Interleukin 2 (IL-2) is a cytokine that is released by Th1-type helper T cells during inflammation and is essential for the activation and proliferation of cytotoxic T cells as part of the cell-mediated immune response to infection. IL-2 is currently used therapeutically in patients with cancer or HIV infection to enhance the immune response (Elias et al., 1995; de Boer et al., 2003). However, its effects on cytochrome P450 activity in human liver have not been well characterized. Two clinical investigations have demonstrated decreased CYP3A protein content (Elkahwaji et al., 1999) and activity (Piscitelli et al., 1998a) in cancer patients and HIV-infected patients treated with IL-2. These findings suggest potential mechanisms whereby cytokines released from infiltrating blood mononuclear cells might modulate intercellular signaling and controls on hepatocyte function by various cell types that reside in liver.

This research was supported by a grant from the Society of Infectious Diseases Pharmacists.

Address correspondence to: Dr. Angela D. M. Kashuba, UNC School of Pharmacy, Division of Pharmacotherapy, Kerr Hall CB #7360, UNC-CH, Chapel Hill, NC 27599-7360. E-mail: akashuba@unc.edu

Materials and Methods

Materials. Reagents and culture supplies were purchased as indicated: IL-1, IL-6, and IL-2 were obtained from R&D Systems (Minneapolis, MN); col-

Kupffer cell-mediated IL-2 suppression of CYP3A activity in human hepatocytes

Jeffrey A. Sunman, Roy L. Hawke, Edward L. LeCluyse, and Angela D. M. Kashuba

School of Pharmacy, Division of Pharmacotherapy, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina

(Received September 11, 2003; accepted November 25, 2003)

This article is available online at http://dmd.aspetjournals.org

ABSTRACT:

Interleukin (IL)-2 administration has been shown to decrease CYP3A enzyme activity in vivo. To determine whether IL-2 suppression of human hepatocyte CYP3A activity is direct or whether it is facilitated by the presence of Kupffer cells, primary human hepatocytes were cultured alone or cocultured with primary human Kupffer cells at physiologic hepatocyte/Kupffer cell ratios of 10:1 or 10:4. Using proinflammatory cytokines as positive controls, IL-1 (0.2–20 ng/ml) and IL-6 (2–200 ng/ml) exposure resulted in a 70 to 90% decrease in CYP3A activity after 72 h in hepatocyte cultures. In the hepatocyte/Kupffer cell cocultures, an 80% decrease in CYP3A activity was observed with IL-1 (2 ng/ml) or IL-6 (20 ng/ml), suggesting that direct suppressive effects of proinflammatory cytokines on hepatocyte CYP3A activity are not substantially altered by Kupffer cells. In contrast to the effects of these proinflammatory cytokines, no sustained suppression of CYP3A activity was observed with IL-2 (2–200 ng/ml) in hepatocyte cultures. However, in hepatocyte/Kupffer cell cocultures, a concentration-dependent 50 to 70% suppression of CYP3A activity was observed with IL-2 at 72 h. In summary, these data suggest that Kupffer cells are required to reconstitute the suppressive effects of IL-2 on CYP3A activity that are observed in vivo and that hepatocyte/Kupffer cell cocultures may provide a useful model for investigating mechanisms of CYP3A4 regulation by cytokines. Of particular relevance to certain hepatic diseases, these findings suggest potential mechanisms whereby cytokines released from infiltrating blood mononuclear cells might modulate intercellular signaling and controls on hepatocyte function by various cell types that reside in liver.

To further elucidate the mechanism of IL-2’s suppression of CYP3A activity, we investigated the effects of IL-2 treatment in primary cultures of human hepatocytes and in cocultures that included human hepatocytes and Kupffer cells to more closely approximate in vivo conditions. Kupffer cells are specialized liver macrophages that upon stimulation synthesize and release proinflammatory cytokines such as IL-1, IL-6, and tumor necrosis factor-alpha, which are known to directly suppress CYP3A activity (Abdel-Razzak et al., 1993; Muntane-Relat et al., 1995). IL-1 and IL-6 were included in these experiments to evaluate whether the suppressive effects of proinflammatory cytokines on CYP3A activity were altered in the presence of Kupffer cells. We demonstrate that Kupffer cells are required for the sustained suppression of CYP3A activity that is observed in vivo with IL-2. Hepatocyte/Kupffer cell cocultures may provide a useful model to further evaluate the specific mechanisms of IL-2 suppression of CYP3A activity in human hepatocytes and to investigate mechanisms of CYP3A4 regulation during infection. Such information is critically important due to the potential impact of decreased CYP3A-dependent metabolism on concomitant drug therapy in these complicated patient populations.

