Animal Models of Acute Moderate Hypoxia Are Associated with a Down-Regulation of CYP1A1, 1A2, 2B4, 2C5, and 2C16 and Up-Regulation of CYP3A6 and P-glycoprotein in Liver

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Received October 25, 2006; accepted February 12, 2007

ABSTRACT:

In humans, indirect evidence suggests that hypoxia reduces the rate of biotransformation of drugs cleared by cytochrome P450 (P450) subfamilies CYP1A, 2B, and 2C. The aim of this study was to assess whether acute moderate hypoxia modulates the expression of CYP2B4, 2C5, and 2C16 in vivo, and to determine whether the changes in hepatic P450 are conveyed by serum mediators. Moreover, because hypoxia increases the expression of P-glycoprotein in vitro, we examined whether in vivo acute moderate hypoxia modulates the expression of several membrane transporters in the liver. Rabbits and rats were exposed to a fractional concentration of oxygen of 8% for 48 h to generate a stable arterial partial pressure of O2 of 34 ± 1 mm Hg. Compared with rabbits breathing room air, hypoxia in rabbits reduced the amount of CYP1A1, 1A2, 2B4, 2C5, and 2C16 proteins and increased the expression of CYP3A6. Sera of rabbits with hypoxia were fractionated by size exclusion chromatography, the fractions were tested for their ability to modify the expression of P450 isoforms, and serum mediators were identified through neutralization experiments. The serum mediators responsible for the down-regulation of P450 isoforms were interferon-γ, interleukin-1β (IL-1β), and IL-2. In vivo, in rats, hypoxia increased the mRNA and protein expression of P-glycoprotein but did not affect the mRNA of breast cancer resistance protein and organic anion-transporting polypeptide 2. It is concluded that in vivo, hypoxia down-regulates rabbit hepatic CYP1A1, 1A2, 2B4, 2C5, and 2C16 and up-regulates CYP3A6. CYP3A11 and P-glycoprotein were up-regulated in the livers of hypoxic rats.

In patients with cardiorespiratory diseases, acute hypoxemia appears to reduce the ability of cytochrome P450 isoforms to catalyze the biotransformation of xenobiotics. Patients with pulmonary insufficiency who show an impairment of their general state present an increased incidence of adverse effects, e.g., grand mal seizures, associated with the administration of “usual” doses of theophylline. Theophylline clearance is significantly reduced in patients with acute cardiogenic pulmonary edema (Piafsky et al., 1977) and in patients with worsening airway obstruction, severe bronchial obstruction, congestive heart failure, and pneumonia (Vozeh et al., 1978).

In humans, at therapeutic concentrations, the biotransformation of theophylline is primarily catalyzed by CYP1A2, whereas CYP2D6, 2E1, and 3A4 exhibit low affinity and variable capacity (Ha et al., 1995). Rabbits exposed to a fractional concentration of inspired O2 (FiO2) of 10% for 24 h demonstrate a reduced clearance of theophylline and decreased expression of CYP1A1 and 1A2, although the expression of CYP3A6 is increased (Kurdi et al., 1999). In rabbits subjected to acute moderate hypoxia, hepatic down-regulation of CYP1A1 and 1A2 is triggered by serum mediators, e.g., interferon-γ (IFN-γ), interleukin 2 (IL-2), and IL-1β; the up-regulation of CYP3A6 is, at least in part, associated with erythropoietin (Epo) (Fradette et al., 2002). In vivo, the increase in CYP3A6 by hypoxia is associated with the increased expression and nuclear translocation of hypoxia-inducible factor-1 (HIF-1), activator protein 1 (AP-1), and constitutive androstane receptor (Fradette and du Souich, 2003).

