CYTOCHROME P450 DOWN-REGULATION BY SERUM FROM HUMANS WITH A VIRAL INFECTION AND FROM RABBITS WITH AN INFLAMMATORY REACTION

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ABSTRACT:

Serum from humans with an upper respiratory viral infection (HSURVI) and from rabbits with a turpentine-induced acute inflammatory reaction (RSTIAR) reduces the activity of hepatic cytochrome P450 (P450) following 4 h of incubation. The aim of the present study was to assess the effect of HSURVI and RSTIAR on P450 activity and expression following 24 h of incubation with hepatocytes from control (HCONT) and rabbits with a TIAR (HINFLA). RSTIAR incubated with HCONT for 24 h reduced P450 content and activity, and CYP3A6 by 45%, without changing CYP1A1 and 1A2; when incubated with HINFLA, RSTIAR decreased P450 content and activity without affecting CYP1A1 or 1A2. HSURVI incubated for 4 h with HCONT decreased P450 activity without affecting the amounts of CYP1A1, 1A2, or 3A6, although when incubated for 24 h, P450 activity and CYP3A6 amount decreased. HSURVI incubated with HINFLA for 4 h reduced P450 content and activity, and incubated for 24 h reduced activity, P450 content, and amount of CYP1A1 and 1A2 proteins. The present study demonstrates that 1) the effect of RSTIAR and HSURVI depends upon the susceptibility of the hepatocyte, i.e., HCONT or primed HINFLA; 2) P450 down-regulation is preceded by a decrease in P450 activity; 3) the nature of the inflammatory reaction determines the repercussions on P450 activity and expression; and 4) CYP3A6 is more vulnerable than CYP1A1 and 1A2 to the down-regulation provoked by an inflammatory challenge.

In human, viral and bacterial infections as well as influenza and BCG vaccinations decrease the clearance of exogenous substances such as theophylline and antipyrine, secondary to a decrease in activity of multiple isoforms of the cytochrome P450 (P450) (Morgan, 1997). As a consequence, bacterial and viral inflammatory reactions are cause of severe drug toxicity, essentially in pediatric and geriatric populations (Hendeles et al., 1977; Ziment, 1982; Koren and Greenwald, 1985). In animal models, infectious and noninfectious acute inflammatory reactions, such as those induced by endotoxin and turpentine, also diminish the rate of metabolism of xenobiotics (Parent et al., 1992; Morgan, 1997).

In response to viral infections, the concentration in blood of many cytokines is increased (Ramshaw et al., 1997). It has been assumed that in vivo cytokines are responsible for P450 depression because in vitro cytokines can depress multiple hepatic P450 isoforms and their mRNAs. For instance, INF-γ depresses CYP1A2, 2A6, 2B6, and 3A4; IL-6 depresses CYP1A1, 1A2, 2D, 3A4, and 4A1; and IL-1β down-regulates 1A2, 2C11, 2D6, 2E1, and 3A (Fukuda et al., 1992; Trautwein et al., 1992; Donato et al., 1997; Parmentier et al., 1997). Furthermore, it has been reported that IFN-α (Stanley et al., 1991), IL-1 (Peterson and Renton, 1986), and TNF-α (Paton and Renton, 1998) can also reduce the activity of several P450 isoforms.

In vitro, following an incubation period of 4 h with hepatocytes, serum from humans with an upper respiratory tract viral infection (HSURVI) and serum from rabbits with a turpentine-induced acute inflammatory reaction (RSTIAR) decreases the activity of CYP1A1 and 1A2 without affecting the amount of these proteins (El-Kadi et al., 1997). The mediators responsible for the decrease in P450 activity are INF-γ, IL-6, and IL-1β in HSURVI and IL-6 in RSTIAR (Bleau et al., 2000), demonstrating that in vivo, infectious, and noninfectious inflammatory reactions generate serum mediators, namely, cytokines, capable to reduce P450 activity.

