among the three donors.[21] IL-6 treatment of cryopreserved human hepatocytes also significantly decreased enzyme activities of all six CYP isoforms examined (CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4), ranging from the least at 22% for CYP1A2 to the greatest at 76% for CYP3A4.[10] The CYP mRNA expression reduction was similar to the decrease in enzyme activity, except that CYP2D6 enzyme activity decreased by 39%, but the mRNA level increased by 2.4-fold.[10]

IL-6 treatment with Caco-2 cells, a model of the intestinal epithelial barrier, had reduced CYP3A4 mRNA expression by ~15% (Table 1), and increased P-gp mRNA expression by ~20% (Table 2).[22] Whereas, in the human hepatoma cell line HuH7, IL-6 treatment significantly reduced P-gp mRNA level ~35% and transporter activity by ~20%, but did not impact P-gp mRNA, protein expression or transporter activity in HepG2 cells.[23] IL-6 treatment with primary human hepatocyte modestly reduced the P-gp mRNA level by ~35% but had no impact on the protein level.[24] Incubation of IL-6 with hPBMCs had no significant impact on CYP2B6, CYP3A4, and P-gp mRNA levels but slightly reduced CYP2B6 and CYP3A4 protein levels, and increased the P-gp protein level.[17] In the human brain endothelial cell line hCMEC/D3 cell line, in vitro model of the human blood–brain barrier (BBB), IL-6 treatment slightly decreased the P-gp mRNA level by 21% and had no significant impact on protein expression, and transporter activity.[25] Similar findings were observed in another study, IL-6 treatment with hCMEC/D3 cell line

TABLE 2 The impact of cytokines on P-gp.

Assay system	IL-2	IL-6	IFN-γ	TNF-α	IL-10	References
Human colon carcinoma cell lines Lo VO, HT115, SW480	↓ mRNA		↓ mRNA	↓ mRNA		14
Human colon carcinoma cell line LS174T	↔ mRNA		↔ mRNA	↔ mRNA		14
Human colon carcinoma cell line HCT15, HCT116	↓ mRNA and protein (transient)					15
hPBMCs	↑ mRNA (~4.7-fold) ↑ activity (~2-fold)	↔ mRNA ↑ protein (~40%)	↑ mRNA (>50-fold) ↑ protein (~2-fold)	↑ mRNA ↑ protein	↔ mRNA ↔ protein	17
hPBMCs	↑ mRNA and activity					18
Primary human hepatocyte		↓ mRNA (~40%)				21
HepaRG cells		↓ mRNA (~20%)		↓ mRNA (~12%)		21
Caco-2 cells		↑ mRNA (~20%)	↑ mRNA (~20%)	↔ mRNA		22
HuH7 cells		↓ mRNA (~35%) ↓ protein (~20%) ↓ activity (~20%)		↓ mRNA (~25%) ↓ protein (~35%) ↓ activity (~35%)		23
HepaG2 cells		↔ mRNA, protein, and activity		↓ mRNA (~25%) ↓ protein (~35%) ↓ activity (~35%)		23
Primary human hepatocyte		↓ mRNA (~35%) ↔ protein		↔ mRNA ↔ protein		24
hCMEC/D3 cells		↓ mRNA (21%) ↔ protein, and activity		↑ mRNA (49%) ↑ protein ↔ activity		25
hCMEC/D3 cells		↓ mRNA (43%) ↔ activity				26
hPBMC derived macrophages			↑ mRNA ↑ activity			28
Primary lymphocytes and monocytic cell lines			↔ mRNA			28
Caco-2 cells			↑ mRNA (2.5-fold) ↑ protein (~50%) ↔ activity	↓ mRNA (~56%) ↓ activity (~20%)		29
Caco-2 cells			↑ protein (~2-fold)			30
iHBMEC and pHBMEC			↑ mRNA ↔ protein ↓ activity	↑ mRNA ↔ protein ↓ activity		31

Abbreviations: hPBMCs, human peripheral blood mononuclear cells; iHBMEC, immortalized human brain microvascular endothelial cell lines; NS, not significant; pHBMEC, primary human brain microvascular endothelial cell lines.

reduced the P-gp mRNA level by 43%, but only had mild modification of P-gp transporter activity.[26]

IFN-γ

IFN-γ is a dimerized cytokine, secreted primarily by T helper cell type 1 (Th1) cells, CD8+ T cells, B cells, NK cells, and antigen presenting cells (monocytes, macrophages, and dendritic cells).[27] The monomer has a molecular weight of 20–25 kDa, and it is composed of a core of six-α-helix-bundle and an extended C-terminal region. IFN-γ plays an important role in host defense by mediating both innate and adaptive immune responses, also, IFN-γ can exert both antitumor and pro-tumor effects.