There is indirect evidence that in addition to CYP1A1 and 1A2, hypoxemia down-regulates other cytochrome P450 isoforms. Antipyrene half-life was effectively increased 120% in a group of patients who predominantly had chronic hypoxemia (PaO2 < 55 mm Hg) from a variety of causes, compared with other subjects without severe hypoxia (Cunning, 1976). In humans, antipyrene is metabolized by numerous hepatic P450 isoforms, e.g., CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, and 3A4 (Engel et al., 1996; Sharer and Wrighton, 1996).

ABBRVIATIONS: FiO2, fractional concentration of inspired O2; AhR, aryl hydrocarbon receptor; AP-1, activator protein-1; Arnt, aryl hydrocarbon receptor nuclear translocator; BCRP, breast cancer resistance protein; Epo, erythropoietin; HCONT, hepatocytes from control rabbits; HHYPO, hepatocytes from rabbits with hypoxia; HIF-1, hypoxia-inducible factor 1 transcriptional activator; HPLC, high performance liquid chromatography; IFN-γ, interferon-γ; IL, interleukin; MDR1, multidrug resistance protein 1; Mr, relative molecular mass; NK-κB, nuclear factor κB; OATP2, organic anion transporting polypeptide 2; P450, cytochrome P450; PXR, pregnane X receptor; SCONT, serum from control rabbits; SHYPO, serum from rabbits with hypoxia.
The present study aimed to explore whether acute moderate hypoxia down-regulates CYP2B4, 2C5, and 2C16 in vivo, the rabbit’s orthologs of human CYP2B6, 2C9 and 2C19, respectively, because of their relative importance in the biotransformation of drugs, and to identify ex vivo the serum mediators implicated in the in vivo down-regulation of these hepatic isoforms. In contrast, P-glycoprotein is modulated by constitutive androstane receptor (Burk et al., 2005) and by the presence of reactive oxygen species (Wartenberg et al., 2003), which are increased by acute moderate hypoxia (Fradette and Du Souich, 2004); moreover, the multidrug resistance protein 1 (MDR1) gene contains a binding site for HIF-1 (Wartenberg et al., 2003). Thus, we hypothesized that in vivo, hypoxia would increase the expression of the P-glycoprotein MDR1 (multidrug resistance gene product, ABCB1). Therefore, the present study also aimed to document in vivo the effect of moderate hypoxia on several efflux and influx membrane transporters.

Materials and Methods

Animals and Collection of Hepatocytes. Male New Zealand White rabbits (1.8–2.2 kg) were obtained from Ferme Charles Rivers (St. Constant, QC, Canada). Rabbits were maintained on Purina Laboratory Chow and water ad libitum for at least 7 days before any experimental work was undertaken. To induce hypoxia, rabbits were placed in a Plexiglas chamber (0.75 × 1.20 × 1.25 m) with a FiO2 of 8%, adjusted with an oxygen monitor (OM-15; Healthcare BioSciences Inc. (Baie d’Urfe, QC, Canada) as described previously (Fradette et al., 2002). In brief, the flow rate of the mobile phase was set at 0.3 ml min⁻¹ and column pressure was maintained between 9 and 12 bar with an LKB (Bromma, Sweden) 2150 HPLC pump. Absorbance was measured at 280 nm with a Waters 490E spectrophotometric detector (Millipore, Billerica, MA). The eluant buffer included 115 mM NaCl, 5 mM KCl, 1 mM KH₂PO₄, 1 mM HEPES, 25 mM EGTA, and 5.5 mM glucose; the pH of the solution was adjusted to 7.4 and filtered through a 0.22-µm membrane. Serum aliquots of 300 µl were injected into the column and fractions of 1.2 ml were collected with a fraction collector (LKB 2211 Supercarr). To calculate the Mi of the serum proteins contained in each HPLC fraction, a calibration curve was established by injecting 300 µl of the buffer containing a mixture of six standard proteins (100 µg ml⁻¹): β-glutamic dehydrogenase (55,600), aldolase (39,200), triosephosphate isomerase (26,600), trypsin inhibitor (26,600), cytochrome c (12,500), and aprotinin (6500). The fractions containing proteins with Mi between 9000 and 14,000, 15,000 and 23,000, 24,000 and 31,000, 32,000 and 44,000, 45,000 and 64,000, and 65,000 and 94,000 were separated and collected. Based on the size exclusion range of the column, proteins isolated in the first fraction were assumed to have a Mi greater than 95,000. To increase the inhibitory activity of the fractions collected, these were concentrated on Microsep 3K membranes (Pall Filtron, Northborough, MA) that retain proteins greater than 3000. Three milliliters of the fractions were added to the sample reservoir and centrifuged at 75,000g to reduce the volume and hence concentrate serum fractions 1.25 times.

