Temporal changes in CYP3A4 mRNA and activity following treatment of cultured human hepatocytes with interleukin-6 (IL-6): Implications for study design and endpoint selection

Michelle McBride, Jason Neat, David Buckley, Jennifer Simpson, Bradley Klaus, Janet Sawi, Paul Bolliger and Andrew Parkinson
XenoTech, LLC, Lenexa, KS 66219

INTRODUCTION

In recent years the focus of pharmaceutical drug development (once dominated by small molecule (NCE) therapies) has shifted and is now shared with a significant number of new therapies emerging from biological (New Biological Entities or NBE’s) development. Since the approval of the first biotechnical treatment in the United States (recombinant insulin, 1982), more than 250 biologics have reached the market, representing roughly one-quarter of all new drugs approved by the U.S. and European Union authorities (Thussheim et al, 2010). Biologics include a broad range of therapies (including but not limited too) vaccines, cell or gene therapies, therapeutic protein hormones, cytokines and tissue growth factors and monoclonal antibodies. The impact, both in vitro and in vivo of biologics, either that act directly (cytokines or interleukins, as in inflammation or disease states) or those that act indirectly (cytokine modulation), on drug metabolizing enzymes (DMEs) has been well documented and this presents a potential new area of concern surrounding safety testing and the disposition of concomitantly administered drugs (Morgan, 2001; Renton KW, 2004; Mammood I and Green MD, 2007; Morgan et al, 2001; Kacwienia M et al, 2008). Currently, draft guidance released by the Food and Drug Administration (FDA 2006), European Medical Agencies (EMA 2010) and other regulatory bodies, primarily focus on the drug-drug interaction potential for two or more concomitantly administered small molecule drugs, but provides little or no recommendations on the methods of evaluating possible interactions between small molecule drugs and therapeutic proteins (TPPs), or biologics. However, the FDA suggests that biologics should be investigated, either in vitro, in vivo, or both; for the potential to cause biologic-drug interactions (BDIs) (Huang SM et al, 2010).

Like their small molecule counterparts, therapeutic proteins, such as cytokines, monoclonal antibodies and soluble receptors, can increase systemic exposure to concomitantly administered small drug molecules. Biologics primarily act by suppressing hepatic cytochrome P450 levels through activation of transcription factors like NFκB either directly (as occurs with IL-1α), IL-6, TNFα and TGFβ or indirectly, by stimulating the release of these pro-inflammatory cytokines from Kupffer (liver) or peripheral blood mononuclear cells (PBMCs) (Zhou et al, 2006; Gu et al, 2006; Mammood I and Green MD, 2007).

In order to assess the robustness of plated human hepatocytes to a pro-inflammatory cytokine, namely IL-6, and to provide guidelines for both endpoint selection and duration of test article exposure; we examined the time course of changes in CYP activity (and mRNA levels) in primary cultures of human hepatocytes treated for either 24 hours or once daily for three consecutive days with IL-6.

METHODS

Chemicals and reagents: The sources of the reagents used in this study have been described previously (Maddum et al, 2003). Rifampin and interleukin 6 (IL-6) were purchased from Sigma Aldrich and EMD Chemicals, respectively.

Hepatocyte Cell Culture: Primary human hepatocytes were isolated and plated on collagen-coated dishes at XenoTech, LLC, Lenexa, KS 66219 and were then treated with IL-6 for three consecutive days caused a decrease in CYP3A4 activity and mRNA expression; however, the decreases were more pronounced than those following a single treatment with IL-6. The decrease in CYP3A4 activity following a 3-day treatment with IL-6 was progressive by day and was most pronounced at 72 hours (31.4% of control). In a similar manner, the decrease in CYP3A4 mRNA expression occurred prior to the subsequent decrease in enzyme activity.

RESULTS

Table 1 and Table 2 show that treatment of three individual cultures of human hepatocytes with IL-6 for three consecutive days caused a decrease in CYP3A4 activity and mRNA expression; however, the decreases were more pronounced than those following a single treatment with IL-6. The decrease in CYP3A4 activity following a 3-day treatment with IL-6 was progressive by day and was most pronounced at 72 hours (31.4% of control). In a similar manner, the decrease in CYP3A4 mRNA expression occurred prior to the subsequent decrease in enzyme activity.

Table 2 and Figure 4 illustrate that treatment of three individual cultures of human hepatocytes with IL-6 for 3 consecutive days caused a decrease in CYP3A4 activity and mRNA expression; however, the decreases were more pronounced than those following a single treatment with IL-6. The decrease in CYP3A4 activity following a 3-day treatment with IL-6 was progressive by day and was most pronounced at 72 hours (31.4% of control). In a similar manner, the decrease in CYP3A4 mRNA expression was progressive by day and the decrease was most dramatic at 72 hours (72.7% of control).

CONCLUSIONS

1. Treatment of hepatocytes with a single or multiple applications of IL-6 suppressed CYP3A4 mRNA levels and, to a lesser extent, CYP3A4 activity in all three hepatocyte cell lines.

2. Following a single application of IL-6, suppression of CYP3A4 mRNA was maximal after 24 hours whereas suppression of CYP3A4 activity was maximal after 48 hours. Likewise, three daily applications of IL-6 recovery of CYP3A4 mRNA levels was greater the recovery of CYP3A4 activity.

3. Compared with a single application, multiple applications of IL-6 (once a day for 3 days) caused greater suppression of CYP3A4 activity with no recovery over the course of treatment (the study (2) hrs following first exposure).

4. These data suggest that for a single application of pro-inflammatory cytokine, mRNA may be most sensitive when examined after 24 hours whereas CYP activity may best be evaluated 48 hours post-treatment.

5. Consequently, the selection of a treatment regimen (single vs. multiple daily treatments) is an important factor in the selection of both time points and endpoints (mRNA and CYP activity) when evaluating therapeutic proteins for the potential to modulate CYP3A4 expression.

REFERENCES


