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Cytochrome P450 inactivation by serum from humans with a viral infection and serum from rabbits with a turpentine-induced inflammation: the role of cytokines

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1 Serum from humans with an acute upper respiratory viral infection and from rabbits with turpentine-induced inflammation reduce the catalytic activity of hepatic cytochrome P450 (P450). The aim of this study was to identify the serum mediators responsible for the decrease in P450 activity.

2 Rabbit and human sera were fractionated by size exclusion chromatography and the fractions tested for their ability to reduce the activity and amount of P450 after 4 h of incubation with hepatocytes from turpentine-treated rabbits (H_{INF}). Rabbit and human sera decreased P450 activity by around 40% without any change in the amount of CYP1A1 and 1A2 apoproteins.

3 In rabbit serum, the fraction containing proteins of M_r 23–15 kDa decreased P450 content by 41%, but did not alter the amount of the apoproteins. Anti-IL-6 antibody added to the M_r 23–15 kDa fraction restored P450 content to 97% of control values, while anti-IL-1β, TNF-α and IFN-γ antibodies had no effect. Supporting the role of IL-6, incubation of H_{INF} in the presence of IL-6 for 4 h reduced P450 content by 40%.

4 In human serum, the fraction containing proteins of M_r >95 kDa lowered P450 content by 43% without modifying the amounts of CYP1A1/2. Neutralization experiments showed that IFN-γ, IL-6, and IL-1β contributed to the decrease in P450 content.

5 In conclusion, the present results demonstrate that IL-6, and IFN-γ, IL-6 and IL-1β are the serum mediators released *in vivo* by a turpentine-induced inflammatory reaction in the rabbit and an upper respiratory viral infection in humans, respectively, inactivating hepatic P450.
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Abbreviations: H_{CONT}, hepatocytes from control rabbits; H_{INF}, hepatocytes of rabbits with a turpentine-induced inflammatory reaction; HPLC, high-performance liquid chromatography; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interleukin-6; M_r, molecular mass, relative; P450, cytochrome P450; SDS, sodium dodecyl sulphate; TNF-α, tumour necrosis factor-α; WME, William's medium E

Introduction

In humans, inflammation and infection modify the function of the liver, i.e. there is an increase in the synthesis and secretion of acute phase proteins (Schreiber *et al*., 1982), and a decrease in the synthesis of other proteins, such as albumin and enzymes of the cytochrome P450 (P450) (Morgan, 1997). As a consequence, the rate of drug metabolism may be reduced in the presence of an inflammatory reaction or an infection (Kobusch *et al*., 1986), a situation that may cause drug toxicity (Chang *et al*., 1978). In animal models, non-infectious inflammatory reactions, such as those induced by turpentine, also down-regulate several hepatic P450 isoforms (Parent *et al*., 1992; Morgan, 1989). We have reported that serum from humans with an acute upper respiratory tract viral infection and from rabbits with a turpentine-induced acute inflammatory reaction contain mediators that reduce the catalytic activity of the P450 of cultured hepatocytes, effect that is detected within 4 h of incubation (El-Kadi *et al*., 1997).

Numerous reports have proposed that pro-inflammatory cytokines and mediators of the hepatic acute-phase response, notably interleukin-1β (IL-1β), interleukin-6 (IL-6), interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α), may be major contributors to the decline of hepatic P450 content (Abdel-Razzak *et al*., 1993; Chen *et al*., 1995; Clark *et al*., 1995). The ability of these cytokines to depress hepatic P450 has been documented *in vivo* after their administration to animal models or *in vitro* following their incubation with hepatocytes; these cytokines appear to act mainly on P450 gene expression at a transcription level (Morgan, 1997).

Despite the fact that viral infections and a turpentine-induced acute inflammatory reaction enhance plasma levels of many cytokines (Neuzil & Graham, 1996; Yamashita *et al*., 1994), there is no direct *in vivo* evidence supporting that under these two conditions, cytokines are the serum mediators affecting the expression of P450 isoforms. Furthermore, there is no evidence that the cytokines contained in the serum from humans or rabbits with an inflammatory reaction can rapidly inactivate hepatic P450.

The aims of this study were to assess how serum mediators in patients with an upper respiratory tract viral infection and in rabbits with a turpentine-induced acute inflammatory reaction reduce P450 content and activity, and to document whether these serum mediators are cytokines, more specifically IL-1β, IL-6, IFN-γ and TNF-α. For this purpose, P450 content and amount of CYP1A1/2 and 3A6 were assessed after 4 h of

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incubation of the sera with hepatocytes. In addition, mediators in sera were isolated by size exclusion high-performance liquid chromatography and cytokines identified by direct neutralization with antibodies.

