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Down-regulation of the hepatic cytochrome P450 by an acute inflammatory reaction: implication of mediators in human and animal serum and in the liver

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1 Infection and inflammation trigger a cascade of mediators that eventually will down-regulate the hepatic cytochrome P450 (P450). The present study aimed to characterize the mediators contained in the serum of rabbits with an acute inflammatory reaction (AIR) induced by the s.c. injection of turpentine (5 ml), and in the serum of humans with an acute upper respiratory tract viral infection.

2 Hepatocytes from control (H_{CONT}) rabbits and rabbits with an AIR (H_{INFLA}) were isolated and cultured. Compared with H_{CONT} in H_{INFLA} the production of theophylline metabolites, 3-methylxanthine (3MX), 1-methyluric acid (1MU), and 1,3-dimethyluric acid (1,3DMU) was reduced as was the amount of total P450, while lipid peroxidation was increased. Incubation of H_{INFLA} with serum of rabbits with an AIR (RS_{INFLA}) for 4 h further reduced the formation of the metabolites of theophylline as well as the amount of P450, and enhanced the lipid peroxidation. RS_{INFLA} obtained 6, 12 and 24 h after the injection of turpentine showed the same ability to down-regulate hepatic P450 as the serum obtained at 48 h.

3 The efficacy (E_{max}) of RS_{INFLA} to inhibit the formation of theophylline metabolites differed, i.e. 1,3DMU > 1MU > 3MX, and the potency of serum mediators (IC_{50}) was similar for 3MX and 1MU, but lower for 1,3DMU.

4 Incubation of serum of human volunteers (HS_{INFLA}) with a viral infection with H_{CONT} or H_{INFLA} reduced the production of theophylline metabolites, as well as the amount of P450, and increased the lipid peroxidation. HS_{INFLA} depressed 1,3DMU more efficiently than 3MX and 1MU. HS_{INFLA} reduced 3MX with greater efficacy than did RS_{INFLA}. Potency was very variable but not different from rabbits.

5 It is concluded that the serum of rabbits with an AIR or of humans with a viral infection contain several mediators that inhibit noncompetitively various isoenzymes of the hepatic P450. The decrease in P450 induced by HS_{INFLA} or RS_{INFLA} is closely associated with the increase in lipid peroxidation ($r^2 = 0.8870$) suggesting that lipid peroxidation could directly or indirectly be involved in the P450 down-regulation.

Keywords: Cytochrome P450; inflammation; human serum mediators; rabbit serum mediators; turpentine; viral infection

Introduction

In man, acute viral infections of the upper respiratory tract (Chang *et al*., 1978), bacterial pneumonia (Vozeh *et al*., 1978; Sonne *et al*., 1985), and BCG vaccination are able to reduce the clearance of theophylline (Renton *et al*., 1981; Gray *et al*., 1983) by down-regulating multiple isoforms of the hepatic cytochrome P450 (P450). In animals, non-infectious inflammatory reactions, i.e. induced by turpentine, carrageen or Freund's adjuvant, diminish the amount of hepatic P450 and reduce the rate of biotransformation of multiple substrates and drugs, such as tolbutamide and theophylline (Beck & Whitehouse, 1974; Belpaire *et al*., 1989; Ishikawa *et al*., 1991; Parent *et al*., 1992; Barakat & du Souich, 1996).

Theophylline is extensively metabolized by several isoenzymes of the hepatic P450 (Robson *et al*., 1987). In man, theophylline 8-hydroxylation is catalysed by CYP1A2, 2D6, 2E1 and 3A4 yielding 1,3-dimethyluric acid (1,3DMU) as the major metabolite (Figure 1). Theophylline 1-demethylation is carried out by CYP1A2 yielding 3-methylxanthine (3MX). Theophylline 3-demethylation is catalysed by CYP1A1 and 1A2 yielding 1-methylxanthine, which is hydroxylated at C-8 by xanthine oxidase, to generate 1-methyluric acid (1MU) (Sarkar & Jackson, 1994; Ha *et al*., 1995; Zhang & Kaminsky, 1995). Since in man and in animals, an inflammatory reaction has the ability to reduce the clearance of theophylline, it can be assumed that the inflammatory reaction down-regulates multiple isoenzymes of the hepatic P450.