IFN-γ significantly reduced CYP2C8, 3A4, and 2B6 by ~50% to 90%, but had no significant effect on 2C9 and 2C19 mRNA in primary human hepatocytes (Table 1).[20] IFN-γ also reduced CYP2B6, 2C9, and 3A4 protein levels by ~60–80%.[20] Incubation of IFN-γ with hPBMCs resulted in reduced CYP2B6 and CYP3A4 mRNA and protein levels; in contrast, IFN-γ dramatically increased P-gp mRNA and protein levels, and resulted in reduced cellular accumulation of digoxin by 26% and saquinavir by 30%.[17]

IFN-γ significantly reduced P-gp mRNA expression in Lo VO, HT115, and SW480 cells, but had no effect on P-gp expression in LS174T cells (Table 2).[14] IFN-γ upregulated P-gp expression and increased P-gp transport activity in a dose- and time-dependent manner in human peripheral blood monocyte-derived macrophages. The upregulation of P-gp by IFN-γ is the specific response of primary macrophages, as it has no impact on P-gp expression in primary lymphocytes and monocytic cell lines.[28] IFN-γ treatment with Caco-2 cells had no significant impact on CYP3A4 mRNA expression, but modestly increased P-gp mRNA expression by ~20%.[22] In another study with Caco-2 cells, IFN-γ treatment increased the P-gp mRNA to 2.5-fold, and a time-dependent increase of protein level with maximum increase ~50% at 24 h, but did not impact transporter activity.[29] Similarly, Dixit et al.[30] reported that IFN-γ increased P-gp protein level in a concentration- and time-dependent manner in Caco-2 cells with maximum increase by approximately twofold. In both immortalized and primary human brain microvascular endothelial cell lines (iHBMEC and pHBMEC), another in vitro BBB model, IFN-γ treatment increased P-gp mRNA expression transiently with maximum effect observed at 24 h but had no effect on the P-gp protein level. In addition, IFN-γ treatment transiently decreased P-gp transporter activity with a maximum effect observed at 12 h.[31]

TNF-α

TNF-α exits as two forms, a 26 kDa transmembrane form and a soluble 17 kDa form, which is the extracellular domain cleaved from the transmembrane form.[32] Both forms are biologically active but require trimerization for the activity. TNF-α is predominantly produced by macrophages and monocytes. TNF-α plays important roles in acute and chronic inflammation, immunostimulation, resistance to infection agents, antitumor response, sleep regulation, embryonic development, and inducing necrotic or apoptotic cell death.[33]

In primary human hepatocytes, TNF-α significantly reduced the mRNA levels of CYP2C8 and 3A4 by 60–80%, but had no significant effect on CYP2C9, 2C19, and 2B6 mRNA (Table 1).[20] TNF-α also reduced CYP2B6, 2C9, and 3A4 protein levels by ~60–80%.[20] In cryopreserved human hepatocytes, TNF-α treatment also significantly decreased enzyme activities of all six CYP isoforms examined (CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4), with greater reduction (>70%) for CYP1A2/2C19/3A4 and relatively smaller reduction (<50%) for CYP2B6/2C9/2D6.[10] The reduction of CYP mRNA expression levels was similar to the decrease in enzyme activities for CYP1A2/2D6/3A4, whereas CYP2B6/2C9/2C19 mRNA levels were relatively unchanged.[10] Similarly, in HepaRG cells, TNF-α treatment significantly reduced all major P450s isoform mRNA levels by at least 40%, but only slightly reduced P-gp mRNA by ~12%; the enzymatic activities of P450s 1A2, 2B6, 2C8/9/19, and 3A4 were also reduced with most at greater than 80% reduction except about 30% reduction for CYP2C8.[21] In contrast, incubation of TNF-α with hPBMCs significantly increased the mRNA and protein levels of CYP2B6 and P-gp, but had no impact on CYP3A4 mRNA and protein levels.[17]