Methods

Hepatocyte isolation and culture

Male New Zealand rabbits (2–2.3 kg) ($n=13$) from the Ferme Cunicole (St. Valérien, QC, Canada) were housed in separate cages for at least 7 days before use. A local inflammatory reaction was induced by the s.c. injection of 5 ml of turpentine at two distinct sites of the back of the rabbits. The severity of the inflammatory reaction was assessed by measuring the 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 48 h after the injection of turpentine by means of the two step liver perfusion method of Seglen (1976), with minor modifications (El-Kadi *et al*., 1997). Rabbits were anaesthetized with sodium pentobarbital 30 mg kg^{-1}, and after a laparotomy, the portal and inferior cava veins were cannulated. The liver was first perfused *in situ via* the portal vein with a washing solution containing (mM): NaCl 115, KCl 5, KH_2PO_4 1, HEPES 25, EGTA 0.5, glucose 5.5 and 56.8 mg ml^{-1} heparin, followed by perfusion with a solution of 0.013% collagenase, $CaCl_2$ (1 mM) and trypsin inhibitor (0.25 mM). Living cells were isolated on a 40% Percoll gradient. Viability was >90% as assessed by trypan blue exclusion, and the cell concentration was adjusted to 4×10^6 ml^{-1} with William's medium E (WME) supplemented with 10% calf serum and 1 mM insulin. Aliquots of 2 ml of the hepatocytes in suspension were transferred into 12-well plastic culture plates (Falcon, Becton Dickinson Labware, Rutherford, NJ, U.S.A.) coated with type I rat tail collagen and incubated for 4 h at 37°C in an atmosphere of 95% O_2/5% CO_2.

Rabbit and human serum preparation

A blood sample (10 ml) was withdrawn from the rabbits 48 h after the s.c. injection of turpentine in a sterile Vacutainer Brand SST (Becton Dickinson, Mississauga, ON, Canada). Human blood was obtained from volunteers ($n=6$) at the apex of the clinical symptomatology of a common cold, usually 24 h after overt manifestations of a viral infection of the upper respiratory tract, such as rinorrhea, sneezing, nasal congestion, sore throat, cough and systemic signs of malaise in absence of purulent secretions. Blood samples were allowed to clot at room temperature for 2 h, and thereafter were centrifuged at 2500 r.p.m. for 5 min, and the serum was decanted and stored frozen at −20°C in 1 ml aliquots until use.

Fractionation of serum proteins

Serum proteins were separated by size exclusion high-performance liquid chromatography (HPLC) on a Superose 12 HR column from Pharmacia Biotech (Baie d'Urfé, QC, Canada). Column pressure was maintained between 8 to 10 bar, flow rate was set at 0.3 ml min^{-1} with a LKB 2150 HPLC pump (Bromma, Sweden) and absorbance was measured at 280 nm with a Waters 490E spectrophotometric detector (Millipore, Milford, MA, U.S.A.). The eluent buffer contained (mM): NaCl 115, KCl 5, KH_2PO_4 1, HEPES 1, EGTA 25 and glucose 5.5; this solution was adjusted to pH 7.4 and filtered through a 0.22 μm membrane. To calculate the M_r of the serum proteins contained in each HPLC fraction, a calibration curve was established by injecting 300 μl of buffer containing a mixture of six standard proteins (100 μg ml^{-1}): L-glutamic dehydrogenase (55.6 kDa), aldolase (39.2 kDa), triosephosphate isomerase (26.6 kDa), trypsin inhibitor (26.6 kDa), cytochrome c (12.5 kDa) and aprotinin (6.5 kDa). Serum aliquots of 300 μl were injected into the column and fractions of 1.2 ml were collected with a fraction collector (LKB 2211 Superrac). Since the column could not separate proteins in the high M_r region, the proteins contained in the first fraction were designated as M_r >95 kDa.

In order to increase the sensitivity, collected fractions were concentrated on Microsep 3K membranes (Pall Filtron, Northborough, MA, U.S.A.) which retain proteins of more than 3 kDa. In the first set of experiments, the whole fraction (1.2 ml) was transferred into the sample reservoir and centrifuged at $75{,}000\times g$ for approximately 30 min, until 600 μl remained on top of the membrane. The retentate was repeatedly pulled in and out of a micropipette to remove the proteins adsorbed onto the membrane. This provided the equivalent of a serum diluted 1:2. The same procedure was used to obtain more concentrated fractions, i.e. 3 ml of the fraction were added to the sample reservoir, and the volume was reduced to 600 μl to concentrate serum fractions 1.25 times.

Determination of cytochrome P450 content

The efficacy of the serum and HPLC fractions to reduce hepatic P450 content was tested by incubating for 4 h 200 μl of serum or the HPLC fractions with hepatocytes of rabbits with a turpentine-induced inflammatory reaction (El-Kadi *et al*., 1997). Hepatic P450 content was measured spectrophotometrically as described by Omaru & Sato (1964). Amount of proteins in hepatocytes was measured by the method of Lowry *et al*. (1951).

