DIRECT AND CYTOKINE-MEDIATED EFFECTS OF ALBUMIN-FUSED HUMAN GROWTH HORMONE, TV-1106, ON CYP ENZYME EXPRESSION IN HUMAN HEPATOCYTES *IN VITRO*

Maciej Czerwinski,[1] Paul Bolliger, [1] Victor Piryatinsky, [2] Hussein Hallak, [2] Yousif Sahly, [2] Immaculate Amunom[1] and David Buckley[1]

[1]XenoTech, LLC, 16825 W. 116th St., Lenexa, KS, USA [2]Teva Pharmaceutical Industries, Ltd., 5 Basel St., Petach Tikva, Israel

Introduction

Drug-drug interactions involving therapeutic proteins that can modulate effects of cytokines and potentially impact cytochrome P450 (CYP) enzymes have been of increased interest to regulatory agencies and pharmaceutical industry sponsors in recent years. The well-documented therapeutic protein DDI mechanism involves pro-inflammatory, cytokine-mediated changes in drug-metabolizing enzymes. Multiple *in vitro* and a number of *in vivo* human studies have demonstrated the effect of individual cytokines and their modulators on P450s and transporters (Evers et al., 2013).

TV-1106 is recombinant human albumin (rhA), genetically fused at its C-terminus to recombinant human growth hormone (rhGH) to prolong systemic circulation of rhGH and improve its therapeutic activity (Osborn et al., 2002; Sleep, 2014). Phase 1 clinical trial of TV-1106 demonstrated that the drug is well tolerated, has a prolonged half-life in the circulation, and is biologically active in adults with GH deficiency.

GH also enhanced pro-inflammatory cytokines IL1-alpha, IL-6 and TNF-alpha production by lipopolysaccharide (LPS)-activated monocytes in whole blood and its administration at high doses to critically ill adults was associated with an increase in morbidity and mortality (Uronen-Hansson et al., 2003). GH has been shown to be a major determinant of hepatic CYP expression in rats (Morgan et al., 1998). An *in vitro* study showed an increased CYP3A4 gene expression in cultured human hepatocytes after exposure to GH (Liddle et al., 1998). In healthy elderly men GH induces CYP1A2 and, to a lesser extent, inhibits CYP2C19, but it exerts no effects on CYP2D6 and CYP3A4 enzymes (Jurgens et al., 2002).

An evaluation of TV-1106 effects on plasma cytokines and subsequently on hepatic drug metabolism is warranted by the fact that rhGH is known to modulate plasma cytokines in GH-deficient and normal children of short stature (Bozzola et al., 2003; Pagani et al., 2005).

An *in vitro* test system to examine direct- and cytokine-mediated effects of therapeutic proteins on hepatic drug metabolism that is based on treatment of whole blood with the drug, followed by separation of plasma and application of that plasma to cultured hepatocytes, has been recently developed (Czerwinski et al., 2015). Here we evaluated the ability of TV-1106 to stimulate cytokine release in whole blood and the effect of up to 50% plasma from TV-1106-treated blood on CYP expression in cultured human hepatocytes and compared the direct effects of treating primary cultures of human hepatocytes with TV-1106 and rhGH on the expression of CYP enzymes.

Materials & Methods

Chemicals and reagents: TV-1106, rhGH and sterile drug vehicle were provided by Teva. LPS was purchased from Sigma-Aldrich (St. Louis, MO). Blood was donated by healthy volunteers who gave inform consent to participate in the study. Interleukin 6 (IL-6) and TRIzol were purchased from EMD Biosciences (Jolla, CA) and Invitrogen (Grand Island, NY), respectively.

Cytokine release assay: Drug vehicle (1% v/v), LPS (50 µg/mL), rhGH (1.25 µg/mL), or TV-1106 (5 µg/m concentration equimolar to rhGH) were added to blood, gently mixed and incubated at 37°C for 24 h. Plası was separated from whole blood by centrifugation at 600 x g for 10 min, aliquoted and stored at -80°C. Cytokines were measured in the plasma by sandwich immunoassay with electrochemiluminescence detec (Human Proinflammatory 9-Plex Ultra-Sensitive Kit, Meso Scale Discovery, Gaithersburg, MD).