TNF-α significantly reduced P-gp mRNA expression in Lo VO, HT115, and SW480 cells, but had no effect on P-gp expression in LS174T cells (Table 2).[14] Mixed results were reported on the impact of TNF-α treatment on Caco-2 cells, Bertilsson et al.[22] indicated that TNF-α treatment had no significant impact on CYP3A4 and P-gp mRNA expression; whereas, Belliard demonstrated that TNF-α treatment resulted in a time-dependent decrease of the P-gp mRNA level, with maximum reduction ~56% at 48 h, and also had significant but moderate (~20%) reduction in transporter activity.[29] In the human hepatoma cell line, HuH7 and HepG2 cells, TNF-α treatment significantly reduced the P-gp mRNA level ~25% and transporter activity by ~35%.[23] TNF-α treatment with primary human hepatocyte had no impact on either the P-gp mRNA level or the protein level.[24] In the human hCMEC/D3 cell line, TNF-α treatment significantly increased P-gp mRNA level by 49% and protein expression, but had no effect on transporter

activity.[25] In another in vitro human BBB model, iHBMEC and pHBMEC cells, TNF-α treatment also increased P-gp mRNA expression transiently with maximum effect observed at 24 h in iHBMEC and consistently increased P-gp mRNA expression in pHBMEC, but had no effect on the P-gp protein level in both cell lines.[31] Interestingly, TNF-α treatment transiently decreased P-gp transporter activity with maximum effect observed at 12 h in iHBMEC cells, and resulted in more consistent reduction of P-gp transporter activity in pHBMEC cells.[31]

IL-10

IL-10 is a dimerized anti-inflammatory cytokine, secreted primarily by monocytes, type-II T helper cells (TH2), CD4 + CD25+ T regulatory cells, γδT cells, and activated B cells.[34] The monomer has a molecular weight of ~18 kDa, consisting of a core of six-α-helix-bundle. IL-10 plays an immunoregulatory role and has inhibitory effects on pro-inflammatory cytokine production and function.

In general, incubation of IL-10 with hPBMC had no significant impact on the mRNA and protein levels of CYP2B6, CYP3A4, and P-gp, except that there is a transient slight increase in the CYP3A4 mRNA level (Tables 1 and 2).[17] In addition, IL-10 treatment in 12 healthy volunteers did not significantly alter CYP1A2, CYP2C9, and CYP2D6 activities, but slightly reduced CYP3A activity by ~12%.[35]

Clinical DDI studies involving TPs with pro-inflammatory activity

Peginterferon alfa-2a, a covalent conjugate of recombinant alfa-2a interferon with a polyethylene glycol (PEG) chain, was approved for chronic hepatitis B and C. Peginterferon alfa-2a was found to have no significant effect on the pharmacokinetics (PKs) of representative drugs metabolized by CYP2C9 (tolbutamide), CYP2C19 (mephenytoin), CYP2D6 (debrisoquine), or CYP3A4 (dapsone); and was associated with an inhibition of CYP1A2 and increased theophylline AUC 25% in healthy volunteers.[36,37]

Peginterferon alfa-2b, a covalent conjugate of recombinant alfa-2b interferon with a PEG chain, was approved for chronic hepatitis C. Peginterferon alfa-2b increased caffeine (CYP1A2 substrate) AUC 18–39% in healthy and chronic hepatitis C subjects depending on the dose regimen, increased dextromethorphan (CYP2D6 substrate) AUC 103% in healthy subjects but had no impact in chronic hepatitis C subjects, and increased desipramine (CYP2D6 substrate) AUC 30% in healthy subjects.[38,39] Peginterferon alfa-2b had no significant impact on the PKs of tolbutamide (CYP2C9 substrate), midazolam (CYP3A4 substrate), and dapsone (N-acetyltransferase substrate).[38,39] In another report, high-dose interferon alfa-2b was found to reduce CYP1A2 activity by 60% in patients with high-risk melanoma.[40]