The effect of the serum from rabbits ($n=6$) and humans ($n=6$) on the activity of CYP1A1 and 1A2 was determined by measuring the ability of P450 to metabolize theophylline and generate 3-methylxanthine (3MX), 1-methyluric acid (1MU) and 1,3-dimethyluric acid (1,3DMU) (Sarkar & Jackson, 1994; Kurdi *et al*., 1999). Theophylline was dissolved in serum-free WME, and 100 μl were added to each well containing the hepatocytes to attain a final concentration of 176 μM. At time zero, 350 μl of the supernatant were collected from each well (control sample), and followed 4 h of incubation, the remaining incubation medium was collected and frozen at −20°C until theophylline, 3MX, 1MU, and 1,3DMU were assayed by high performance liquid chromatography (du Souich *et al*., 1989).

Western blot analysis

Proteins were separated by SDS-polyacrylamide gel electrophoresis (7.5% polyacrylamide) under non-reducing conditions (Smith, 1994). Separated proteins were electrophoretically transferred to a nitro-cellulose membrane using a semi-dry transfer process (Bio-Rad, Hercules, CA, U.S.A.). CYP1A1 and 1A2 proteins were detected with a specific polyclonal anti-rabbit CYP1A1, and visualized with an alkaline phosphatase (AP) conjugated secondary goat antibody using nitro blue tetrazolium as the substrate (Kruger,

1994). CYP3A6 protein was detected with a monoclonal anti-rat CYP3A1 and a horseradish peroxidase-conjugated secondary antibody, and chemiluminescence was visualized by autoradiography (Thorpe *et al*., 1985). The intensities of the bands were measured with a software Alphaimager version 3.24.

Immuno-neutralization of cytokines

One polyclonal and four monoclonal antibodies against cytokines were used in an attempt to neutralize the decrease in P450 content induced by the serum and HPLC fractions. To immuno-neutralize rabbit serum mediators, the following antibodies were used: a goat anti-rabbit interleukin-1β (anti-rbIL-1β antibody), monoclonal antibodies against human interleukin-6 (anti-IL-6) and human interferon-γ (anti-IFNγ), and a sheep anti-human tumour necrosis factor-α (anti-TNF-α). The antibodies against human cytokines were used to neutralize the homologous rabbit cytokines because of the known inter-species reactivity of these antibodies (Herbertson *et al*., 1995; Huang *et al*., 1997; Muscettola *et al*., 1995). The same antibodies against human cytokines as well as a monoclonal anti-human interleukin-1β (anti-hIL-1β antibody) were used to neutralize the mediators in human serum fractions. These antibodies were used because no significant cross-reactivity or interference is observed among them or with other cytokines as indicated by the supplier. An irrelevant monoclonal antibody (IgG to *Pseudomonas aeruginosa*) served as control. Aliquots of 2 μg of each antibody were added individually to 200 μl of the HPLC fractions showing a P450 inhibitory activity, and incubated at 37°C for 1 h. The combination of the four antibodies was incubated only with the serum from humans and from rabbits with an inflammatory reaction. Antibody-treated sera or HPLC fractions were added to the hepatocytes at the beginning of the 4 h-incubation period.

Effect of IL-6 on P450 content

To confirm that IL-6 has the capacity to inactivate hepatic P450, human recombinant IL-6 (10 ng ml^{-1}) was incubated for 4 h with hepatocytes from rabbits with a turpentine-induced inflammatory reaction ($n=5$). The effect of IL-6 was assessed by measuring P450 content (Omura & Sato, 1964). The concentration of IL-6 used was similar to that assayed in the plasma of rabbits with a turpentine-induced inflammatory reaction (Turnbull *et al*., 1999).

Drugs and chemicals

The Percoll gradient, William's medium E, calf serum, type I rat tail collagen, NaCl, KCl, KH_2PO_4, HEPES, EGTA, glucose, theophylline, 3MX, 1MU and 1,3DMU were purchased from Sigma Chemicals (Sigma, St. Louis, MO, U.S.A.), insulin from Boehringer Mannheim Biochemica (Mannheim, Germany), and L-glutamic dehydrogenase, aldolase, triosephosphate isomerase, trypsin inhibitor, cytochrome c and aprotinin from Pharmacia Biotech (Baie d'Urfé, QC, Canada). Human recombinant IL-6 was obtained from Sandoz Canada Inc. (Markham, ON, Canada), the polyclonal anti-rabbit CYP1A1 and the monoclonal anti-rat CYP3A1 from Oxford Biochemical Research (Oxford, MI, U.S.A.), the goat anti-rabbit IL-1β from Cedar Lane (Hornby, ON, Canada), the monoclonal antibodies against human IL-6 and human IFN-γ from R&D Systems (Minneapolis, MN, U.S.A.). The sheep anti-human TNF-α antibody was graciously given by Protherics Inc. (formerly Therapeutic Antibodies, Nashville, TN, U.S.A.). Finally, the monoclonal antibody to *Pseudomonas aeruginosa* was kindly given by Dr J. Lagacé (Université de Montréal).