The medium of the cultured hepatocytes was replaced with fresh Williams’ medium E supplemented with 10% calf serum and 1 µM insulin, and hepatocytes (HCONT and HYPO) were then incubated with 200 µl of serum from control rabbits (SCONT), 200 µl of serum from hypoxic rabbits (SYPHO), 200 µl of the HPLC fractions of SCONT and SYPHO, or 200 µl of saline, i.e., in the absence of serum or its fractions, for 24 h. After 24 h of incubation, the effect of serum or the HPLC fractions was assessed by measuring the expression of CYP1A1/1A2, 2B4, 2C5, 2C16, and 3A6.

The identification of the serum mediators was performed by immunoneutralization. The selection of the antibodies used for the immunoneutralization of cytokines was based 1) upon the Mi of the proteins incorporated in the HPLC fractions having the ability to change P450 isoform expression, and 2) according to the cytokines released during hypoxia (Naldini et al., 1997). To prevent the changes in P450 isoform expression induced by the HPLC fractions, the following antibodies were used: a goat anti-rabbit IL-1β (anti-IL-1β) polyclonal antibody, and anti-human IL-2 (anti-IL-2), IFN-γ (anti-IFN-γ), IL-6 (anti-IL-6), and Epo (anti-Epo) monoclonal antibodies. The antibodies against human proteins were used to neutralize the homologous rabbit proteins because of the known interspecies reactivity of these antibodies (Fradette et al., 2002). An IgG monoclonal antibody to Pseudomonas aeruginosa served as control. Aliquots of 2 µg of each antibody were added individually to 200 µl of the HPLC fractions that were able to decrease the expression of P450 isoforms, and were incubated at 37°C for 1 h. The antibody and the HPLC fractions were incubated with the hepatocytes for 24 h when P450 isoform expression was assessed. The amount of 2 µg of antibodies was used because this amount is effective to immunoneutralize IL-1β, IL-6, and IFN-γ in the sera of rabbits and of humans with an inflammatory reaction (Fradette et al., 2002).

Measure of P450 Isoforms by Immunoblot. Hepatocytes were washed, harvested in ice-cold phosphate-buffered saline, and centrifuged at 1500g for 5 min. The pellet was resuspended in ice-cold lysis buffer (10 mM HEPES, pH 7.9, 10 mM KCl, 1 mM EDTA, 0.1 mM EGTA, 1 mM dithiobiotitin, protease inhibitor mixture), and cells were allowed to swell on ice for 15 min and vortexed for 30 s. The amount of protein in hepatocytes was measured in cell lysate by the method of Lowry et al. (1951). For Western blot analysis, 50 µg of cell lysate were separated by SDS-polyacrylamide gel electrophoresis (Fradette et al., 2002). Separated proteins were electrophoretically transferred to a nitrocellulose membrane using a semidry transfer process (Bio-Rad, Hercules, CA). CYP1A1 and 1A2 were detected with a polyclonal anti-rabbit CYP1A1 (Oxford Biomedical Research, Oxford, MA) diluted 1:100 in 5% nonfat milk in Tris-buffered saline/Tween 20 (0.1% Tween 20) and visualized with an alkaline phosphatase-conjugated secondary antibody using nitro blue tetrazolium as substrate. CYP2B4, 2C5, 2C16, and 3A6 proteins were detected with rabbit anti-human antibodies, which recognize rabbit isoforms, against CYP2B4 diluted 1:500 (Research Diagnostics, Flanders, NJ), CYP2C5 diluted 1:100 (Research Diagnostics), CYP2C16 diluted 1:100 (Research Diagnostics), and CYP3A6 diluted 1:500 (Oxford Biomedical Research), using a
secondary antibody conjugated with a chemiluminescence reagent (horseradish peroxidase enzyme) and visualized by autoradiography. As reference protein, in each gel, 50 μg of proteins extracted from the same control hepatocytes, with constant amounts of CYP1A1, 1A2, 2B4, 2C5, 2C16, and 3A6, were used. The assays were linear in the range of protein amounts assessed under the actual experimental conditions, and the results are presented as a ratio of the P450 isoform to the reference protein.