No clinical DDI information have been reported for the other approved therapeutic proteins with pro-inflammatory activity, including interferon alfacon-1, interferon gamma-1b, interferon beta-1b, TNF-α, aldesleukin, denileukin diftitox, blinatumomab, tebentafusp-tebn, mosunetuzumab, and teclistamab-cqyv.[41–51] However, interferon gamma-1b had label language describing that the interferon gamma had demonstrated reduction in hepatic CYP450 concentrations in rodents, which could lead to suppression of the hepatic metabolism of certain drugs.[42] Interferon beta-1b also had label language describing caution when interferon beta-1b in combination with medicinal products that have a narrow therapeutic index and largely dependent on the hepatic cytochrome P450 system for clearance, as interferon was reported to be associated with reduced activity of hepatic CYP450 enzymes.[44] Blinatumomab, tebentafusp, mosunetuzumab, and teclistamab had label languages describing transient release of cytokines that may suppress CYP450 enzymes which could result in DDI risk.[48–51]

Cocktail studies

Clinically validated cocktails involving CYP mediated DDIs

Nine clinically validated (drugs within the cocktail have no mutual interactions in clinical study) cocktails with at least five CYP enzymes were shown in Table 3, including the publication time for the final version of the cocktail, drugs in the cocktail and their doses/routes, and the applications of the cocktail with no change or minimal changes.

The major CYP enzymes assessed in these cocktails included CYP1A2, 2B6, 2C9, 2C19, 2D6, and 3A4.[52–63] Caffeine was selected as the CYP1A2 probe substrate in all these cocktails. Four CYP2C9 probe substrate drugs were used in these cocktails, including flurbiprofen, losartan, tolbutamide, and warfarin. Mephenytoin and omeprazole were used as the CYP2C19 probe substrates with omeprazole predominantly used. Debrisoquine, metoprolol, and dextromethorphan were used as the CYP2D6 probe substrates. Dapsone, quinine, and midazolam were used as the CYP3A4 probe substrates with midazolam predominantly used. CYP2B6 was only assessed in the Basel cocktail and Geneva cocktail with efavirenz and bupropion as the respective probe substrate.[61,63] In addition, the Pittsburgh

TABLE 3 Clinically validated cocktails involving CYP mediated DDIs.

	Pittsburgh Cocktail[52,53]	Zhu Cocktail[54]	Karolinska Cocktail[55]	Blakey Cocktail[58]	Cooperstown 5+1 Cocktail[56,57]	Inje Cocktail[59,64]	Turpault Cocktail[60]	Basel Cocktail[61]	Geneva Cocktail[62,63]
Publication date	September 2006	November 2001	June 2003	February 2004	November 2003	November 2007	December 2009	March 2014	September 2016
CYP1A2	Caffeine 100 mg	Caffeine 100 mg	Caffeine 100 mg	Caffeine 100 mg	Caffeine 2 mg/kg	Caffeine 93 mg	Caffeine 100 mg	Caffeine 100 mg	Caffeine 50 mg
CYP2B6								Efavirenz 50 mg	Bupropion 20 mg
CYP2C9	Flurbiprofen 50 mg		Losartan 25 mg	Tolbutamide 250 mg	Warfarin 10 mg	Losartan 30 mg	Warfarin 10 mg	Losartan 12.5 mg	Flurbiprofen 10 mg
CYP2C19	Mephenytoin 100 mg	Mephenytoin 100 mg	Omeprazole 20 mg		Omeprazole 40 mg	Omeprazole 20 mg	Omeprazole 20 mg	Omeprazole 10 mg	Omeprazole 10 mg
CYP2D6	Debrisoquine 10 mg	Metoprolol 100 mg	Debrisoquine 10 mg	Debrisoquine 5 mg	Dextromethorphan 30 mg	Dextromethorphan 30 mg	Metoprolol 100 mg	Metoprolol 12.5 mg	Dextromethorphan 10 mg
CYP3A4	Dapsone 100 mg	Midazolam 7.5 mg	Quinine 250 mg	Midazolam 0.025 mg/kg (i.v.)	Midazolam 0.025 mg/kg (i.v.)	Midazolam 2 mg	Midazolam 0.03 mg/kg	Midazolam 2 mg	Midazolam 1 mg
CYP2E1	Chlorzoxazone 250 mg	Chlorzoxazone 200 mg		Chlorzoxazone 250 mg					
Application	85,86				76,80,81,87–93	94–98	82		

Note: The dosing route is oral, if not specified.