Almost a decade ago Ghezzi *et al*. (1986), by conducting serum transfer experiments, observed that the endotoxin-induced depression of P450 was mediated by a serum factor which was assumed to be a cytokine secreted by the monocytes. More recently, Moochhala & Renton, (1991) demonstrated that the depression of P450 induced by interferon could be prevented by puromycin or actinomycin D, implying that certain cytokines, such as interferon depressed the P450 by inducing the formation of another protein. On the other hand, it has been shown that an inflammatory reaction enhances hepatic lipid peroxidation, and this increase in reactive species has been associated with the down-regulation of the P450 (Proulx & du Souich, 1995; Proulx *et al*., 1995).

It is apparent in man and animal models that an acute inflammatory reaction (AIR) will trigger a cascade of mediators that eventually will lead to the down-regulation of hepatic P450. However, whether the ability to down-regulate the hepatic P450 and to increase hepatic lipid peroxidation by the mediators in serum of patients with a viral infection is comparable to the effect of the mediators in serum of animals with a non-infectious AIR remains unknown. In an attempt to characterize further the mediators of human and rabbit serum leading to the down-regulation of the hepatic P450, hepatocytes from control rabbits or from animals with a turpentine-induced AIR were co-cultured with serum of rabbits with a turpentine-induced AIR and with serum of volunteers with an upper respiratory tract viral infection, and their ability to

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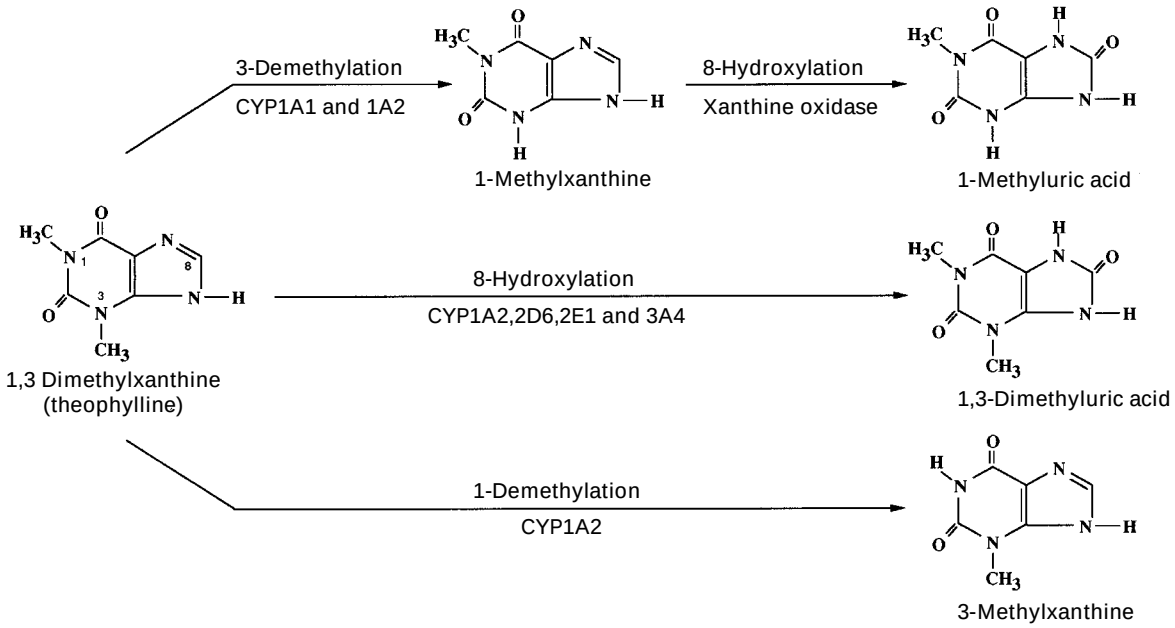

Figure 1 Scheme for the biotransformation of theophylline in human adults and isoforms of the cytochrome P450 involved in the formation of the main metabolites (adapted from Sarkar & Jackson, 1994, Ha *et al*., 1995 and Zhang & Kaminsky, 1995).

down-regulate the P450, as well as to promote the lipid peroxidation was compared.