**Effect of Hypoxia on the Expression of Membrane Transporters.** These experiments were conducted with rats because of the lack of information and commercial availability of antibodies for transporters in rabbit. Preliminary experiments showed that a FiO2 of 8% reduced the arterial partial pressure to levels similar to those measured in rabbits, i.e., around 35 mm Hg, and that after 48 h, the expression of CYP3A1 was induced consistent with that seen in rabbits.

Male Sprague-Dawley rats (250–275 g) were purchased from Charles River Canada (St. Constant, QC, Canada), and studies were conducted in accordance with the guidelines of the Canadian Council on Animal Care. Rats were exposed for 48 h to a FiO2 of 8% in the chamber as described above. Control rats were placed into chambers that contained room air (FiO2 = 21%) for the experiments. Forty-eight hours later, the rats were anesthetized with halothane and the liver was removed, rapidly frozen in liquid N2, and stored at -80°C until used for protein and RNA isolation. The expression of CYP3A1 was measured from the microsomal fraction of the liver as described above. To further validate the rat model, preliminary experiments showed that the effect of 48 h of hypoxia in vivo on the expression of P450 isoforms was not affected by the approach used, e.g., measure of P450 isoforms in microsomes or in cultured hepatocytes.

**Analysis of Mdr, OATP, BCRP, and PXR Expression.** Total RNA was isolated from rat liver using the QuickPrep RNA extraction kit (GE Healthcare BiSciences Inc.) according to the manufacturer’s protocol, and cDNA was synthesized from 2.5 μg of RNA using the First Strand cDNA Synthesis Kit (MBI Fermentas, Flamborough, ON, Canada). Transporter mRNA levels were measured by real-time quantitative polymerase chain reaction using LightCycler technology (Roche Diagnostics, Mannheim, Germany) with LC FastStart DNA Master SYBR Green I for detection. Primer sequences for Mdr1a, Mdr1b, and PXR were previously reported (Kaliszky-Szirés et al., 2004). Primers for breast cancer resistance protein (BCRP) were: forward, 5′-AGTCTCGGAAAACAGCTGAGA-3′, and reverse, 5′-CCCATCACAACGTCTATCTGG-3′; and for OATP2 were: forward, 5′-TTGTTGTGATGGTAGTGACATGGAT-3′ and reverse, 5′-GCCAATGGTCATCTTT-3′. All mRNA levels were normalized to 18S mRNA and the ratios are presented as percentage control.