Abbreviations: DDI, drug-drug interaction; IV, intravenous.

cocktail, the Zhu cocktail, and the Blakey cocktail also included CYP2E1 with chlorzoxazone as the probe substrate.[52,54,58] Despite that some cocktails shared the same probe substrate, the dose amount and dosing route could be different. For example, midazolam was dosed intravenously in the Blakey cocktail and the Cooperstown 5+1 cocktail and was dosed orally in the other cocktails. All the individual tested cocktails showed that there were no mutual metabolic interactions among the included drugs at the respective doses, except that whereas caffeine, losartan, omeprazole, and quinine had no significant change when administered together compared with administered alone, an inhibition of debrisoquine metabolism was observed when concurrent administration of the five drugs in the Karolinska cocktail.[55] The Inje cocktail was further validated by the typical CYP inducer rifampicin or inhibitors cimetidine and fluvoxamine, and the degrees of interaction are consistent with the single agent studies.[64]

Clinically validated cocktails involving transporter mediated DDIs

Three clinically validated cocktails involving transporters were shown in Table S1, including the publication time for the final version of the cocktail, drugs in the cocktail and their doses/routes, and the applications of the cocktail with no change or minimal changes.

A five-drug microdose probe drug cocktail (here named as the Microdose cocktail) consisting of 10 μg midazolam (CYP3A), 375 μg dabigatran etexilate (P-gp), 10 μg pitavastatin (OATP1B1), 50 μg rosuvastatin (BCRP and OATP1B1/1B3), 100 μg atorvastatin (CYP3A, BCRP, OATP1B1/1B3, and P-gp) was qualified by clinical DDI studies with inducer/inhibitors of rifampin, itraconazole, and clarithromycin.[65] The observed DDIs were consistent with historical observed ones and/or in agreement with theoretical expectations, with the exception that the observed DDI for dabigatran was about twofold higher for microdose compared to that of conventional dosing, which could be due to P-gp mediated nonlinearity in the PKs of dabigatran.[65]

Stopfer et al.[66] evaluated a four-drug cocktails (here named as the Stopfer transporter cocktail) consisting of 0.25 mg digoxin (P-gp), 5 mg furosemide (OAT1 and OAT3), 500 mg metformin (OCT2, MATE1, and MATE2-K), and 10 mg rosuvastatin (OATP1B1, OATP1B3, and BCRP) for evaluation of transporter mediated DDI. Approximately 40% increase in rosuvastatin exposure was observed which could be due to the metformin and furosemide had an effect on rosuvastatin.[66] As a result, reduced doses of furosemide and metformin in this four-drug cocktail was further tested, with 0.25 mg digoxin, 1 mg furosemide, 10 mg metformin, and 10 mg rosuvastatin, and no mutual interaction was observed.[67] A follow-up study further evaluated this cocktail with four common transporter inhibitors, rifampin (rosuvastatin inhibitor), probenecid (furosemide inhibitor), cimetidine (metformin inhibitor), and verapamil (digoxin inhibitor). Generally, the DDIs between the transporter inhibitors and substrates were consistent with the historical data, except that the effect of single dose verapamil on digoxin was less than the effect observed from multiple verapamil dose studies.[68]

Trueck et al.[69] evaluated a five-drug transporter cocktails (here named as the Trueck transporter cocktail) consisting of 10 mg adefovir dipivoxil (OAT1), 100 mg sitagliptin (OAT3), 500 mg metformin (OCT2, MATE1, and MATE2-K), 2 mg pitavastatin (OATP1B1), and 0.5 mg digoxin (P-gp), and no significant mutual interactions were observed.

Clinically used cocktails without validation source

In addition to the validated cocktails, other cocktails were also used for evaluation of complicated DDIs, although no clinical data can be identified to illustrate whether there is mutual interaction among the cocktail drugs. The unvalidated cocktails can be found in Table S2.

In silico methods

The cocktail DDI studies could be helpful for evaluating the potential DDI risks for TPs with pro-inflammatory activities with the advantage of simultaneous evaluation of multiple CYP enzymes. In silico methods might also be a viable approach to assess such DDIs.