Methods

Animal studies

Male New Zealand White rabbits (1.8–2.2 kg), were obtained from Ferme Cunipur (St. Valérien, Québec). Rabbits were housed in separate cages and fed water and rabbit chow *ad libitum* for at least 7 days before being used. The inflammatory reaction was induced locally by injecting turpentine (5 ml) s.c. at two distinct sites of the back of the rabbits as described elsewhere (Ashton *et al*., 1970; Parent *et al*., 1992). All the experiments were conducted according to the Canadian Council on Animal Care guidelines for use of laboratory animals. The hepatocytes were isolated 48 h after the induction of the inflammatory reaction.

Three solutions were utilized for the isolation of rabbit hepatocytes. Solution A contained (mM): NaCl 115, KCl 5, KH_2PO_4 1, HEPES 25, EGTA 0.5, glucose 5.5 and heparin 56.8 μg ml^{-1} in deionized water. Solution B was Solution A with $CaCl_2$ 1 mM, trypsin inhibitor 0.25 μM and collagenase 0.005%. Solution C contained 100 ml of solution B supplemented with $MgSO_4$ 1.2 mM and 1 ml of Williams' medium E (WME). Solutions A and B were adjusted to pH 7.4 by adding HCl 1 N and filtered through a 22 μm membrane before use.

Rabbit hepatocytes were isolated according to the two step liver perfusion method of Seglen (1976), with minor modifications. Overnight fasted rabbits were anaesthetized with sodium pentobarbitone, 30 mg kg^{-1}, a midline laparotomy was performed and the portal, the suprahepatic and the inferior cava veins were cannulated. All tubing and solutions were maintained at 37°C and saturated with 100% O_2. The liver was perfused via the portal vein with 400 ml of solution A, by use of a peristaltic pump (Harvard Apparatus Co., Inc., U.S.A.) at a flow rate of 38–42 ml min^{-1} for 5–8 min, then with 300 ml of solution B at a flow rate of 25–30 ml min^{-1} for 6–10 min, until the liver appeared completely blanched and softened. The liver was maintained wet with saline during the entire period of perfusion.

After *in situ* perfusion, the gall bladder and liver were removed and placed in a Petri dish containing 100 ml solution C. The capsule was stripped away from one side of the liver, and the cells were detached by brushing the liver with a plastic comb and filtered through nylon filter (253 μm). The suspension of cells was incubated in a shaker water bath at 37°C for 20 min with 100% O_2, filtered through a nylon filter (80 μm) and divided into two aliquots which were placed on ice. Once the temperature of 4°C was reached, the cells were centrifuged at 340 r.p.m. for 3 min, the supernatant was aspirated and the sediment was resuspended in WME and recentrifuged at 340 r.p.m. for 3 min, an operation that was repeated twice. Finally, the sediment was centrifuged on a 40% percoll gradient at 4000 r.p.m. for 10 min. The supernatant was discarded and the sediment resuspended in WME to obtain 4×10^6 cells ml^{-1}.

Each well of the plastic culture plates (12-well, Falcon, Becton Dickinson Labware, New Jersey, U.S.A.) was coated with 600 μl of Type I rat-tail collagen (50 μg ml^{-1} acetic acid 0.02 N), and one hour later, the wells were rinsed with deionized water and washed once with serum-free WME before the hepatocytes (4×10^6 ml^{-1} per well) were added in WME supplemented with 10% calf serum and insulin 1 μM. The plastic culture plates were incubated at 37°C in a humidifier with 95% O_2 and 5% CO_2. Viability was assessed before and after the incubation period by the trypan blue (0.2%) exclusion method, and in both instances the viability was over 90%.