Hepatocyte culture and treatments: Hepatocytes from three non-transplantable livers were isolated by a two-step collagenase perfusion method and plated on collagen-coated 24-well plates, as described previously (Madan et al., 2003). This isolation procedure routinely resulted in a co-culture of hepatocytes and liver macrophages (Lambert Li et al., 2013). Cells were treated according to published protocols (Robertson et al., 2000; Paris et al., 2009). Briefly, the confluent co-cultures were achieved with approximately 4 x 105 cells per well, overlaid with Matrigel 2-3 hours after seeding and were adapted to culture conditions for two to three days with daily changes of medium. Following the adaptation period cells were treated once daily for three consecutive days with modified Chee's medium (MCM+), containing 0.1% v/v DMSO (vehicle, negative control), IL-6 (10 ng/mL, positive control), 0.5% v/v drug vehicle, TV-1106 (2.5 µg/mL) or one of two CYP enzyme inducers, namely, omeprazole (100 µM) and rifampin (10 µM). In addition, hepatocytes were treated with plasma from drug vehicle-, LPS-, rhGH-, or TV-1106-treated blood. The plasmas were added to cultured cells at 10%, 20% or 50%, v/v. Following last treatment, the cell culture medium was removed and the cells were washed with 1X PBS. Fresh incubation medium without plasma was added containing phenacetin (100 µM), S-mephenytoin (400 µM) or midazolam (30 µM). After 30-min incubation with these probe substrates, 150 µL of the medium was mixed with an equal volume of acetonitrile containing an appropriate internal standard, as described in Paris et al.(2009). Metabolite formation was analyzed by LC-MS/MS for the formation of acetaminophen (CYP1A2), 4′-hydroxymephenytoin (CYP2C19) or 1´-hydroxymidazolam (CYP3A). Additional cells were lysed with TRIzol for isolation of mRNA.

Cytoxicity assessment: Leakage of lactate dehydrogenase (LDH) in hepatocyte cultures was determined by the method provided by Roche Diagnostics. LDH release into the cell culture medium at 24, 48 and 72 h was lower in the cultures treated with TV-1106 than rhGH for all three hepatocyte donors (data not shown). Daily light microscopic examinations of the cultures throughout culturing period did not reveal signs of toxicity.

mRNA Analysis: Total RNA was phase extracted with TRIzol followed by purification with an RNeasy Mini Kit (Qiagen, Valencia, CA). Purified RNA was reverse transcribed to cDNA with the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA) and the Applied Biosystems 7300 Real-Time PCR System (AB7300). Quantitative PCR was performed with the AB7300 with the Applied Biosystems Universal Master Mix and TaqMan® Gene Expression Assays. CYP mRNA levels were normalized to the levels of GAPDH mRNA.

Results

Figure 1 presents the experimental design of the study. This design is a subject of the US Patent 8846576.