Since in vivo, an inflammatory reaction depresses the expression of multiple isoforms of the P450, it was of interest to establish whether HSURVI and RSTIAR are capable to down-regulate the expression of selected P450 isoforms in hepatocytes. Specifically, the aims of the present study were 1) to assess the effect of RSTIAR and HSURVI on the activity and expression of hepatic CYP1A1, 1A2, and 3A6 following 24-h incubation periods; and 2) to compare the differences in effect between RSTIAR and HSURVI as a function of the source of the hepatocytes, i.e., hepatocytes harvested from control rabbits (HCONT) and hepatocytes from rabbits with a TIAR (HINFLA). The use of HINFLA was justified because the density of surface receptors to...
cytokines is greater than in \( H_{\text{CONT}} \) (Dinarello, 1994). Theophylline was used to assess the activity of some isoforms of the P450. In the rabbit, theophylline is primarily metabolized by CYP1A2 and CYP1A1, and to a minor degree by CYP3A6; CYP1A1, and CYP1A2 both contribute to the formation of theophylline three metabolites 3-methylxanthine (3 MX), 1-methyluric acid (1 MU), and 1,3-dimethyluric acid (1,3DMU) (Kurdi et al., 1999).

**Materials and Methods**

**Collection of Hepatocytes and Serum from Rabbits.** Male New Zealand White rabbits (1.8–2.2 kg) were obtained from Ferme Charles Rivers (St-Constant, Québec, Canada). A local inflammatory reaction was induced by the s.c. injection of 5 ml of turpentine (Recochem, Montréal, Québec) distributed into four distinct sites of the back of the rabbits. Forty-eight hours later, blood (20 ml) was withdrawn from the central artery of an ear of the rabbits, controls and with a TIAR; the rabbits were anesthetized, and hepatocytes were isolated. Blood samples were allowed to clot at room temperature for 2 h, centrifuged at 2500 rpm for 5 min, and the serum was decanted and stored frozen at \(-20^\circ\text{C}\) in 1-ml aliquots until use. The severity of the inflammatory reaction was assessed by measuring serum concentrations of seromucoids (Parent et al., 1992). All the experiments were conducted according to the Canadian Council on Animal Care guidelines for use of laboratory animals.

Hepatocytes were isolated according to the two-step liver perfusion method of Seglen (1976), with minor modifications (El-Kadi et al., 1997). Viability was over 90% as assessed by trypan blue exclusion, and cell concentration was adjusted to 4 \times 10^6/ml with Williams’ medium E supplemented with 10% calf serum. Aliquots of 2 ml of the hepatocytes, i.e., 8 \times 10^6 cells, in suspension were transferred into each well of 12-well plastic culture plates (Falcon; Becton Dickinson Labware, Rutherford, NJ) coated with type I rat tail collagen and incubated with serum from control rabbits (RSCONT) and RS TIAR for 4 and 24 h at 37°C in an atmosphere of 95% O\(_2\), 5% CO\(_2\). Cell culture was conducted under sterile conditions.

**Collection Serum from Humans.** Blood (10 ml) was withdrawn from humans (\( n = 8 \)) with an inflammatory reaction secondary to an upper respiratory viral infection, at the apex of clinical symptomatology, i.e., 24 h after the appearance of overt manifestations of an upper respiratory tract viral infection, such as rhinorrhea, sneezing, nasal congestion, sore throat, cough, and systemic signs of malaise, including fever, in absence of purulent secretions. Blood samples were allowed to clot at room temperature for 2 h, centrifuged at 2500 rpm for 5 min, and the serum was decanted and stored frozen at \(-20^\circ\text{C}\) in 1-ml aliquots until use.

Five of the volunteers with an upper respiratory viral infection, once the blood sample was withdrawn, took 300 mg of theophylline orally, and urine was collected for 24 h. At least 2 months later, in absence of any sign of infectious disease, a blood sample of 10 ml was withdrawn from the same five volunteers who subsequently received orally a second dose of 300 mg of theophylline, and urine was collected for 24 h. Serum from healthy volunteers (HCONT) and HSURVI were incubated with \( H_{\text{CONT}} \) and \( H_{\text{INFLA}} \) and its effect on total P450 content and theophylline metabolism was assessed. Theophylline and its metabolites were assayed in the 24-h urine collections.