In all experiments, the efficacy of serum mediator(s) to down-regulate hepatic P450 was characterized by culturing hepatocytes with serum for 4 h and assessing the catalytic activity of the P450 by measuring the concentration of theophylline metabolites generated. Theophylline was selected because it is metabolized by several P450 isoforms (Sarkar & Jackson, 1994; Ha *et al*., 1995; Zhang & Kaminsky, 1995), and that allows monitoring of different isozymes of the P450. Theophylline was dissolved in serum-free WME and 50 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 following 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 (h.p.l.c.) (du Souich *et al*., 1989). In addition, the effect of the serum mediator(s) was determined by measuring the amount of total P450 in the hepatocytes 4 h after the co-incubation with serum (Omura & Sato, 1964). The protein content in the hepatocytes was determined by the method of Lowry *et al*. (1951). Finally, the effect of the serum mediator(s)

on the promotion of lipid peroxidation in the hepatocytes was investigated by measuring the amount of malondialdehyde formed in the hepatocytes with the thiobarbituric acid reaction (Ohkawa *et al*., 1979). Viability of the hepatocytes was assessed always before and after the incubation period and was found to remain always greater than 90%.

A control experiment was conducted to discount the possibility that hepatocytes of rabbits with an AIR ($n=5$) contain a factor(s) able to depress hepatic P450. For this purpose, 0.5 ml of WME containing 2×10^6 hepatocytes of control rabbits (H_{CONT}) ($n=5$) were co-cultured with 0.5 ml of WME containing 2×10^6 hepatocytes of rabbits with an AIR (H_{INFLA}).

To determine whether the mediator(s) in serum was able to depress hepatic P450, serum (100 μl) of control rabbits (RS_{CONT}) ($n=5$) or of rabbits with an AIR (RS_{INFLA}) ($n=5$) were added to H_{CONT} or H_{INFLA}. In addition, the ability of RS_{INFLA} to depress the P450 of H_{INFLA} was characterized as a function of the time elapsed after the induction of the inflammatory reaction to rabbits ($n=5$). For this purpose, blood was withdrawn at 6, 12, 24 and 48 h after the induction of the inflammatory reaction and 100 μl of serum were incubated with H_{INFLA}.

Finally, to assess whether the down-regulation produced by serum mediator(s) was dose-dependent, dose-response curves were obtained by measuring the ability of a wide range of volumes (5 to 200 μl) of RS_{INFLA} ($n=3$) to depress the P450 of H_{INFLA}.

Studies in man

The ability of human serum to down-regulate hepatic P450 was assessed by incubating 100 μl of serum of healthy volunteers (HS_{CONT}) ($n=5$) or of the same subjects but with an upper respiratory tract viral infection (HS_{INFLA}) ($n=5$) with H_{CONT} or H_{INFLA} of rabbits. For this purpose, 48 h after the beginning of the clinical manifestations of the viral infection, 10 ml of blood were drawn from an antecubital vein and the serum decanted; the serum (100 μl) was added to the hepatocytes and incubated for 4 h with theophylline (176 μM) to assess how the presence of serum affected the concentration of the metabolites produced. The upper respiratory tract viral infection was diagnosed by the presence of rhinorrhea, sneezing, nasal congestion, sore throat, cough and systemic signs of malaise in the absence of purulent secretions.

The ability of human serum to down-regulate hepatic P450 was further characterized by studying the response to different volumes, i.e. performing dose-response curves measuring the ability of different volumes (5 to 200 μl) of HS_{INFLA} of 3 volunteers to inhibit the formation of theophylline metabolites in H_{INFLA}.

Drugs and chemicals

Insulin was purchased from Boehringer Mannheim GmbH (Germany), while HEPES, EGTA, WME, trypsin inhibitor, type IV collagenase, type I rat-tail collagen, calf serum, theophylline and its metabolites, and other chemicals were purchased from Sigma Chemical Company (St. Louis, U.S.A.).