Statistical analysis

All data are reported as means ± s.e.mean. Comparisons between treatment groups were carried out using one-way ANOVA followed by Newman-Keuls *post hoc* test. The differences were considered statistical significantly with a probability $P<0.05$.

Results

Effect of the inflammatory reaction on P450 content and activity and amount of P450 isoforms

In the group of rabbits ($n=6$) with a turpentine-induced inflammatory reaction, mean seromucoid concentration was 83.5 ± 2.5 mg dl^{-1} compared with 21.9 ± 2.5 mg dl^{-1} in control rabbits ($P<0.05$). In hepatocytes of control rabbits, P450 content was 0.47 ± 0.09 nmol mg^{-1} of protein. In H_{INF}, P450 content was reduced to 0.20 ± 0.03 nmol mg^{-1} of protein ($P<0.05$). Compared with control rabbits, the turpentine induced inflammatory reaction diminished the amount of CYP1A1 and CYP1A2 apoproteins by 39 and 44%, respectively, and the amount of CYP3A6 apoprotein to almost undetectable levels (Figure 1). The reduction in CYP1A1 and 1A2 is reflected by a decrease in the amount of theophylline metabolites produced (Table 1).

Effect of serum from rabbits with a turpentine-induced inflammatory reaction and HPLC fractions on P450 content and activity

Incubation of hepatocytes from control rabbits with serum from rabbits with an inflammatory reaction or with the HPLC fractions did not modify P450 content. In contrast, following 4 h of incubation of H_{INF} with serum from rabbits with an inflammatory reaction ($n=6$), P450 content decreased by 30% ($P<0.05$) (Figure 2). In parallel, the amount of theophylline metabolites formed by H_{INF} decreased in presence of serum from rabbits with an inflammatory reaction ($n=6$) (Table 1).

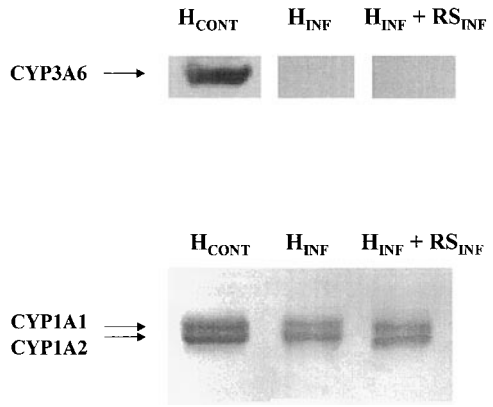

Figure 1 Effect of a turpentine-induced inflammatory reaction on the relative amounts of hepatic CYP1A1, 1A2 and 3A6 immunoreactive proteins in rabbits, 48 h after the s.c. injection of turpentine. H_{CONT} and H_{INF} are hepatocytes of control rabbits and rabbits with an inflammatory reaction, respectively.

Table 1 Effect of serum from rabbits and humans on the ability of the hepatocytes to biotransform theophylline

	*3MX** (μg ml^{-1})	*1MU* (μg ml^{-1})	*1,3DMU* (μg ml^{-1})
$H_{CONT}+RS_{CONT}$	0.093±0.026†	0.079±0.034	1.498±0.419
$H_{CONT}+HS_{CONT}$	0.085±0.008	0.086±0.008	1.350±0.255
$H_{INF}+RS_{CONT}$	0.011±0.003‡	0.053±0.006‡	0.710±0.057‡
$H_{INF}+RS_{INF}$	0.008±0.002	0.027±0.006#	0.527±0.049#
$H_{INF}+HS_{CONT}$	0.015+0.004+	0.052+0.008+	0.881+0.106+
$H_{INF}+HS_{INF}$	0.009+0.004	0.030+0.004§	0.598+0.068§

Hepatocytes from control rabbits (H_{CONT}) and hepatocytes from rabbits with a turpentine -induced inflammatory reaction (H_{INF}) were incubated for 4 h with serum from control rabbits (RS_{CONT}) ($n=6$), serum from rabbits with a turpentine-induced inflammatory reaction (RS_{INF}) ($n=6$), serum from healthy volunteers (HS_{CONT}) ($n=6$), and serum from humans with a respiratory tract viral infection (HS_{INF}) ($n=6$) to assess the effect on the formation of theophylline metabolites. *3 MX, 1MU and 1,3DMU are 3-methylxanthine, 1-methyluric acid and 1,3-dimethyluric acid, respectively. †Data are mean±s.e.mean. ‡$P<0.05$ compared with $H_{CONT}+RS_{CONT}$. #$P<0.05$ compared with $H_{INF}+RS_{CONT}$. +$P<0.05$ compared with $H_{CONT}+HS_{CONT}$. §$P<0.05$ compared with $H_{INF}+HS_{INF}$.