The hepatic crude membrane fraction was isolated as described previously (Teng and Piquette-Miller, 2005) and measured by the Bradford method (Bradford, 1976) using the Bio-Rad protein assay. For the immunoblotting, 10 μg of protein was loaded and run on a 10% acrylamide gel and transferred onto a Hybond ECL nitrocellulose membrane (GE Healthcare BioSciences Inc.). Membranes containing transporter protein were then cut in half and the upper portion (mol. wt. >78,000) was incubated overnight with 2 μg of the P-glycoprotein monoclonal antibody, C219 (ID Labs Inc., London, ON, Canada), followed by sheep anti-mouse IgG (Amersham). To control for variability in protein loading, the lower portion of the membrane (mol. wt. <78,000) was incubated with anti-β-actin clone AC15 antibody (Sigma-Aldrich, St. Louis, MO) followed by sheep anti-mouse IgG (GE Healthcare BioSciences Inc.). Bands were detected using an ECL Western blotting analysis system (GE Healthcare BioSciences Inc.), imaged on Bioflex MSi film (Corion Corp. and InterSciences Inc., Markham, ON, Canada), and quantified using Kodak Digital Science 1D Image Analysis software (Eastman Kodak, Rochester, NY).

**Drugs and Chemicals.** Percoll gradient, Williams’ medium E, calf serum, type I rat-tail collagen, NaCl, KCl, KH2PO4, Heps, EGTA, and glucose were purchased from Sigma Chemical (St. Louis, MO). Insulin was acquired from Roche Diagnostics (Laval, QC, Canada), and L-glutamic dehydrogenase, aldolase triosephosphate isomerase, trypsin inhibitor, cytochrome c, and apotinin were obtained from GE Healthcare BiSciences Inc.). The polyclonal anti-rabbit CYP1A1 and the monoclonal anti-rat CYP3A1 antibodies were purchased from Oxford Biomedical Research; the anti-CYP2B4, anti-CYP2C5, and anti-CYP2C16 antibodies from Research Diagnostics; the anti-IL-1β antibody from Cedar Lane (Hornby, ON, Canada); and the anti-IL-2, anti-IL-6, and anti-IFN-γ antibodies from R&D Systems (Minneapolis, MN).

**Statistical Analysis.** All results are presented as mean ± S.E. The comparison of the results from the various experimental groups and their corresponding controls was carried out by a one-way analysis of variance, followed by the Newman-Keuls post hoc test. Differences were considered significant when p < 0.05.

**Results**

**Effect of Hypoxia in Vivo on Hepatic P450 Isoform Expression.** Exposure of rabbits to an 8% FiO2 atmosphere for 48 h generated a stable hypoxemia, with an average PaO2 of 34 ± 1 mm Hg, without influencing the arterial partial pressure of CO2 (pH 7.47 ± 0.05). Compared with rabbits breathing a 21% FiO2 (i.e., room air), hypoxia reduced the amount of CYP1A1, 1A2, 2B4, 2C5, and 2C16 proteins by 37, 40, 55, 75, and 82%, respectively (p < 0.05, n = 6 per group). On the other hand, 48 h of hypoxia increased the amount of CYP3A6 by 71% (Fig. 1).

**Effect of SCONT and SHYPO on Hepatic P450 Isoform Expression in HCONT and in HYPO.** Compared with HCONT incubated with SCONT, incubation of HYPO with SCONT for 24 h reduced the amount of CYP2C5 and 2C16 by 15% and 17%, respectively (p < 0.05). Moreover, SHYPO increased the amount of CYP3A6 by 31% (p < 0.05 compared with control). No changes in CYP1A1, 1A2 and 2B4 proteins were observed (Fig. 2A).

In HYPO incubated with SCONT, the expression of CYP1A1, 1A2, 2B4, 2C5, and 2C16 was reduced by 33, 35, 42, 70, and 77%, respectively (p < 0.05, n = 6), and that of CYP3A6 was increased by approximately 70% (p < 0.05), compared with HCONT (Fig. 2B). After 24 h of incubation of HYPO with SHYPO, the amount of CYP1A1 and 1A2 proteins was further reduced by an average of 27 and 30%, respectively (p < 0.05), whereas the amount of CYP2B4, 2C5, and 2C16 decreased to an undetectable level. On the other hand, incubation of HYPO with SHYPO increased the expression of CYP3A6 by an average of 50% (p < 0.05) (Fig. 2B).