Statistical analysis

All results are presented as mean $\pm$ 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 (ANOVA) followed by Newman-Keuls *post hoc* tests. The differences were considered significant when $P\leqslant0.05$.

Results

Incubation of H_{CONT} with theophylline for 4 h generated substantial concentrations of 1,3DMU and smaller amounts of 3MX and 1MU. However, when H_{INFLA} were incubated with theophylline, the formation of 3MX, 1MU and 1,3DMU was reduced by 50, 68 and 36% ($P<0.05$), respectively (Table 1). By comparison to control rabbits, the amount of total P450 was reduced by 54% ($P<0.05$) in H_{INFLA}, while lipid peroxidation, expressed in terms of generated malondialdehyde, was increased by 149% ($P<0.05$) (Table 1). H_{INFLA} co-cultured with H_{CONT} did not modify the formation of theophylline metabolites, the amount of total P450 or the lipid peroxidation in H_{CONT} (Table 1).

The biotransformation of theophylline by H_{CONT}, the amount of total P450 or hepatic lipid peroxidation were not altered by the addition of RS_{CONT} or RS_{INFLA} to the incubation medium (Table 2). Similarly, the addition of RS_{CONT} to the incubation media did not depress further the metabolism of theophylline by H_{INFLA}. However, the incubation of H_{INFLA} with RS_{INFLA} resulted in a further reduction in the formation of 3MX, 1MU and 1,3DMU by 40, 25 and 36% ($P<0.05$), respectively, as well as a reduction in the amount of total P450, and an increase in hepatic lipid peroxidation (Table 2). The viability of H_{CONT} or H_{INFLA} was not decreased following the 4 h incubation with RS_{INFLA}.

Compared with the concentrations of the metabolites of theophylline generated by H_{INFLA} in the presence of RS_{CONT}, the serum obtained 6 h after the injection of turpentine further depressed ($P<0.05$) the ability of H_{INFLA} to biotransform theophylline. Effectively, the concentrations of 3MX, 1MU and 1,3DMU generated by H_{INFLA} in the presence of RS_{CONT} were 0.15 ± 0.03, 0.13 ± 0.01 and 8.35 ± 0.70 μg ml^{-1}, respectively, and those generated by H_{INFLA} in presence of RS_{INFLA} obtained at 6 h were 0.09 ± 0.01, 0.10 ± 0.01 and 5.47 ± 0.40 μg ml^{-1}, $P<0.05$, respectively. The ability of the serum obtained 12, 24 and 48 h after the induction of the AIR to depress the P450 was similar to that observed with the serum obtained at 6 h.

The depression of P450 induced by the serum mediator(s) in the RS_{INFLA} was closely associated with the volume of RS_{INFLA} added to the H_{INFLA}. The volume required to produce the maximal depression depended upon the metabolic route, i.e. 30 μl were sufficient to depress maximally the formation of 3MX, while 40 and 100 μl were required to inhibit maximally the formation of 1MU and 1,3DMU, respectively. As a con-

Table 1 Effect of an *in vivo* turpentine-induced inflammatory reaction in rabbits on the ability of the hepatocytes to biotransform theophylline

	3MX (μg ml^{-1})	*1MU* (μg ml^{-1})	*1, 3DMU* (μg ml^{-1})	*Cytochrome P450* (nmol mg^{-1} protein)	*Lipid peroxidation* (pmol mg^{-1} protein)
H_{CONT}	0.24 ± 0.02	0.44 ± 0.10	14.97 ± 1.12	0.35 ± 0.03	4.70 ± 0.8
H_{INFLA}	0.12 ± 0.03¶	0.14 ± 0.01¶	9.55 ± 1.65¶	0.16 ± 0.01¶	11.7 ± 2.5¶
$H_{CONT}+H_{INFLA}$	0.15 ± 0.02*	0.24 ± 0.04*	10.33 ± 0.54*	0.20 ± 0.01*	8.10 ± 0.2*