Figure 1

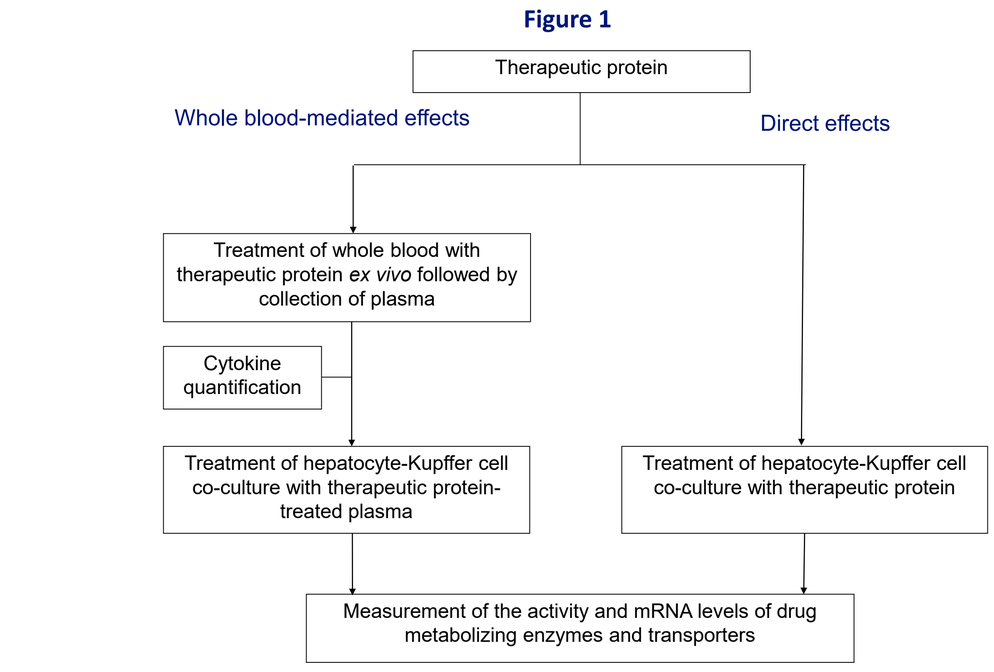


Figure 2 illustrates the effects of vehicle, LPS, rhGH or TV-1106 on the release of cytokines in whole blood. Treatment of blood with LPS caused the release of all cytokines examined. Treatment of blood with rhGH also stimulated cytokine release but to lower extent than LPS with exception of IL-12p70. Treatment of whole blood with TV-1106 increased 6 cytokines, compared to the vehicle-treated controls.