**Cytochrome P450 Content and Activity.** The efficacy of the serum to reduce hepatic P450 content was tested by incubating 200 \( \mu \text{g} \) of serum with 2 \( \mu \text{g} \) of theophylline \( \text{mg} \) of protein in \( H_{\text{CONT}} \) and \( H_{\text{INFLA}} \) for 4 and 24 h of incubation (Kurdi et al., 1999). Theophylline was used to assess the activity of some isoforms of the P450. In the rabbit, theophylline is primarily metabolized by CYP1A2 and CYP1A1, and visualized with an alkaline phosphatase-conjugated secondary goat antibody using nitro blue tetrazolium as the substrate (Kruger, 1994). CYP3A6 protein was detected with a monoclonal anti-rabbit CYP3A1 and a horseradish peroxidase-conjugated secondary antibody; chemiluminescence was visualized by autoradiography (Thorpe et al., 1985). The intensities of the bands were measured with a software Un-Scan-Gel (Silk Scientific Inc., Orem, UT).

**Drugs and Chemicals.** The Percoll gradient, Williams’ medium E, calf serum, type I rat tail collagen, NaCl, KCl, KH\(_2\)PO\(_4\), HEPES, EGTA, glucose, theophylline, 3 MX, 1 MU, and 1,3DMU were purchased from Sigma (St. Louis, MO) and insulin was from Roche Molecular Biochemicals (Mannheim, Germany).

**Statistical Analysis.** All data are reported as means ± S.E. The comparison of the results from the various experimental groups and their corresponding controls was carried out using a one-way analysis of variance followed by Newman-Keuls post hoc test. The effect of RS TIAR, and HSURVI on the amount of CYP1A1, 1A2, and 3A6 (densitometry values) was compared with the effect of RSCONT and HCONT by using a paired \( t \) test. The differences were considered significant when \( p < 0.05 \).

**Results**

*Effect of a TIAR on P450 Content, Activity, and Amount of P450 Approteins.* Total P450 content in \( H_{\text{CONT}} \) was 0.284 ± 0.014 nmol/mg of protein, a value that was reduced to 0.152 ± 0.012 nmol/mg of protein in \( H_{\text{INFLA}} \) (\( p < 0.05 \)). Compared with \( H_{\text{CONT}} \), when theophylline was incubated for 4 h with \( H_{\text{INFLA}} \), the formation of 1,3DMU, 3 MX, and 1 MU was reduced by 43, 85, and 43%, respectively (\( p < 0.005 \)), reflecting essentially a decrease in CYP1A1 and 1A2 activity (Table 1). After 24 h of incubation, the formation of 1,3DMU, 3 MX, and 1 MU was reduced by a percentage similar to that reported after 4 h incubation (data not shown). Compared with \( H_{\text{CONT}} \), the TIAR diminished the amount of CYP1A1 by 47%, i.e., densitometry values were 391,687 ± 41,243 in \( H_{\text{CONT}} \) and 207,492 ± 30,617 in \( H_{\text{INFLA}} \) (\( n = 3 \), \( p < 0.05 \)). CYP1A2 densitometry values were 309,098 ± 57,205 in \( H_{\text{CONT}} \) and 205,618 ± 35,456 in \( H_{\text{INFLA}} \) (\( n = 3 \), \( p > 0.05 \)). The amount of CYP3A6 apoprotein was reduced to an undetectable level (Fig. 1).

*Effect of RSCONT and RS TIAR on P450 of HCONT Following 4 and 24 h of Incubation.* Incubation of \( H_{\text{CONT}} \) with \( H_{\text{CONT}} \) did not modify P450 content and ability to biotransform theophylline (\( n = 7 \)) (Table 1). Compared with \( H_{\text{CONT}} \), RS TIAR did not modify P450 content, and did not reduce the formation of 3 MX, 1 MU, and 1,3DMU (\( p > 0.05 \)) (Table 1). Neither RS CONT nor RS TIAR affected the amount of CYP1A1, 1A2, and 3A6 apoproteins in \( H_{\text{CONT}} \) (data not shown).