The data were assesed by measuring the concentration of theophylline metabolites, the amount of hepatic total cytochrome P450 and hepatic lipid peroxidation. 3MX, 1MU and 1, 3DMU are 3-methylxanthine, 1-methyluric acid and 1,3-dimethyluric acid, respectively. H_{CONT}, H_{INFLA} are hepatocytes of control ($n=5$) and of rabbits with an inflammatory reaction ($n=5$), respectively. ¶$P<0.05$ compared with H_{CONT}. *$P>0.05$ compared with sum of H_{CONT} and H_{INFLA} divided by 2.

sequence, the estimated IC_{50} values required to inhibit the formation of theophylline metabolites differed substantially, i.e. 3MX < 1MU << 1,3DMU (Table 3). The maximal effect (E_{max}) attained was also specific for each metabolite (Table 3).

The incubation of 100 μl of human serum (HS) of healthy volunteers (HS_{CONT}) with hepatocytes of rabbits, H_{CONT} or H_{INFLA}, for 4 h did not modify the ability of the hepatocytes to biotransform theophylline (Table 4). However, HS_{INFLA} of volunteers with an acute upper respiratory tract viral infection reduced the rate of formation of 3MX and 1,3DMU by 29 and 46% ($P<0.05$), respectively in H_{CONT}, and further reduced the formation of all three metabolites, 3MX, 1MU and 1,3DMU by 46, 31 and 53% ($P<0.05$), respectively, in H_{INFLA} (Table 4). By comparison with the control group (H_{CONT} or H_{INFLA} with HS_{CONT}), HS_{INFLA} further reduced the amount of hepatic P450 and increased lipid peroxidation in both H_{CONT} and H_{INFLA} (Table 4).

The severity of the depression of the P450 by human serum was associated with the volume of HS_{INFLA} added to the incubation medium. The E_{max} for 3MX was similar to that estimated for 1MU, although slightly smaller than the E_{max} for 1,3DMU ($P>0.05$). The estimated IC_{50} values for inhibition of the formation of theophylline metabolites were rather similar for 3MX and 1MU but greater for 1,3DMU ($P<0.05$) (Table 3).

Discussion

The present results demonstrate that 48 h after the induction of an AIR by the s.c. injection of turpentine, the amount and activity of P450 is depressed in hepatocytes by around 50%. These results confirm other studies where it was shown that a turpentine-induced inflammatory reaction reduced the amount of P450 (Mahu & Feldmann, 1984; Kobusch *et al*., 1986; Proulx & du Souich, 1995), and decreased *in vivo* the clearance of tolbutamide and theophylline (Parent *et al*., 1992; Barakat & du Souich, 1996). *In vivo*, the serum mediator(s) must play a predominant role in the down-regulation of the P450 induced by the AIR, since turpentine does not depress directly hepatic P450 (Kobusch *et al*., 1986).

Mediator(s) in the serum of rabbits with an AIR are able to depress further by almost 40% the amount and activity of hepatic P450 of rabbits with an AIR. However, the serum mediator(s) of rabbits with an AIR do not depress the P450 of hepatocytes of control rabbits. The down-regulation of P450 of hepatocytes of rabbits with an AIR shows some peculiarities: firstly, the depression is evident following 4 h of incubation, and secondly, the ability of the serum to depress the P450 is apparent 6 h after the induction of the AIR. The fact that the serum mediator(s) only down-regulated the P450 of he-

Table 2 Effect of serum of control rabbits or of rabbits with a turpentine-induced inflammatory reaction on the ability of hepatocytes from control rabbits and rabbits with a turpentine-induced inflammatory reaction to biotransform theophylline