Machavaram et al.[70] developed a physiologically-based pharmacokinetic (PBPK) model for IL-6 incorporating in vitro IL6 CYP suppression kinetics from hepatocytes and evaluated the impact of IL-6 on CYP3A4 substrates (simvastatin and cyclosporine). A steady-state concentration of 100 pg/mL IL-6 was associated with increased exposure of simvastatin, and the predicted increase in AUC is comparable with the observed data (59% vs. 58%, respectively) in patients with rheumatoid arthritis (RA; patients with RA had elevated IL-6 around 100 ng/mL).[70,71] Whereas, a steady-state concentration of 500 pg/mL IL-6 resulted in cyclosporine exposure increase and the predicted increase in AUC was consistent with the observed (45% vs. 39%, respectively) in patients with bone marrow transplant (BMT; patients with BMT had higher IL-6 around 500 pg/mL).[70,72] However, in another set of patients with

BMT, the predicted cyclosporine increase by IL-6 was less than clinically observed (1.6–1.7-fold vs. 3–5-fold). Machavaram et al. further used this model with modification to predict the change of the exposure of several CYP probe substrates, CYP3A4 (simvastatin and midazolam), CYP1A2 (caffeine), CYP2C9 (S-warfarin), CYP2C19 (omeprazole), and CYP2D6 (dextromethorphan) in subjects with RA (steady-state IL6 concentrations of 50 or 100 pg/mL were used) and compared with healthy subjects and found reasonable agreement.[73,74]

Xu et al.[75] developed a PBPK model to predict the DDI potential of blinatumomab, a TP with pro-inflammatory activity which can transiently elevate multiple cytokines, including IL-6, IL-10, and IFN-gamma. The model focused on IL-6 mediated DDI and time-concentration profile of IL-6 from patients with non-Hodgkin's lymphoma receiving blinatumomab was simulated and in vitro IL-6 CYP suppression kinetics were incorporated into the model. The predicted exposure increases for sensitive substrates of CYP3A4 (simvastatin and midazolam), CYP1A2 (theophylline and caffeine), and CYP2C9 (S-warfarin) ranged from 1.2- to 1.9-fold, and lasted less than 1 week, indicating transient and weak DDI potential for blinatumomab.[75]

The exposure of several CYP probe substrate drugs were altered in patients with RA at pre- and post-sirukumab (an anti-IL-6 monoclonal antibody) treatment.[76] Midazolam, omeprazole, and S-warfarin exposure reduced about 33%, 40%, and 18%, respectively; whereas caffeine exposure increased ~25%.[76] A PBPK model was used to predict the impact of elevated IL-6 and sirukumab on CYP enzymes in patients with RA. The predicted exposure of several CYP probe substrates, caffeine (CYP1A2), S-warfarin (CYP2C9), omeprazole (CYP2C19), and midazolam (CYP3A4) in patients with RA pre- and post-sirukumab treatment (IL-6 assumed to be 50 and 0 pg/mL for pre- and post-sirukumab treatment, respectively) were consistent with the observed from the sirukumab clinical TP-DDI study.[74]

The PBPK model was also used to simulate IL-6 induced P-gp activity reduction in BBB and examine its impact on P-gp substrate digoxin exposure in the brain.[26] The simulation showed that the digoxin brain exposure is marginally affected with IL-6 treatment (a nonsignificant 4% increase of AUC_{inf}).

DISCUSSION

There is an increasing number of TPs, including TPs with pro-inflammatory activities in the different stages of drug development. In this review, we examined the DDI potential for TPs with pro-inflammatory activities via cytokine-drug interaction. Specifically, several key cytokines and their impact on major CYP enzymes and P-gp, the clinical DDI information of TPs with pro-inflammatory activities, drug cocktails for clinical DDI studies, and in silico methods for evaluating clinical DDI potential were summarized.