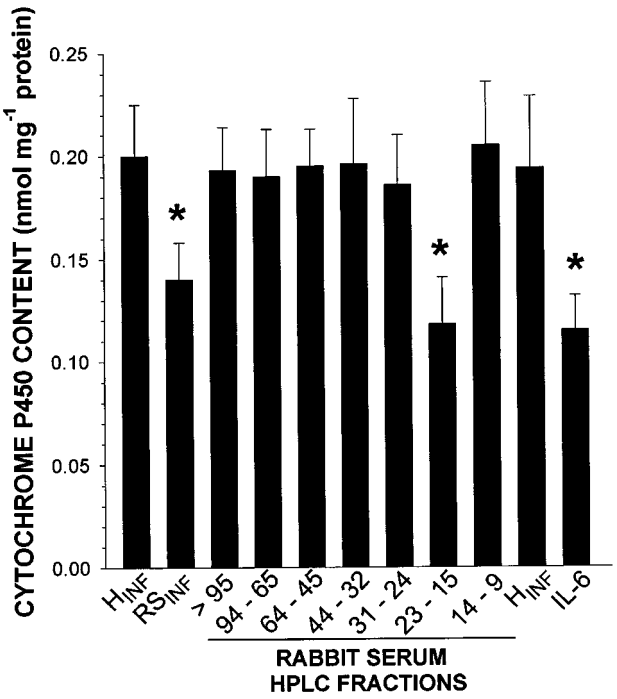

Figure 2 Effect of concentrated ×1.25 HPLC fractions of serum from rabbits with a turpentine-induced inflammatory reaction (RS_{INF}) ($n=6$) on cytochrome P450 content in hepatocytes of rabbits with a turpentine-induced inflammatory reaction (H_{INF}) following 4 h incubation. HPLC fractions are designated by the M_r of proteins contained in each fraction. *$P<0.05$ compared with P450 content in H_{INF}.

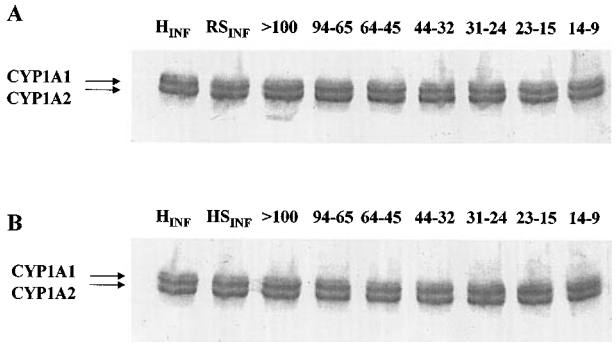

Figure 3 Effect of serum and concentrated HPLC fractions on the amount of CYP1A1 and 1A2 apoproteins of hepatocytes from a representative rabbit with a turpentine-induced inflammatory reaction (H_{INF}). H_{INF} were incubated for 4 h in the presence of serum and concentrated (×1.25) HPLC fractions from turpentine-treated rabbit (RS_{INF}) (A), and of serum from one representative human with an upper respiratory tract viral infection and HPLC fractions (HS_{INF}) (B). HPLC fractions are designated by the M_r of proteins contained in each fraction.

Because of the inherent dilution effect of HPLC, the serum components eluted in each fraction were diluted approximately 1:4 compared with their original serum concentration. Incubation of H_{INF} with the diluted HPLC fractions did not affect P450 content, although the fraction corresponding to the M_r of 23–15 kDa induced a decrease of 21% in P450 content, reduction that did not reach statistical significance ($P>0.05$). When the HPLC fractions were concentrated to obtain the equivalent of a serum diluted 1:2, the fraction including proteins with a M_r of 23–15 kDa decreased P450 content by 39%, even though this diminution did not reach statistical significance. The incubation of more concentrated fractions, i.e. equivalent to 1.25 times the serum ($n=6$), demonstrated that the fraction including proteins with a M_r of 23–15 kDa diminished P450 content by 41% ($P<0.05$) (Figure 2).

The addition of serum from rabbits with an inflammatory reaction or the corresponding HPLC concentrated ×1.25 fractions ($n=3$) to H_{INF} did not decrease the amount of CYP1A1 and CYP1A2 apoproteins (Figure 3A). It was not possible to assess the effect of serum on CYP3A6 apoprotein since the baseline amount was barely detectable.

Effect of serum from humans with a viral infection and HPLC fractions on P450 content and activity

Human serum from individuals with an upper respiratory tract viral infection reduced the formation of theophylline metabolites in H_{INF} by around 40% ($P<0.05$) (Table 1). In diluted HPLC fractions, the ability to decrease P450 content was present in the fractions containing proteins of M_r >95 kDa and 23–15 kDa. The HPLC concentrated fraction (×1.25) ($n=6$), including proteins with a M_r >95 kDa, reduced P450 content in H_{INF} by 43% ($P<0.05$). On the other hand, the fraction containing proteins with a M_r of 23–15 kDa tended to decrease P450 content, but this effect did not reach statistical significance ($P>0.05$) (Figure 4). The addition of serum from human with a viral infection or the concentrated ×1.25 M_r >95 kDa fraction to H_{INF} did not decrease the amount of CYP1A1 and CYP1A2 apoproteins (Figure 3B).