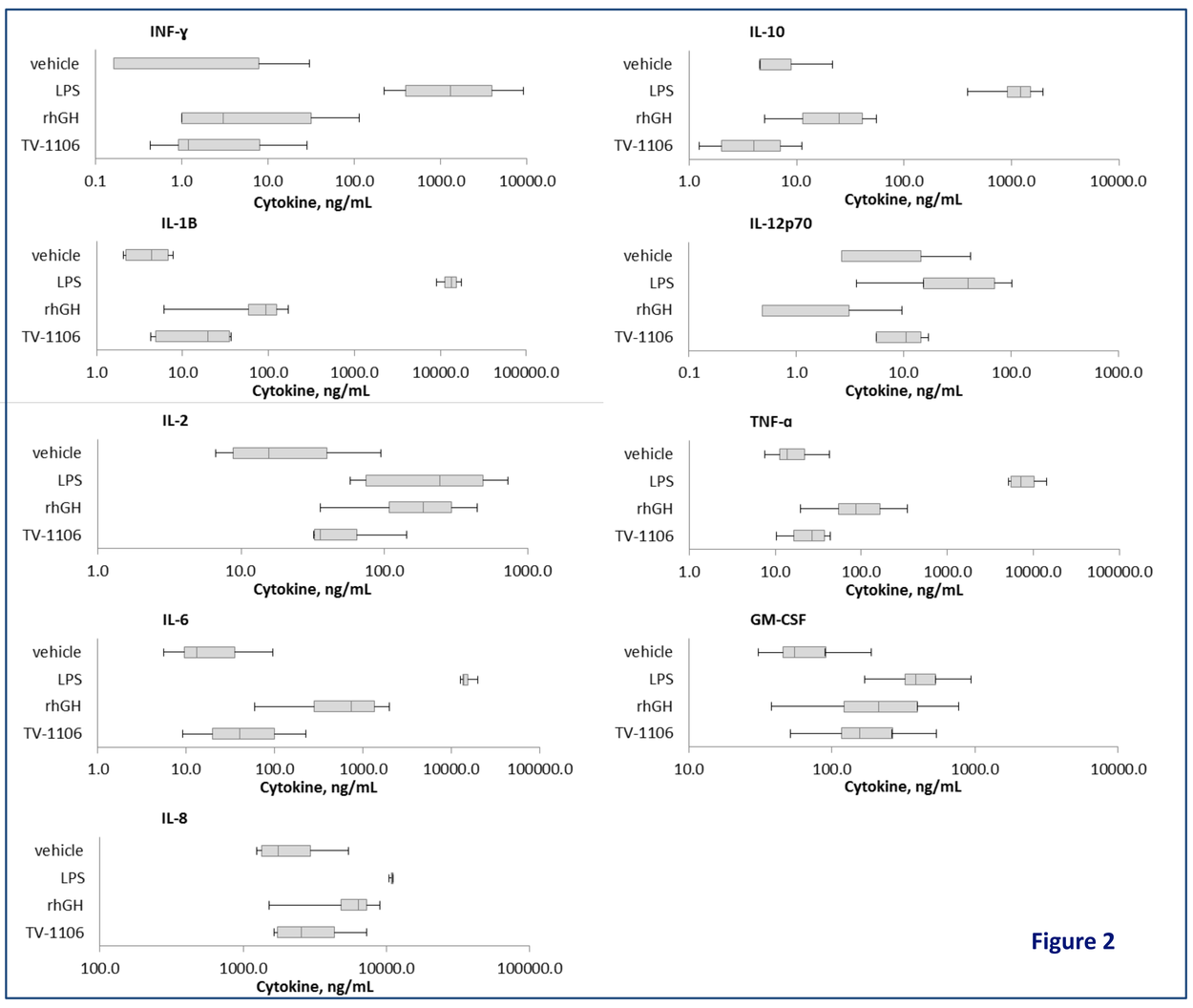


Figure 2

The effects of treating cultured human hepatocytes with drug vehicle, TV-1106, IL-6, DMSO, omeprazole or rifampin on CYP mRNAs expression and enzyme activity *in vitro* are shown in **Figure 3**. Treatment of cultured hepatocytes with the enzyme inducers omeprazole or rifampin resulted in expected changes in CYP1A2, CYP2C19 and CYP3A4 mRNA expression and enzyme activities. Treatment of human hepatocytes with IL-6 caused suppression of CYP1A2, CYP2C19 and CYP3A4 mRNA expression and enzyme activities, respectively.

Treatment of human hepatocytes with TV-1106 caused little or no change in CYP1A2, CYP2C19 or CYP3A4 mRNA expression and enzyme activities. In one donor TV-1106 was 34% as effective as rifampin at inducing CYP3A enzyme activity.