**Effect of SHYPO, HPLC Fractions on P450 Isoform Expression in HYPO.** Compared with the SCONT, incubation of the SHYPO 15- to
23-kDa HPLC fraction with HHYPO did not elicit any effect on P450 isoform expression, despite increasing the amount of antibody to 4 μg (data not shown).

Whereas the SHYPO 32- to 44-kDa fraction significantly increased CYP3A6 expression by approximately 80%, coinoculation of an anti-Epo antibody (n = 6) to the 32- to 44-kDa fraction attenuated CYP3A6 protein induction by 40% (p < 0.05). However, CYP3A6 expression was still greater (p < 0.05) than that observed in the SCONT-treated HHYPO cells (Fig. 4).

Effect of Moderate Hypoxia on the Expression of Membrane Transporters. Compared with control rats (n = 6), the expression of CYP3A1 increased by 75% (p < 0.05, n = 9) in hypoxic rats exposed to a FiO2 of 8% for 48 h (Fig. 5). Levels of CYP3A1 mRNA were also similarly induced in hypoxic rats (data not shown). Levels of mdr1b mRNA were significantly induced in hypoxic rats (p < 0.05, n = 9); however, hypoxia did not affect the expression of Mdr1a, OATP2, and BCRP mRNAs (Fig. 6). Likewise, protein levels of P-glycoprotein were significantly increased by 77% in hypoxic rats (p < 0.05, n = 9) (Fig. 7). Hypoxia tended to increase PXR mRNA (p > 0.05, n = 9) (Fig. 6).

Discussion

The present study demonstrates that besides CYP1A1 and 1A2, acute moderate hypoxia down-regulates CYP2B4, 2C5, and 2C16, the orthologs of human CYP2B6, 2C9, and 2C19, respectively. The serum mediators contributing to the decrease in expression of these isoforms are IFN-γ, IL-1β, and IL-2. This study confirms that acute moderate hypoxia as well as serum from rabbits with hypoxemia up-regulates the expression of CYP3A6, in part, because of Epo (Fradette et al., 2002). Hypoxia in vivo and SHYPO induce the expression of CYP3A6 through a protein tyrosine kinase- and extracellular signal-regulated kinase 1/2-dependent activation of HIF-1 and AP-1 nuclear translocation (Fradette and du Souich, 2003). The decrease in CYP1A1 and 1A2 expression by hypoxia occurs at the transcriptional level (Fradette et al., 2002).

The serum mediators responsible for the down-regulation of CYP1A1, 1A2, 2B4, 2C5, and 2C16 are primarily IFN-γ and IL-1β, with IL-2 playing a minor role (Table 1). It is noteworthy that the cytokines modulating P450 isoforms depend upon the experimental condition. For instance, in hypoxia, IL-6 does not contribute to the decrease in expression of P450 isoforms, contrasting with the turpen-
time-induced inflammatory reaction, where IL-6 is the most important serum mediator involved in the down-regulation of CYP1A1, 1A2, and 3A6 (Bleau et al., 2003). Moreover, IFN-γ is the predominant mediator in acute moderate hypoxia and in the serum from humans with an upper respiratory viral infection, whereas IFN-γ does not modulate the expression of P450 isoforms in the turpentine-induced inflammatory reaction (Bleau et al., 2003). IFN-γ does not appear to be selective since it contributes to the down-regulation of all P450 isoforms investigated. On the other hand, in the serum of rabbits with hypoxia, IL-1β contributes to the down-regulation of CYP1A1, 2B4, and 2C5, whereas in the serum of rabbits with a turpentine-induced inflammatory reaction, IL-1β reduces CYP1A2 and 3A6, and in the serum from humans with an upper respiratory viral infection, IL-1β diminishes only CYP3A6. Moreover, the effect of serum from hypoxic rabbits depends upon the source of hepatocytes; for example, the down-regulation of CYP1A1, 1A2, 2B4, 2C5, and 2C16 and the up-regulation of CYP3A6 after 24 h of incubation with serum from rabbits with hypoxia is marginal in hepatocytes from control rabbits but extensive in hepatocytes from rabbits with hypoxia. Similar differences occur when serum from rabbits with an aseptic inflammatory reaction is incubated with hepatocytes from control rabbits or with hepatocytes from rabbits with an inflammatory reaction. The differences in response to serum fade when incubations are conducted for longer periods, e.g., 48 h (El-Kadi et al., 1997; Bleau et al., 2003). The differences in response to serum may be associated with the expression of cytokine membrane receptors, which is enhanced by increased serum concentrations of cytokines secondary to the inflammatory reaction and hypoxia (Geisterfer et al., 1993; Naldini et al., 1997).