After a 24-h period of incubation with \( H_{\text{CONT}} \), compared with RS CONT, RS TIAR did not modify P450 content, but reduced the formation of 1 MU and 1,3DMU by 27 and 28%, respectively (\( p < 0.05 \), \( n = 8 \)) (Table 1). Following 24 h of incubation with RS TIAR, the amount of CYP1A1 and 1A2 remained unchanged, i.e., densitometry values for CYP1A1 were 283,835 ± 17,818 incubated with RS CONT and 277,457 ± 19,912 with RS TIAR, and for CYP1A2 densitometry values were 305,303 ± 60,486 with RS CONT and 327,771 ± 79,745 with RS TIAR. On the other hand, by reference to RS CONT (densitometry value 492,585 ± 98,749), CYP3A6 decreased by 45% when \( H_{\text{CONT}} \) was incubated with RS TIAR (284,232 ± 68,265) (\( n = 4 \), \( p < 0.05 \)) (Fig. 2).

*Effect of RSCONT and RS TIAR on P450 of HINFLA Following 4 and 24 h of Incubation.* Following 4 h of incubation, and compared with RS CONT, RS TIAR did not reduce P450 content in \( H_{\text{INFLA}} \), but reduced the formation of 1 MU and 1,3DMU by 34 and 26%, respectively (\( p < 0.005 \), \( n = 7 \)) (Table 2). On the other hand, the
amount of CYP1A1 and 1A2 proteins was not affected in \( H_{\text{INFLA}} \) (data not shown).

Following 24 h of incubation, \( RS_{\text{TAR}} \) decreased P450 content by 36% (Table 2), and reduced the rate of formation of 3 MX, 1 MU, and 1,3DMU by 36, 49, and 36%, respectively (\( p < 0.005, n = 7 \)) (Table 2). Following 24 h of incubation with \( RS_{\text{TAR}} \), the amount of CYP1A1 and 1A2 remained unchanged, i.e., densitometry values for CYP1A1 were 204,011 ± 15,778 incubated with \( HS_{\text{CONT}} \) and 201,612 ± 19,225 with \( RS_{\text{TAR}} \), and for CYP1A2 densitometry values were 218,437 ± 27,978 with \( HS_{\text{CONT}} \) and 228,921 ± 40,541 with \( RS_{\text{TAR}} \) (\( n = 4 \)). Since in \( HS_{\text{CONT}} \), the amount of 3A6 apoprotein was not measurable, it was not possible to assess the effect of \( RS_{\text{TAR}} \) (data not shown). The percentage of reduction for each metabolite is greater at 24 h than at 4 h (\( p < 0.05 \)).

**TABLE 1**

Effect of serum from rabbits with an inflammatory reaction on P450 content and ability to biotransform theophylline incubated for 4 and 24 h with hepatocytes from control rabbits

Hepatocytes from control rabbits (\( H_{\text{CONT}} \)) (\( n = 8 \)) and hepatocytes from rabbits with a turpentine-induced inflammatory reaction (\( H_{\text{INFLA}} \)) (\( n = 8 \)) were incubated with NaCl 0.9% for 4 h or with serum from control rabbits (\( RS_{\text{CONT}} \)) and serum from rabbits with an inflammatory reaction (\( RS_{\text{TAR}} \)) for 4 and 24 h.

<table>
<thead>
<tr>
<th>4-h Incubation</th>
<th>( H_{\text{CONT}} + \text{NaCl} )</th>
<th>( H_{\text{INFLA}} + \text{NaCl} )</th>
<th>( H_{\text{CONT}} + \text{RS}_{\text{CONT}} )</th>
<th>( H_{\text{INFLA}} + \text{RS}_{\text{TAR}} )</th>
<th>( H_{\text{CONT}} + \text{RS}_{\text{TAR}} )</th>
<th>( H_{\text{INFLA}} + \text{RS}_{\text{TAR}} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytochrome P450</td>
<td>204,011 ± 15,778</td>
<td>201,612 ± 19,225</td>
<td>197,137 ± 27,978</td>
<td>214,747 ± 40,541</td>
<td>204,011 ± 15,778</td>
<td>201,612 ± 19,225</td>
</tr>
<tr>
<td>3 MX</td>
<td>0.084 ± 0.012</td>
<td>0.091 ± 0.013</td>
<td>0.096 ± 0.024</td>
<td>0.130 ± 0.0232</td>
<td>0.084 ± 0.012</td>
<td>0.091 ± 0.013</td>
</tr>
<tr>
<td>1 MU</td>
<td>0.052 ± 0.005</td>
<td>0.052 ± 0.005b</td>
<td>0.073 ± 0.014</td>
<td>0.117 ± 0.033</td>
<td>0.052 ± 0.005</td>
<td>0.052 ± 0.005b</td>
</tr>
<tr>
<td>1,3DMU</td>
<td>1.404 ± 0.186</td>
<td>1.404 ± 0.186</td>
<td>1.390 ± 0.400</td>
<td>1.170 ± 0.333</td>
<td>1.404 ± 0.186</td>
<td>1.404 ± 0.186</td>
</tr>
</tbody>
</table>