Figure 3

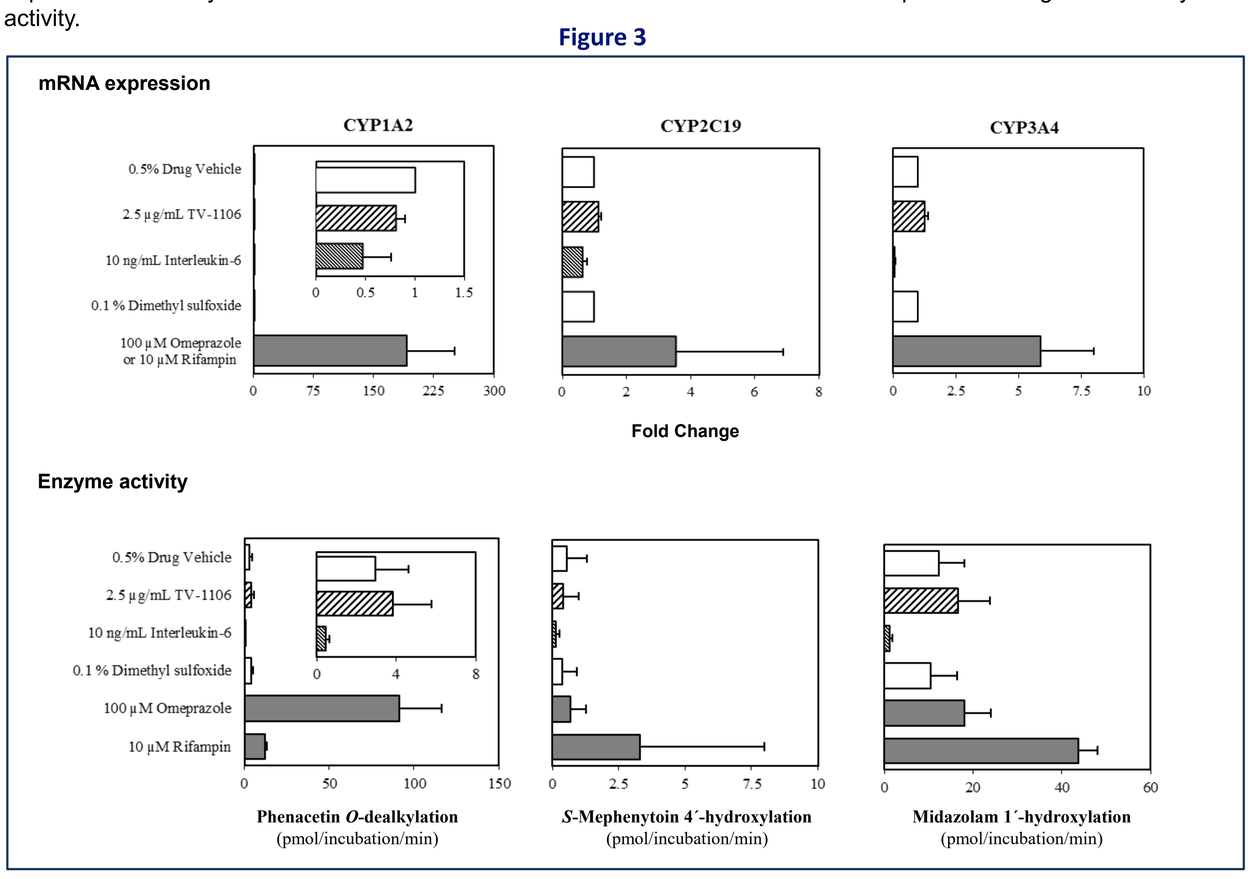
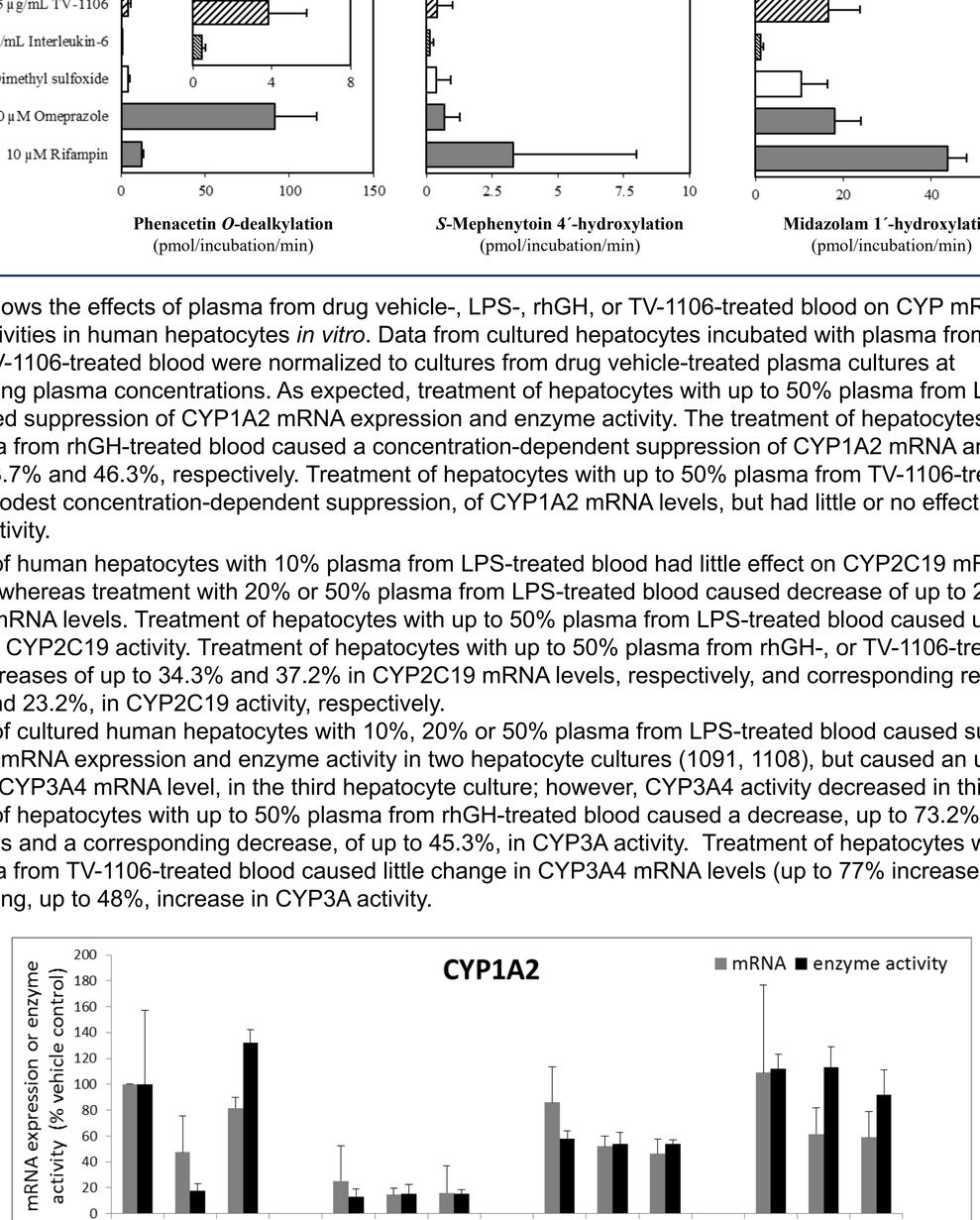