Among the pro-inflammatory cytokines examined, IL-6 exhibited consistent and profound effect in reducing the major CYP enzymes mRNA and protein levels as well as enzymatic activities across different assay systems, except that CYP2D6 mRNA in cryopreserved human hepatocyte was increased and CYP2B6 and CYP3A4 mRNA in hPBMCs were not changed. However, CYP2D6 activity in cryopreserved human hepatocytes and CYP2B6 and CYP3A4 protein levels in hPBMCs were reduced with IL-6 treatment. Compared to IL-6, IFN-γ and TNF-α showed slightly less effect but also significantly reduced the mRNA, protein levels, and enzymatic activities of the major CYP enzymes in most assay systems. IL-2 had the least activity in suppression of CYP enzymes, and the mechanism in suppression of CYP enzymes for IL-2 might be different from IL-6, IFN-γ, and TNF-α, because IL-2 had minor or only transient effect on CYP enzymes in primary or cryopreserved human hepatocytes but significantly reduced CYP3A4 activity in primary human hepatocyte/Kupffer coculture; in contrast, IL-6, IFN-γ, and TNF-α had similar effect on CYP enzymes in primary or cryopreserved human hepatocytes with/without Kupffer coculture. Therefore, IL-2 might suppress CYP enzymes in hepatocytes indirectly through inducing IL-6 and other pro-inflammatory cytokines in Kupffer cells.[11] In addition to the in vitro assay systems, patients with hepatic metastases treated with IL-2 resulted in CYP enzyme reduction in a clinical study.[13] For comparison purpose, we also examined the effect of an anti-inflammatory cytokine, IL-10, on CYP enzymes. Not surprisingly, IL-10 had negligible effect on CYP enzymes in hPBMCs and in humans from a clinical study.

In addition to the CYP enzymes, the impact of cytokines on transporter P-gp was also examined. The impact of cytokines on transporter P-gp is varied and dependent on assay systems. In general, the pro-inflammatory cytokines IL-2, IL-6, IFN-γ, and TNF-α increased P-gp mRNA and protein levels in hPBMC; whereas the impact of different cytokines on the other assay systems, including human colon carcinoma cell lines, Caco-2 cells, hepatocyte, HepaG2 cells, and in vitro BBB model cells were varied and could be a reduction, no change, or increase. In addition, IL-10 had no impact on P+gp. In this review, we only focused on P-gp as a prototypical transporter. However, other transporters can also be affected by the pro-inflammatory cytokines. For instance, IL-6 and TNF-α significantly reduced BCRP mRNA level and activity in the human hCMEC/D3 cell line.[25] TNF-α

and IL-2 downregulated NTCP, OATP1B1, OATP1B3, OATB2B1, OCT1, and OAT2 mRNA levels, and reduced NTCP, OATP1B1 protein levels and NTCP, OATP, and OCT1 transporter activities; IL-6 decreased mRNA level of MDR1, MRP2, and BCRP, and also MRP2 and BCRP protein expression in primary human hepatocyte.[24] TNF-α but not IL-6 reduced BSEP mRNA level and increased BCRP protein expression, and TNF-α and IL-6 increased MRP3 protein level.[24]

The current review focused on several key pro-inflammatory cytokines (IL-2, IL-6, TNF-α, and IFN-γ) and anti-inflammatory cytokine IL-10 for comparison. Other pro-inflammatory cytokines could also have impact on the CYP enzymes and transporters. For example, theophylline (a CYP1A2 probe substrate) was shown to have a reduced clearance by ~26% in patients with chronic hepatitis C treated with interferon beta[77]; whereas, interferon beta had no significant effect on exposure of mephenytoin (CYP2C19 probe substrate) and debrisoquine (CYP2D6 probe substrate) in patients with multiple sclerosis.[78] In addition, incubation of Hepa-RG cells with IL-1β resulted in significant reduction of the mRNA levels (>80%) and enzyme activities (>60%) for key CYP enzymes 1A2, 2B6, 2C19, 2C8, 2C9, and 3A4 as well as P-gp mRNA level ~55%.