\( a \) \( p < 0.05 \) \( RS_{\text{TAR}} \) compared with \( RS_{\text{CONT}} \).

\( b \) \( p < 0.05 \) \( H_{\text{INFLA}} \) compared with \( H_{\text{CONT}} \).

*Fig. 1.* Effect of a turpentine-induced inflammatory reaction on the amount of CYP1A1, 1A2, and 3A6 apoproteins in hepatocytes from control (\( H_{\text{CONT}} \)) and rabbits with a turpentine-induced inflammatory reaction (\( H_{\text{INFLA}} \)) 48 h after the injection of turpentine.

Numbers indicate densitometry values.

*Fig. 2.* Effect of serum from control (\( RS_{\text{CONT}} \)) and rabbits with a turpentine-induced inflammatory reaction (\( RS_{\text{INFLA}} \)) on the amount of CYP1A1, 1A2, and 3A6 apoproteins in hepatocytes from control rabbits (\( H_{\text{CONT}} \)) and rabbits with a turpentine-induced inflammatory reaction (\( H_{\text{INFLA}} \)) after a 24-h period of incubation.

Numbers indicate densitometry values.
Effect of serum from rabbits with an inflammatory reaction on P450 content and ability to biotransform theophylline incubated for 4 and 24 h with hepatocytes from rabbits with a turpentine-induced inflammatory reaction

Hepatocytes from rabbits with a turpentine-induced inflammatory reaction (H_{INFLA}) (n = 7) were incubated with serum from control rabbits (RS_{CONT}), and serum from rabbits with an inflammatory reaction (RS_{TIAR}) (n = 7) for 4 and 24 h. Data are means ± S.E.

<table>
<thead>
<tr>
<th>Cytochrome P450</th>
<th>3 MX</th>
<th>1 MU</th>
<th>1,3DMU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytomembrane P450</td>
<td>0.164 ± 0.021</td>
<td>0.011 ± 0.003</td>
<td>0.053 ± 0.005</td>
</tr>
<tr>
<td>Liver P450</td>
<td>0.125 ± 0.021</td>
<td>0.008 ± 0.002</td>
<td>0.035 ± 0.003</td>
</tr>
</tbody>
</table>

*p < 0.05 compared with H_{INFLA} + RS {CONT}.

Discussion

The present study demonstrates that RS_{TIAR} does not affect P450 activity or bile output when incubated for 4 h with H_{CONT}, however, it decreases P450 activity in H_{INFLA}. When RS_{TIAR} is incubated with H_{CONT} for 24 h, P450 activity is reduced, as well as the amount of CYP3A6 protein. Incubation of RS_{TIAR} with H_{INFLA} for 24 h decreases total P450 content and activity, without affecting CYP1A1 or 1A2. Keeping in mind that theophylline is primarily biotransformed by CYP1A2 (Kurdi et al., 1999), these results show that RS_{TIAR} elicits a dual effect on P450: it reduces the activity of CYP1A1 and 1A2, and down-regulates CYP3A6. RS_{TIAR} effects on P450 depend upon the length of incubation, and the state of the hepatocytes, i.e., control or primed.

The RS_{TIAR}-induced decrease in P450 activity could be a general phenomenon since the down-regulation of P450 isoforms by lipopolysaccharides is preceded by their inactivation (Sewer et al., 1998). Nitric oxide may have a pivotal role in P450 inactivation by both, the binding of carbon monoxide used for the spectrophotometric determination. In parallel, nitric oxide binding to Fe^{2+} and as a consequence, it reduces the theophylline biotransformation.