Figure 4 shows the effects of plasma from drug vehicle-, LPS-, rhGH, or TV-1106-treated blood on CYP mRNAs and enzyme activities in human hepatocytes *in vitro*. Data from cultured hepatocytes incubated with plasma from LPS-, rhGH- or TV-1106-treated blood were normalized to cultures from drug vehicle-treated plasma cultures at corresponding plasma concentrations. As expected, treatment of hepatocytes with up to 50% plasma from LPS-treated blood caused suppression of CYP1A2 mRNA expression and enzyme activity. The treatment of hepatocytes with up to 50% plasma from rhGH-treated blood caused a concentration-dependent suppression of CYP1A2 mRNA and activity levels by 53.7% and 46.3%, respectively. Treatment of hepatocytes with up to 50% plasma from TV-1106-treated blood caused a modest concentration-dependent suppression, of CYP1A2 mRNA levels, but had little or no effect on CYP1A2 activity.

Treatment of human hepatocytes with 10% plasma from LPS-treated blood had little effect on CYP2C19 mRNA expression whereas treatment with 20% or 50% plasma from LPS-treated blood caused decrease of up to 29.6% in CYP2C19 mRNA levels. Treatment of hepatocytes with up to 50% plasma from LPS-treated blood caused up to 78.1% decrease in CYP2C19 activity. Treatment of hepatocytes with up to 50% plasma from rhGH-, or TV-1106-treated blood caused decreases of up to 34.3% and 37.2% in CYP2C19 mRNA levels, respectively, and corresponding reduction, up to 43.9% and 23.2%, in CYP2C19 activity, respectively.

Treatment of cultured human hepatocytes with 10%, 20% or 50% plasma from LPS-treated blood caused suppression of CYP3A4 mRNA expression and enzyme activity in two hepatocyte cultures (1091, 1108), but caused an unexpected increase in CYP3A4 mRNA level, in the third hepatocyte culture; however, CYP3A4 activity decreased in this culture. Treatment of hepatocytes with up to 50% plasma from rhGH-treated blood caused a decrease, up to 73.2% in CYP3A4 mRNA levels and a corresponding decrease, of up to 45.3%, in CYP3A activity. Treatment of hepatocytes with up to 50% plasma from TV-1106-treated blood caused little change in CYP3A4 mRNA levels (up to 77% increase) and a corresponding, up to 48%, increase in CYP3A activity.

Figure 4



Conclusions

We compared TV-1106, an albumin-fused GH, and nascent GH ability to stimulate cytokine secretion in whole human blood and the effects of the plurality of the released cytokines on hepatic drug metabolism *in vitro*.

We found that GH genetically fused to human albumin attenuated peptide cytokine response measured with a panel of nine pro-inflammatory cytokines in whole human blood.

The cytokines stimulated by TV-1106 had only minor effects on CYP1A2, CYP2C19 and CYP3A4 expression and enzyme activity in fresh plated human hepatocytes from three healthy donors.

From the drug safety perspective, the *in vitro* cytokine and CYP response to TV-1106 demonstrates weaker-to-no DDI potential of the drug compared to rhGH.

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