In vivo system test to evaluate drug-drug interactions with biologics
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INTRODUCTION

Inflammation, infection, vaccination, and some marketed therapeutic proteins (biologics) are associated with cytokine-mediated suppression (down-regulation) of drug-metabolizing enzymes (DME). Biologics, such as monoclonal antibodies, can trigger the release of pro-inflammatory cytokines (e.g. IL-1, IL-6, TNFα, IFN-γ), extreme cases of which are known as cytokine storms. Protein therapeutics may change the clearance of small molecule drugs (SMO's) by affecting DME expression and thereby precipitate drug-drug interactions (DDIs). Potential of biologics to cause DDI is a safety concern recognized by the FDA and discussed for biologics linked to cytokine storms (Figure 1). In the present study we developed an in vitro method to evaluate the potential of biologics to elicit DDIs with SMOs via alteration of DME expression. This method involves treating human blood with a biologic to stimulate the release of pro-inflammatory cytokines from peripheral blood mononuclear cells (PBMCs), after which plasma is prepared and added to primary human hepatocytes co-cultured with Kupffer cells to evaluate effects of biologics on cytochrome P450 (CYP) enzyme expression. E. coli lipopolysaccharide (LPS) and murine anti-CD28 monoclonal antibody were evaluated for their potential to perturb DMI with SMOs.

MATERIALS & METHODS

Ex-vivo stimulation of whole blood: Four healthy donors gave informed consent to participate in the study. Blood was drawn into 10 ml sodium heparin vacutainers (Becton-Dickinson, Franklin Lakes, NJ), transferred into sterile 5 ml polypropylene tubes (BD Biosciences, San Diego, CA) and aliquoted into sterile polypropylene micro tubes (Sarstedt, Newton, NC) for incubation. Endotoxin removal was obtained from normal saline (XenoTech, LLC) with the Endotoxin Removal Gel (Pierce Biotechnology, Rockford, IL). The 5 μg/ml stock solution of lipopolysaccharides from E. coli (Sigma-Aldrich, St. Louis, MO) was prepared with saline. Type B CpG synthetic oligonucleotide (InvivoGen, San Diego, CA) was reconstituted with saline at 1 mM. Anti-CD28 antibody, ANC28.1 (murine IgG1 isotype, and isotype-control antibody were purchased from Ancell Corp. (Bayport, MN). The stimuli were gently mixed with the whole blood and incubated for 24 hrs at 37°C in the atmosphere of 5% CO2. Plasma was separated from the cell-free supernatant at 600 x g for 10 min, aliquoted and stored at -80°C.

Cytokine quantification: Levels of GM-CSF, IL-1β, IL-6, IL-8, IL-10, IL-12p70 and TNFα were measured in the plasma samples with a human cytokine assay kit (Mesoscale Discovery, Galthouse, MD) according to the manufacturer’s protocol.

Hepatocyte cultures: Primary human hepatocytes were isolated by a two-step collagenase perfusion method and plated on collagen-coated dishes. Briefly, the hepatocytes were allowed to adapt to culture medium (MD) according to the manufacturer’s protocol. After 72 hr of treatment CYP enzymatic activities were analyzed in situ and the cells were lysed with 1 ml of 1% Triton X-100. The lysates were centrifuged at 12,000 rpm for 15 min at 4°C. The supernatants were transferred to a new Eppendorf tube and stored at -80°C. Total RNA was phase extracted with Trizol® reagent followed by purification with ethanol. The media was recovered with purified saline at 1 mM.

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Figure 2 illustrates effects of plasma obtained from the LPS- or cytokine-stimulated whole blood on the activity and mRNA expression of selected CYP enzymes in cultured human hepatocytes.

○ Treatment of human hepatocytes with plasma from LPS-stimulated whole blood reduced both enzymatic activity and mRNA expression of CYP1A2, CYP2B6 and CYP3A4, as compared to the plasma from the control-stimulated blood. CYP3A4 activity and mRNA expression were significantly suppressed (by up to 75% and >99%, respectively) by the "LPS-stimulated plasma". LPS applied directly to the cell culture media suppressed CYP3A4 activity and mRNA expression by up to 70% and 90%, respectively. The observed reduction in CYP activity and mRNA expression following treatment of hepatocyte cultures with LPS represents a direct effect of LPS on the hepatocytes in vitro. However, the suppression of CYP enzymes in hepatocytes following treatment with plasma may be mediated also by the activated hepatic macrophages (i.e. Kupffer cells). This route of CYP suppression was supported by staining of human Kupffer cells in cultures of fresh human hepatocytes.

○ Treatment of hepatocytes with plasma from the anti-CD28 antibody-stimulated whole blood reduced both enzymatic activity and mRNA expression of CYP1A2 and CYP3A4. Interestingly, the same plasma caused a significant dose-dependent increase of CYP2B6's bupropion hydroxylase activity and the mRNA.

○ Antigen-presenting cells (APC), such as dendritic cells and monocytes, are capable of producing IFN-γ to activate T-cells to exert its effects indirectly on drug metabolism.

○ In this method, fresh human hepatocytes, co-cultured with Kupffer cells, respond to structurally diverse biologics (e.g. lipopolysaccharide, or a monoclonal antibody), in a way that reflects biologics’ effects on drug metabolism in vivo. Biologics released by whole blood cultures in response to stimulation with biologics (e.g. lipopolysaccharide, C6-glycolipidocetate or a monoclonal antibody) retain their activity following cryopreservation and elicit the anticipated biological response (i.e. suppression of CYP activity and mRNA expression) from cultured human hepatocytes under standard cell culture conditions.

The methods presented here involving ex vivo stimulation of whole blood combined with in vitro culture of human hepatocytes has the potential to identify biologics that can precipitate DDI with a small molecule drug through signaling by pro-inflammatory cytokines.

REFERENCES


In conclusion, the present study shows that the addition of LPS or cytokines to human blood in vitro can elicit pro-inflammatory cytokine release, which can then be used to perturb drug-metabolizing enzyme expression in cultured human hepatocytes, thus providing a new in vitro system to evaluate drug-drug interactions with biologics.