Considering CYP1A1 and 1A2, at least three mechanisms may contribute to their decrease in expression by hypoxia. In vivo, hypoxia induces the production of reactive oxygen species in the liver (Fradette and Du Souich, 2004), which may activate nuclear factor-κB (NF-κB), which can, in turn, interact with the aryl hydrocarbon receptor (AhR). This leads to a mutual functional repression of AhR by NF-κB and, consequently, to a reduction of the expression of CYP1A1 and CYP1A2 (Tian et al., 1999).

Hypoxia also activates transcription factors that regulate adaptive responses in cells (Semenza, 2000). Cells adapt to low partial pressure of oxygen by up-regulating the transcription of multiple genes, such as vascular endothelial growth factor, Epo, and several glycolytic en-
zmes. These target genes are induced by a common transcription factor, HIF-1 (Aragones et al., 2001). Hypoxia, cytokines, and ROI stabilize HIF-1α, which translocates to the nucleus, where it dimerizes with HIF-1β or aryl hydrocarbon receptor nuclear translocator (Arnt) to form HIF-1. Because Arnt is also a heterodimerization partner of AhR, hypoxia will decrease the availability of Arnt and so cause a down-regulation of CYP1A1 and 1A2 (Chan et al., 1999). Supporting the idea that hypoxia reduces CYP1A1 and 1A2 by decreasing the availability of Arnt is the fact that stabilization of HIF-1α with cobalt chloride decreases CYP1A1 and 1A2 expression (Fradette and Du Souich, 2003).

AP-1 is activated by hypoxia by the increase in intracellular calcium. Hypoxia increases intracellular calcium, which activates extracellular signal-regulated kinase 1/2 with the subsequent induction of the immediate-early response genes c-fos, c-jun, and erg-1 (Michiels et al., 2000). Dimerization of these early response genes forms AP-1, which binds to DNA to modulate gene transcription. CYP1A1 and 1A2 negative regulatory element contains AP-1 motifs (Chung and Bresnick, 1997). There is evidence that CYP1A1 down-regulation by lead acetate increases its nuclear translocation as well as the down-regulation of CYP1A1 and CYP1A2 in hepatocytes (Fradette and Du Souich, 2003). All these data suggest that AP-1 could contribute to the CYP1A1 and 1A2 down-regulation.

Hypoxia promotes the release of cytokines including IL-1β, IL-2, IL-4, IL-5, IL-6, tumor necrosis factor α, and IFN-γ (Naldini et al., 1997). The increase in IL-1β, IL-2, and IFN-γ activates transcription factors, such as NF-κB, AP-1, and c-myc (Wesselsborg et al., 1997; Ellery and Nicholls, 2002). There is evidence that in response to IL-1β, the p50 subunit of NF-κB binds to the negative κB response element 1 of the CYP2C11 gene, entailing a down-regulation of CYP2C11 (Iber et al., 2000). Moreover, c-myc diminishes the expression of P450 isoforms by decreasing CCAAT-enhancer binding protein-α-mediated P450 transactivation (Tinel et al., 2003). Therefore, multiple mechanisms probably contribute to the modulation of CYP1A1, 1A2, 2B4, 2C5, and 2C16 expression during hypoxia.