Materials and Methods

Materials. Reagents and culture supplies were purchased as indicated: IL-1, IL-6, and IL-2 were obtained from R&D Systems (Minneapolis, MN); col-

This research was supported by a grant from the Society of Infectious Diseases Pharmacists.

Address correspondence to: Dr. Angela D. M. Kashuba, UNC School of Pharmacy, Division of Pharmacotherapy, Kerr Hall CB #7360, UNC-CH, Chapel Hill, NC 27599-7360. E-mail: akashuba@unc.edu

359
To evaluate the effects of IL-1, IL-6, and IL-2 on CYP3A activity, human hepatocytes (A), and Kupffer cells (B) were isolated from healthy human livers. Cells were viewed at a magnification of 40 (A and B) or 20 (C and D).

**Fig. 1. Primary human hepatocyte and Kupffer cell cultures.**

Lagenaese, fetal calf serum, dexamethasone, testosterone, insulin, and Williams’ E medium (WEM) were purchased from Sigma-Aldrich (St. Louis, MO); Dulbecco’s modified Eagle’s medium and bovine serum albumin were from Invitrogen (Carlsbad, CA); Percoll came from Amersham Biosciences Inc. (Piscataway, NJ); and penicillin streptomycin and ITS + were obtained from BD Biosciences Discovery Labware (Bedford, MA).

**Isolation of Human Hepatocytes.** Human hepatocytes were isolated from healthy human liver tissue, obtained from discarded resection tissue or non-transplantable organs, using a modification of the two-step collagenase digestion method previously described by Tippin et al. (2003). Hepatocyte preparations with viability >85%, as determined by trypan blue exclusion, were used in these studies.

**Isolation of Human Kupffer Cells.** Kupffer cells were isolated by a modified separation technique based on isopycnic sedimentation in Percoll as described by Smedsrod and Pertoft (1985). Viability (>90%) was determined by trypan blue exclusion. Selective adherence was performed to further purify the cell population as described by Valatas et al. (2003). The cells were also characterized by their buoyant density (1.035–1.052) using density marker beads (Pharmacia AB, Uppsala, Sweden), and by phagocytic uptake of Fluoresbrite microspheres (Polysciences, Warrington, PA) (Sun et al., 1998).

**Primary Culture of Human Hepatocytes.** Hepatocytes (1–1.5 × 10^6 cells) were added to 6-well collagen-coated culture dishes (Biocyte Ltd., Wolverhampton, UK) in 1 ml of WEM and allowed to attach for 2 to 3 h. Hepatocytes were then maintained for 36 to 48 h in supplemented WEM (0.1 μM dexamethasone, 1.0% ITS +, and 100 U/ml penicillin G/100 μg/ml of streptomycin) with medium changes every 24 h to allow cultures to stabilize before cytokine treatment.

**Cocultures of Human Hepatocytes and Kupffer Cells.** Hepatocytes and Kupffer cells from the same liver preparation were cocultured in 6-well dishes at ratios of 10:1 and 10:4 (hepatocytes/Kupffer cells) to approximate physiological ratios during “basal” and “inflammatory” states, respectively (Fig. 1). Kupffer cells were allowed to attach for 30 min before the addition of hepatocytes. Supplemented WEM was used during culture and changed every 24 h.

**Cytokine Treatment.** Cytokines in sterile saline were added to supplemented WEM and cultures treated for 72 h in triplicate wells for each cytokine concentration. Concentrations of IL-1 (0.2, 2.0, or 20.0 ng/ml), IL-6 (2.0, 20.0, or 200.0 ng/ml), or IL-2 (2.0, 20.0, or 200.0 ng/ml) were used to determine the effects of cytokines on CYP3A activity in cultures. Experimental modifications are specified in Fig. 2 and Fig. 3 legends.