The results show that RS_{TIAR} affects theophylline metabolism in a biphasic manner, with an initial increase followed by a decrease in theophylline biotransformation. This biphasic effect suggests that RS_{TIAR} alters the balance between pro-inflammatory and anti-inflammatory mediators.

Table 3: Effect of serum from control subjects (HS_{CONT}) and volunteers with an upper respiratory tract viral infection reaction (HS_{URVI}) on the ability of hepatocytes from control rabbits (H_{CONT}) and rabbits with a turpentine-induced inflammatory reaction (H_{INFLA}) to biotransform theophylline after a 4- and 24-h period of incubation

Results are mean ± S.E.

<table>
<thead>
<tr>
<th></th>
<th>3 MX</th>
<th>1 MU</th>
<th>1,3DMU</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-h Incubation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H_{CONT} + H_{CONT}</td>
<td>0.075 ± 0.006</td>
<td>0.082 ± 0.007</td>
<td>1.350 ± 0.131</td>
</tr>
<tr>
<td>H_{CONT} + HS_{URVI}</td>
<td>0.058 ± 0.007</td>
<td>0.058 ± 0.008</td>
<td>0.918 ± 0.144</td>
</tr>
<tr>
<td>H_{INFLA} + HS_{URVI}</td>
<td>0.015 ± 0.004</td>
<td>0.051 ± 0.008</td>
<td>0.881 ± 0.106</td>
</tr>
<tr>
<td>24-h Incubation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H_{CONT} + H_{CONT}</td>
<td>0.177 ± 0.016</td>
<td>0.960 ± 0.010</td>
<td>3.550 ± 0.401</td>
</tr>
<tr>
<td>H_{CONT} + HS_{URVI}</td>
<td>0.130 ± 0.013</td>
<td>0.060 ± 0.008</td>
<td>2.260 ± 0.432</td>
</tr>
<tr>
<td>H_{INFLA} + HS_{URVI}</td>
<td>0.058 ± 0.003</td>
<td>0.076 ± 0.009</td>
<td>2.100 ± 0.211</td>
</tr>
<tr>
<td>H_{INFLA} + HS_{URVI}</td>
<td>0.035 ± 0.005</td>
<td>0.034 ± 0.009</td>
<td>1.290 ± 0.201</td>
</tr>
</tbody>
</table>

*a,b p < 0.05 compared with H_{CONT} + HS {CONT} and H_{INFLA} + HS {CONT}, respectively.

Fig. 3. Amount of 3 MX, 1 MU, and 1,3DMU recovered in a 24-h urinary collection from five volunteers with and without an upper respiratory viral infection after oral intake of 300 mg of theophylline.
P450 INACTIVATION AND DEPRESSION BY INFLAMMATION


Calleja C, Eeckhoutte C, Dacasto M, Larrieu G, Dupuy J, Pineau T and Galtier P (1997) Differential effects of serum from volunteers with (HSURVI) and without (HS CONT) an upper respiratory tract viral infection reaction on the amount of CYP1A1, 1A2, and 3A6 apoproteins in hepatocytes from control rabbits (H CONT) and rabbits with a turpentine-induced inflammatory reaction (H INFLA), after a 24-h period of incubation.

Numbers indicate densitometry values.

In conclusion, the present study demonstrates 1) that the effect of RS_TIAR and HS URVI depends upon the susceptibility of the hepatocyte (H CONT or primed H INFLA), emphasizing the importance of the model for an accurate interpretation of the results; 2) that P450 down-regulation is preceded by a decrease in P450 activity; 3) that the differences between RS_TIAR and HS URVI are primarily dependent upon the mediators contained in the serum, indicating that the nature of the inflammatory reaction is of importance to determine the repercussions on P450 activity and expression; and 4) the present results confirm that CYP3A6 is more vulnerable than CYP1A1 and 1A2 to the down-regulation provoked by an inflammatory challenge, a fact that may have practical consequences when it is taken into account the relevance of this isoform in drug metabolism.

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References


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