The present results afford a mechanism to early reports showing that in humans and animals, hypoxia modulates the clearance of drugs. For instance, antipyrine half-life is increased in hypoxic subjects (Cumming, 1976), possibly because antipyrine is biotransformed by CYP1A2, 2B6, 2C8, 2C9, 2C18, and 3A4 (Engel et al., 1996). Acute moderate hypoxia reduces the clearance of phenytoin, which is biotransformed primarily by CYP2C9 and to a minor degree by CYP2C19 (Giancarlo et al., 2001). On the other hand, the clearance of lidocaine and diltiazem, two drugs primarily metabolized by CYP3A4 in rabbits, is not affected by hypoxia (Fradette and Du Souich, 2004).

The present results demonstrate that in vivo, acute moderate hypoxia increases the expression of the efflux transporter P-glycoprotein in hepatocytes as well as Mdr1b mRNA. These results confirm in vitro results showing that hypoxia reduces the sensitivity of EMT6/RO cells to doxorubicin (Adriamycin), 5-fluorouracil, and actinomycin D because of an amplification of the P-glycoprotein gene family (Sakata et al., 1991). Induction of P-glycoprotein as well as BCRP and the organic anion-transporting polypeptide 2 (OATP2) frequently occurs through activation of PXR (Anapolsky et al., 2006); however, this mechanism did not appear to play an important role in hypoxic animals because mRNA levels of BCRP and OATP2 as well as PXR were not affected. Alternatively, it is plausible that binding sites for HIF-1, found on the promoter region of MDR1 (Krishnamurthy et al., 2004), may be involved in the observed hypoxia-mediated induction of P-glycoprotein. Hypoxia-induced resistance to chemotherapy, which is associated with the overexpression of Mdr1 and MRPI genes, is believed to be mediated through HIF-1 activation, depending in part upon signaling via the stress-activated protein kinase c-Jun NH2-terminal kinase (Comerford et al., 2004). Recent reports also indicate that HIF-1α silencing increases chemosensitivity of non-small cell lung cancer and breast carcinoma to cisplatin, doxorubicin, and methotrexate (Song et al., 2006). Overall, these studies suggest that multiple signaling pathways may be implicated in hypoxia-induced drug resistance (Dixit et al., 2005).

Besides systemic hypoxia, there are other experimental pathological conditions that can modulate the expression of membrane transporters. For instance, acute renal and acute hepatic failure (Murakami et al., 2002), as well as local and systemic inflammatory reactions (Ho and Piquette-Miller, 2006), reduces the expression of P-glycoprotein.

### TABLE 1

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<th>Antibody</th>
<th>CYP1A1</th>
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Hepatocytes were incubated with serum from rabbits subjected to 48 h of hypoxia in the presence of an anti-cytokine antibody for 24 h. The + signs indicate the effect of immunoneutralization on the expression of the isoform; _ indicates no effect on the isoform.
The fact that in vivo acute moderate systemic hypoxia increases the expression of P-glycoprotein may have clinical implications concerning drug response in patients presenting acute hypoxia of respiratory or cardiac origin with or without nocturnal desaturation. In conclusion, acute moderate hypoxia diminishes the expression of CYP1A1, 1A2, 2B4, 2C5, and 2C16, an effect mediated by IFN-γ, IL-1β, and IL-2, but increases that of CYP3A6, an effect partially mediated by Epo. In addition, acute moderate hypoxia increases the expression of P-glycoprotein. The net result of these changes on the kinetics of drugs will depend upon the isoform involved in their biotransformation. We may predict that the area under the concentration-time curve will depend on whether these biotransformation. We may predict that the area under the concentration-time curve will depend on whether these drugs are or are not substrates of P-glycoprotein. On the other hand, by enhancing its expression, the pharmacologic response elicited by substrates of P-glycoprotein may be reduced.

Acknowledgment. We are grateful to Lucie Héroux for excellent technical assistance.

References

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