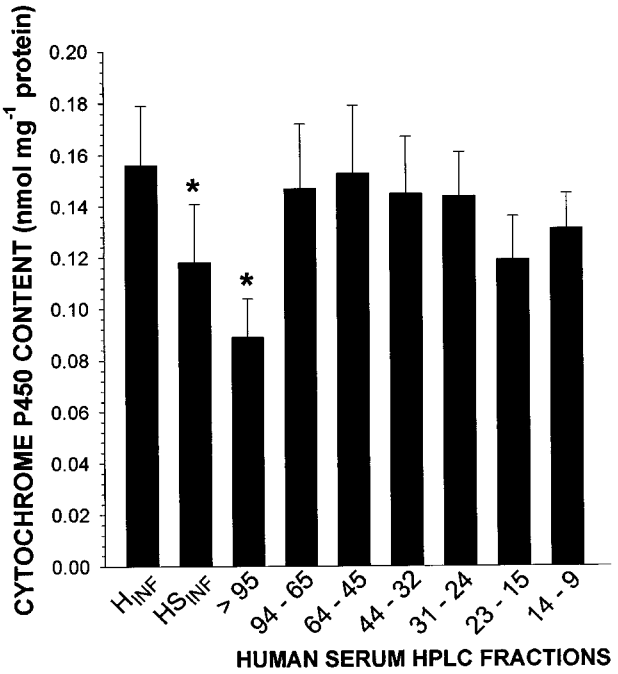

Figure 4 Effect of concentrated $\times 1.25$ HPLC fractions of serum from subjects with an upper respiratory tract viral infection (HS_{INF}) ($n=6$) on cytochrome P450 content in hepatocytes of rabbits with a turpentine-induced inflammatory reaction (H_{INF}) following 4 h incubation. HPLC fractions are designated by the M_r of proteins contained in each fraction. $^*P<0.05$ compared with P450 content in H_{INF}.

Identification of the mediators in serum from rabbits with an inflammatory reaction responsible for the inactivation of the P450

Compared with H_{INF} alone, the addition of serum from rabbits with an inflammatory reaction or the concentrated $\times 1.25$ M_r 23–15 kDa fraction ($n=7$) reduced P450 content in H_{INF} by 22 and 29%, respectively ($P<0.05$) (Figure 5). The addition of anti-IL-1β antibodies to the concentrated $\times 1.25$ M_r 23–15 kDa fraction did not prevent the diminution in P450 content. On the other hand, the addition of anti-IL-6 antibodies restored the P450 content to 97% of the amount measured in H_{INF}. Confirming the role of IL-6, incubation of H_{INF} with recombinant human IL-6 for 4 h elicited a 40% decrease ($P<0.05$) in P450 content (Figure 2). Neither anti-IFN-γ or anti-TNF-α antibodies elicited any protection against the inactivation produced by the concentrated $\times 1.25$ M_r 23–15 kDa fraction.

When all four antibodies were added to the serum from rabbits with an inflammatory reaction ($n=7$), and thereafter incubated with H_{INF}, the extent of the protection did not reach that elicited by the anti-IL-6 antibody alone added to the concentrated $\times 1.25$ M_r 23–15 kDa fraction. i.e. it restored the P450 content back to 89% of that measured with the H_{INF} incubated alone. The addition of the control monoclonal antibody to the concentrated $\times 1.25$ M_r 23–15 kDa fraction did not modify its ability to modify P450 content.

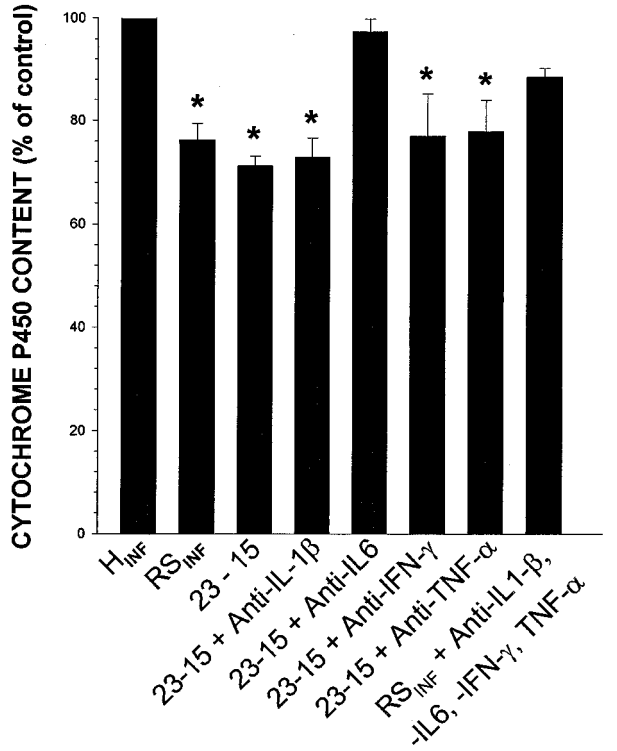

Figure 5 Effect of anti-cytokine antibodies on the ability of serum from rabbits with a turpentine-induced inflammation reaction (RS_{INF}) ($n=7$) and of the M_r 23–15 kDa HPLC fraction to reduce P450 content. H_{INF} are hepatocytes from rabbits with a turpentine-induced inflammatory reaction. $^*P<0.05$ compared with P450 content in H_{INF}.