**Assay of CYP3A Activity during Culture.** CYP3A activity was assayed every 24 h in culture by the addition of 250 μM testosterone to 1 ml of fresh supplemented WEM. After 1 h, the medium was removed for determination of the rate of 6β-hydroxylation of testosterone (expressed as the picomoles of 6β-hydroxytestosterone formed per minute and normalized to hepatocyte protein [adjusted for Kupffer cell protein] in each well), which was used as a specific biomarker assay for CYP3A activity. 6β-Hydroxytestosterone was quantitated by high-performance liquid chromatography as described by Faucette et al. (2001) after the extraction of medium with 5 volumes of methylene chloride and evaporation of the organic layer to dryness under a stream of nitrogen.

**Statistical Analysis.** Data are presented as the mean of triplicate measures for three to six different human liver preparations ± standard error of the mean (S.E.M.). Significance was determined using the Student’s t-test to compare the means between two groups. The one-way analysis of variance was used to test for significance between repeated measures. Tukey’s post hoc test was used after the one-way analysis of variance to determine significant differences within a group. A probability of P < 0.05 was considered statistically significant in all calculations.

In hepatocyte cultures, IL-1 and IL-6, used as positive controls, suppressed CYP3A activity in a time- and concentration-dependent manner (Fig. 2, A and B). Maximum suppression of CYP3A activity by each cytokine was observed after 72 h where IL-1 at 0.2, 2.0, and 20.0 ng/ml produced 68.2 ± 7.66, 84.7 ± 4.26, and 80 ± 4.76% suppression, respectively (Fig. 2A), and IL-6 at 2.0, 20.0, and 200.0 ng/ml produced 87.1 ± 5.61, 86.5 ± 4.48, and 92.1 ± 1.55% suppression, respectively (Fig. 2B).

In both coculture models, IL-1 and IL-6 produced similar suppressive effects to those seen in hepatocyte cultures (Fig. 2, C and D), which indicated that Kupffer cells did not alter the direct suppressive effects of proinflammatory cytokotytes on CYP3A activity. In 10:1 and 10:4 cocultures, IL-1 (2.0 ng/ml) produced 79.7 ± 11.18 and 76.8 ± 10.71% inhibition of CYP3A activity, respectively (Fig. 2C), after 72 h. Similarly, IL-6 (20.0 ng/ml) produced 79.7 ± 11.85 and 74.5 ± 8.07% inhibition of CYP3A activity, respectively (Fig. 2D).

In contrast to the effects observed with proinflammatory cytokotytes, no sustained suppression of CYP3A activity was observed with IL-2 in hepatocyte cultures. After 72 h, IL-2 at 2.0, 20.0, and 200.0 ng/ml produced only -11.40 ± 11.58, 1.60 ± 7.63, and 18.90 ± 17.09% suppression of CYP3A activity, respectively (Fig. 2D). However, in both coculture models, a time- and concentration-dependent suppression of CYP3A activity by IL-2 (Fig. 3, B–D) was observed. In the 10:1 coculture model, IL-2 produced 7.8 ± 2.91, 10.6 ± 2.90, and 51.0 ± 5.42% suppression at concentrations of 2.0, 20.0, and 200.0 ng/ml, respectively, after 72 h. In the 10:4 coculture model, IL-2 produced 27.6 ± 1.41, 45.8 ± 1.42, and 70.1 ± 6.88% suppression at the same increasing concentrations, which suggested that the effects of IL-2 on CYP3A activity are also dependent on the number of Kupffer cells present.

**Discussion**

Several clinical reports have demonstrated that IL-2, a cytokine currently used as a therapeutic agent in a variety of cancers and in HIV infection, decreases CYP3A activity. The studies presented here
evaluated whether these suppressive effects could be reproduced using primary cultures of human hepatocytes. Additionally, to determine whether the presence of Kupffer cells modified the effects of IL-2 on hepatocyte CYP3A activity in vitro, we established cocultures containing two physiologic ratios of hepatocytes and Kupffer cells (10:1 and 10:4), which were also treated with cytokines for comparison.