Despite numerous reports indicating the impact of pro-inflammatory cytokines on CYP enzymes and transporters, limited clinical DDI studies were conducted for the approved TPs with pro-inflammatory activities, with the exception for Peginterferon alfa-2a and Peginterferon alfa-2b. However, most of the approved TPs with pro-inflammatory activities had label languages regarding the potential DDI due to suppression of CYP enzymes. The clinical DDI studies for Peginterferon alfa-2a and Peginterferon alfa-2b adopted cocktail approach, which enables assessment of DDIs for multiple CYP enzymes simultaneously to increase study efficiency and cost saving.[39,79] In addition, due to the cytokine effect on the CYP enzymes, the CYP enzymes are suppressed in certain diseases with elevated pro-inflammatory cytokines, such as plaque psoriasis and RA. Therefore, drugs used to treat these diseases could often result in disease-drug interaction as the treatment normalized the cytokines and the CYP enzymes levels and thus altered the exposure of CYP enzymes substrate drugs post-treatment compared to pre-treatment. Clinical DDI studies have been conducted to evaluate such disease-drug interactions, some using the cocktail approach.[71,76,80–82]

In this review, we summarized the most up-to-date validated cocktails for CYP enzymes and validated cocktails involving transporters, as well as some other cocktails used in the clinical DDI studies, although no validation source can be identified. Almost all the validated cocktails were either for CYP enzymes or for transporters, except that a microdose cocktail included CYP3A4 probe substrate and several transporters probe substrates.[65] Efforts have been made to combine the CYP cocktails with transporter cocktails. For example, Kwon et al.[83] developed and validated a 10-probe drug cocktail, including five major CYP probe substrates and five transporters probe substrates in rat. Bosilkovska et al.[84] explored the possibility to combine the Geneva cocktail (6 CYP enzymes) with fexofenadine (P-gp) and found that the Geneva cocktail co-administration with 25 mg fexofenadine resulted in 49% reduction in maximum concentration and 43% reduction in $AUC_{0\text{-}8}$ for fexofenadine; hence, fexofenadine cannot be combined with the Geneva cocktail. Currently, there is no validated cocktail in the clinic involving major CYP enzymes and transporters, although several unvalidated cocktails consisting of several major CYP enzymes and transporters were used in the clinical studies to assess the clinical DDIs (Table S2).

In silico methods have also been used to evaluate the complicated DDIs, whereas the population PK approach still needs clinical data to assess the DDIs, the bottom-up approach PBPK can directly extrapolate the clinical DDI with in vitro data. Current PBPK models for assessing cytokine-drug DDIs all focus on IL-6, whereas most of the models can reasonably predict the clinical DDIs based on in vitro determined CYP suppression kinetics retrospectively, Machavaram et al.[70] showed that the PBPK model underpredicted exposure of cyclosporine in certain patients with BMT, which might be due to possible contribution of other cytokines (e.g., TNF-α) in the overall suppression of CYP3A4 in these patients, and the PBPK model incorporating IL-6 CYP3A4 suppression alone may underestimate the suppression effect. Therefore, additional efforts may be needed to further evaluate the PBPK models for the other pro-inflammatory cytokines and/or a PBPK model incorporating multiple pro-inflammatory cytokines. Moreover, limited PBPK modeling is available to evaluate the DDI potential of TPs with pro-inflammatory activities with drugs via transporters, and such PBPK models need further development.

Considering the effect of pro-inflammatory cytokines on the major CYP enzymes and P-gp, for TPs with pro-inflammatory activity, a clinical DDI study is recommended for those TPs that can persistently elevate the cytokines and, in this case, the cocktail approach may be used owing to its efficient study design and cost saving; whereas for those TPs transiently elevating cytokines, a clinical DDI study may not be conducted and an in silico method may be used to assess such transient effect, and label language should incorporate the potential DDIs due to cytokine-drug effect.

CONCLUSION

Potential DDI risk for TPs with pro-inflammatory activities via cytokine-drug interaction has been well-recognized. Pro-inflammatory cytokines are generally associated with suppression of CYP enzymes but have varied effects on P-gp expression levels and activities. The approved TPs with pro-inflammatory activities generally contain label languages for potential DDI risks due to cytokine elevation. Cocktail studies have been used to evaluate the complicated DDIs involving multiple CYP enzymes and transporters with the advantage of efficiency and cost saving, and it is a viable approach to assess the DDI for TPs with pro-inflammatory activities. In silico methods are also viable approaches to assess the DDIs for TPs with pro-inflammatory activities.

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Y.Y. was employed at Pfizer when work was conducted and received salary and stock; currently employed at Genentech and owns stock in Roche. D.W. is an employee of Pfizer, Inc. and owns stock in Pfizer Inc. C.H. has no competing interests to declare.

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SUPPORTING INFORMATION

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

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