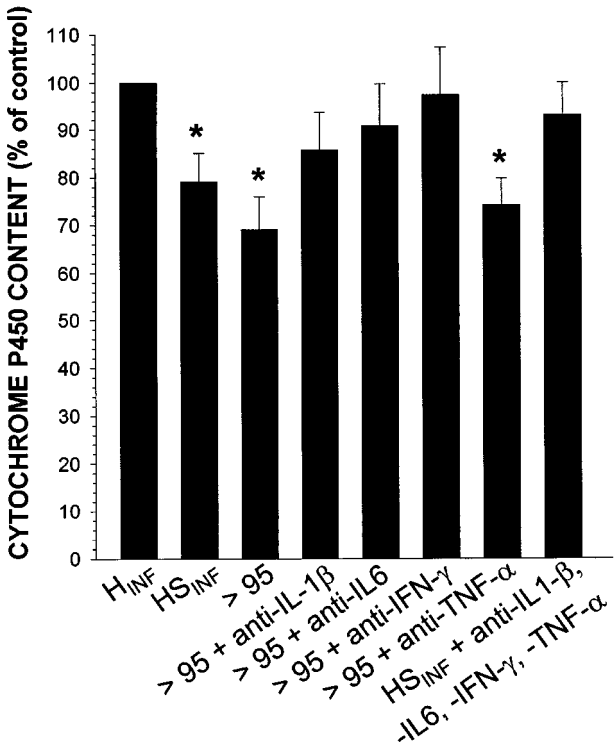

Figure 6 Effect of anti-cytokine antibodies on the ability of serum from humans with an upper respiratory viral infection (HS_{INF}) ($n=8$), and of the M_r >95 kDa HPLC fraction to reduce P450 content. H_{INF} are hepatocytes from rabbits with a turpentine-induced inflammatory reaction. $^*P<0.05$ compared with P450 content in H_{INF}.

Identification of mediators in serum from humans with a viral infection responsible for the inactivation of the P450

Compared with H_{INF} alone, the addition of serum from humans with a viral infection or the concentrated $\times 1.25$ M_r >95 kDa fraction ($n=8$) to H_{INF} reduced P450 content by 21 and 32% respectively ($P<0.05$) (Figure 6). The addition of anti-IL-1β, anti-IL-6 and anti-IFN-γ antibodies individually to the concentrated $\times 1.25$ M_r >95 kDa fraction restored P450 content to 89, 93 and 99%, respectively, of the values measured in H_{INF} alone. The anti-TNF-α antibody and the monoclonal antibody used as control were not able to prevent the reduction in P450 induced by the addition of the concentrated $\times 1.25$ M_r >95 kDa fraction. The mixture of the four antibodies added to serum from human with a viral infection almost completely restored the P450 content value, i.e. back to 93% of that observed in the H_{INF} incubated alone.

Discussion

Incubation of H_{INF} for 4 h with serum from rabbits with an inflammatory reaction or humans with a viral infection reduce P450 content and the formation of theophylline metabolites. The reduction in P450 catalytic activity occurs without any change in CYP1A1 and 1A2 amounts, suggesting that these proteins are inactivated. In the serum from rabbits with an inflammatory reaction, IL-6 accounts for most of the decrease in hepatic P450 content, and incubation of IL-6 with H_{INF} supports that this cytokine is capable to reduce hepatic P450. These results suggest that besides the well-known functions of IL-6 in the turpentine-induced inflammatory reaction, i.e. of transcriptional induction of acute phase response genes in the liver, repression of albumin synthesis (Moshage, 1997), and *in vivo* and *in vitro* repression of P450 at the gene level in human and rat hepatocytes (Abdel-Razzak *et al*., 1993; Chen *et al*., 1995; Clark *et al*., 1995), serum IL-6 is able to induce the inactivation of several P450 isoforms in primed hepatocytes. These results do not discard a role for IL-1β and TNF-α in the turpentine-induced down-regulation of P450 *in vivo*, since these cytokines may be necessary to promote the secretion of IL-6 (Luheshi *et al*., 1997).