The proinflammatory cytokines IL-1 and IL-6 were included in these studies because of their known suppressive effects on hepatocyte CYP3A activity. At all concentrations of IL-1 and IL-6 examined, CYP3A activity was suppressed to $<30\%$ of control values. These results are in agreement with previous reports demonstrating that IL-1 and IL-6 reduce human hepatocyte CYP3A activity in vitro (Abdel-Razzak et al., 1993; Muntane-Relat et al., 1995). The effects of IL-1 or IL-6 were not altered in coculture by a 4-fold change in Kupffer cell concentration, suggesting that Kupffer cells do not contribute to the effects of these proinflammatory cytokines in the coculture systems. However, Kupffer cell enhancement of cytokine-induced CYP3A suppression at lower IL-1 or IL-6 exposures cannot be ruled out.

IL-2 had no sustained suppressive effect on CYP3A activity after long-term treatment in hepatocyte monolayers. A concentration-dependent decrease in CYP3A activity was initially observed after 48 h of treatment, indicating that IL-2 may produce a transient effect on either CYP3A expression or activity that is subsequently overcome during the following 24 h. Previous studies have reported a similar decrease in CYP3A activity in cultures of rat hepatocytes within 24 h of in vitro IL-2 treatment, an effect that was correlated with increased c-myc expression (Tinel et al., 1999). However, these studies did not evaluate treatment times beyond 24 h and, therefore, may not have observed the biphasic response of CYP3A activity seen in our experiments.

In our experiments, the presence of Kupffer cells altered the effects of IL-2 on hepatocyte CYP3A activity in a Kupffer cell concentration-dependent manner, indicating that some of the effects of IL-2 may be indirect. The 50 to 70\% reductions in CYP3A activity observed with the hepatocyte/Kupffer cell cocultures in Fig. 3, C and D, particularly in Fig. 3C using a clinically relevant IL-2 treatment concentration of 20 ng/ml in the 10:4 coculture system (Piscitelli et al., 1998b), were similar to the degree of suppression of CYP3A activity or protein

![Fig. 2. Direct suppression of CYP3A activity by IL-1 and IL-6.](image)
content observed by both Piscitelli et al. (1998a) and Elkahwaji et al. (1999), suggesting that a coculture model can replicate the in vivo effects of IL-2 on CYP3A activity.

Based on previous reports and the results presented here, we suggest that IL-2 may alter human hepatocyte CYP3A activity in one or more ways. First, as suggested by Tinel et al. (1995), IL-2 may produce direct suppression of CYP3A by binding to an IL-2 receptor on hepatocytes resulting in the transient suppression of CYP3A activity. Second, the effects of IL-2 may be indirect, i.e., through effects on Kupffer cells. Continuous infusions of IL-2 result in an increase in the plasma concentration of IL-1, IL-6, and tumor necrosis factor-α (Piscitelli et al., 1998), which are presumably released by activated Kupffer cells to produce suppressive effects on CYP3A activity. Third, it is possible that IL-2 may induce Kupffer cells to release additional mediators, such as prostaglandins, prostacyclins, or eicosanoids, which have been shown to regulate cytochrome P450 activity and may sensitize hepatocytes to the direct actions of IL-2. Our observation that direct effects of IL-2 are lost after 48 h unless hepatocytes are cultured in the presence of Kupffer cells indicates that more than one mechanism must be responsible for IL-2’s effects in human liver.

In summary, we have developed a coculture model which reproduces in vitro the effects of IL-2 on CYP3A activity previously reported in vivo.

The beneficial use of IL-2 in the treatment of cancers and HIV and the alterations in hepatic drug metabolism that subsequently occur make it critically important to understand the nature of CYP3A regulation by IL-2. Cocultures of hepatocytes and Kupffer cells, particularly the inflammatory 4:10 ratio, can be used in the future to evaluate the specific mechanisms by which IL-2 suppresses CYP3A activity in human hepatocytes and determine the contribution of Kupffer cells to this process. Finally, IL-2 and other Th1-associated cytokines are elevated in certain hepatic diseases, such as chronic hepatitis C, due to hepatic infiltration of lymphocytes (Shackel et al., 2002). Therefore, the coculture models described here may also be useful in examining alterations in Kupffer cell controls on hepatocyte apoptotic and proliferative processes following localized changes in the hepatocyte environment that are associated with chronic liver infection.

Acknowledgments. We acknowledge Joelyn Tonkin and Felicia Treadwell for their technical expertise during the course of this study.

References

de Boer AW, Markowitz N, Lane HC, Saravolatz LD, Kolettar SL, Donabedian H, Yoshizawa C,