In humans, influenza A and B, and influenza-like infections trigger the release of IFN-γ (Ennis *et al*., 1981), IL-6, IL-1β and TNF-α (Lehmann *et al*., 1996; Van Reeth *et al*, 1998). The present results show that in serum from humans with a viral infection, IFN-γ, IL-6 and IL-1β contribute to the inactivation of the P450 of H_{INF}. The ability of IFN-γ to inactivate the P450 appears greater than that of IL-6 and IL-1β. Supporting that IFN-γ has a predominant role, in humans, the increase in plasma levels of IFN-γ induced by an acute viral respiratory infection is associated with a reduction in the clearance of antipyrine (Ramshaw *et al*., 1997), and administration of IFN-γ to humans down-regulates P450 (Horsmans *et al*., 1994). Furthermore, in IL-6-deficient mice, symptoms of sickness to influenza infection are still present, suggesting that the role of IL-6 in viral aggression is relatively less important than that of other cytokines (Kozak *et al*., 1997). We can not ascertain that only IFN-γ, IL-6 and IL-1β account for the inactivation of the P450 since other cytokines known to depress P450 *in vitro* have not been tested in the present study, i.e. IL-2, IL-4, oncostatin-M, epidermal growth factor, and transforming growth factor-β1 (Abdel-Razzak *et al*., 1993; Guillen *et al*., 1998; Morgan 1997).

Serum from rabbits with a turpentine-induced inflammatory reaction does not inactivate P450 of H_{CONT}, but inactivates P450 of H_{INF}, and serum from humans with a viral infection inactivates P450 from both H_{CONT} and H_{INF}. Several reasons may explain such differences, but essentially the number of serum mediators and the density of surface receptors available to each mediator. In primed hepatocytes, i.e. those harvested from rabbits with an inflammatory reaction, the density of surface receptors to cytokines is greater than in control hepatocytes (Dinarello, 1994). Rabbit serum presenting a single mediator may require a greater number of surface receptors to this single cytokine than human serum that contains at least three cytokines contributing to P450 inactivation. Supporting such explanation is the fact that human serum has a greater potency to inactivate P450 in H_{INF} than does serum from rabbits (El-Kadi *et al*., 1997).

Contrasting with the active HPLC fraction of serum from rabbits with an inflammatory reaction, the activity of the HPLC fraction of serum from humans with a viral infection resides in the fraction containing proteins with a M_r greater than 95 kDa. α2-Macroglobulin is a 720 kDa homotetramer that functions as proteinase inhibitor and as binding protein for several regulatory polypeptides including active cytokines (Sottrup-Jensen, 1989). IL-6 binds to α2-macroglobulin which acts as a carrier protein; bound IL-6 is protected from hydrolysis by proteases, it retains its activity and its ability to bind to its receptor, and this binding does not interfere with its immunoassay (Matsuda *et al*., 1989). α2-Macroglobulin also binds IL-1β (Borth & Luger, 1989) and IFN-γ (James *et al*., 1992) without inhibiting their biological activity. IFN-γ affinity for native α2-macroglobulin is low (Crookston *et al*., 1994), implying that IFN-γ bound to α2-macroglobulin must be released easily from the complex, and this might explain the effectiveness of IFN-γ to inactivate the P450. On the other hand, cytokines such as IL-6 (May *et al*., 1992), IL-1 (Moshage, 1997) and IFN-γ (Gaillard *et al*., 1993) can also circulate in the blood complexed with the soluble forms of their receptors.

The ability of the cytokines in the serum from rabbits with an inflammatory reaction and in the serum from humans with a viral infection to diminish *ex vivo* the P450 catalytic activity differs from the ability of individual cytokines to down-regulate P450 isoforms when tested *in vitro*. When cytokines are incubated with hepatocytes, IL-1β is the cytokine with the greater ability to depress isoforms such as CYP1A1, 1A2, 2B1, 2C, 2E1 and 4A (Abdel-Razzak *et al*., 1993; Yamashita *et al*., 1994; Chen *et al*., 1995; Clark *et al*., 1995; Parmentier *et al*., 1997). Moreover, according to our results, TNF-α does not contribute directly to the inactivation of the P450, however *in vitro* it depresses CYP1A1/2, 2C, 2D (Abdel-Razzak *et al*., 1993; Clark *et al*., 1995). This apparent discrepancy could be secondary to various factors, such as the experimental model, including the duration of the incubation, differences in the concentrations of the cytokines between *ex vivo* and *in vitro* studies, and the presence of other compounds in the serum affecting P450 activity. This last possibility is supported by the fact that when the four antibodies are added together into the serum, the ability of the serum to inactivate the P450 is less affected than when the individual antibodies are added. We might speculate that the cross-talk between signalling pathways of cytokines with overlapping functions but with selective effects on P450 isoforms (Abdel-Razzak *et al*., 1993; Chen *et al*., 1995; Clark *et al*., 1995) is responsible for this apparent contradiction.

In conclusion, a localized tissue inflammation caused by the injection of turpentine induces the secretion of serum mediators, mainly IL-6, that inactivate hepatic P450 following a 4 h period incubation. On the other hand, an upper respiratory viral infection in humans induces the secretion of several mediators with different abilities to inactivate hepatic P450, i.e. IFN-γ > IL-1β > IL-6. TNF-α does not appear to contribute directly to the inactivation of hepatic P450 in either the turpentine or the viral inflammatory reactions.

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