	3MX (μg ml^{-1})	*1MU* (μg ml^{-1})	*1,3DMU* (μg ml^{-1})	*Cytochrome P450* (nmol mg^{-1} protein)	*Lipid peroxidation* (pmol mg^{-1} protein)
$H_{CONT}+RS_{CONT}$	0.23±0.02	0.23±0.04	13.93±1.70	0.30±0.02	5.40±0.3
$H_{CONT}+RS_{INFLA}$	0.25±0.04	0.27±0.04	14.67±1.40	0.28±0.02	6.10±0.5
$H_{INFLA}+RS_{CONT}$	0.15±0.01¶	0.12±0.01¶	9.00±0.75¶	0.13±0.01¶	10.20±0.6¶
$H_{INFLA}+RS_{INFLA}$	0.09±0.01*	0.09±0.003*	5.73±0.35*	0.08±0.01*	12.70±0.6*

The data were assessed by measuring the concentration of theophylline metabolites, the amount of hepatic total cytochrome P450 and hepatic lipid peroxidation. 3MX, 1MU and 1,3DMU are 3-methylxanthine, 1-methyluric acid and 1,3-dimethyluric acid, respectively. RS_{CONT} and H_{CONT} are serum and hepatocytes of control rabbits ($n=5$). RS_{INFLA} and H_{INFLA} are serum and hepatocytes of rabbits with an inflammatory reaction ($n=5$). ¶$P<0.05$ or *$P<0.05$ compared with $H_{CONT}+RS_{CONT}$ and $H_{INFLA}+RS_{CONT}$, respectively.

Table 3 Maximal inhibitory effect (E_{max}) and volume eliciting half the E_{max} (IC_{50}) of serum of rabbits with a turpentine-induced inflammatory reaction or serum of human volunteers with an upper respiratory tract viral infection on the ability of hepatocytes of rabbits with a turpentine-induced inflammatory reaction to biotransform theophylline

	E_{max} (%)		*IC_{50}* (μl)	
	Rabbit	*Human*	*Rabbit*	*Human*
3-Methylxanthine	35.1±1.1	49.±4.3†	6.9±0.2	14.2±4.0
1-Methyluric acid	50.7±3.9¶	48.0±5.5	9.6±2.0	16.9±3.1
1,3-Dimethyluric acid	70.2±1.5*	64.2±2.2	44.2±15.6	28.9±2.9¶

The data were assessed by measuring the concentration of its metabolites. Dose-response curves were conducted by incubating the hepatocytes with 5, 10, 20, 30, 40, 50, 100, and 200 μl of serum from rabbits with an inflammatory reaction or from humans with a respiratory infection. Values are mean±s.e.; $n=3$. †$P<0.05$ compared with rabbit 3-methylxanthine. ¶$P<0.05$ compared with 3-methylxanthine. *$P<0.05$ compared to 1-methyluric acid and 3-methylxanthine.

Table 4 Effect of serum of healthy volunteers and of subjects with an upper respiratory tract viral infection on the ability of hepatocytes from control rabbits and rabbits with a turpentine-induced inflammatory reaction to biotransform theophylline

	3MX (μg ml^{-1})	*1MU* (μg ml^{-1})	*1,3DMU* (μg ml^{-1})	*Cytochrome P450* (nmol mg^{-1} protein)	*Lipid peroxidation* (pmol mg^{-1} protein)
$H_{CONT}+HS_{CONT}$	0.21±0.02	0.37±0.04	13.90±1.50	0.31±0.02	5.2±0.4
$H_{CONT}+HS_{INFLA}$	0.15±0.01¶	0.35±0.01	7.46±1.80¶	0.16±0.02¶	8.2±0.6¶
$H_{INFLS}+HS_{CONT}$	0.13±0.01	0.13±0.01	6.90±0.31	0.15±0.01	11.6±0.7
$H_{INFLS}+HS_{INFLA}$	0.07±0.003*	0.09±0.006*	3.23±0.47*	0.09±0.01*	14.7±1.1*

The data were assessed by measuring the concentration of theophylline metabolites, the amount of hepatic total cytochrome P450 and hepatic lipid peroxidation. 3MX, 1MU and 1,3DMU are 3-methylxanthine, 1-methyluric acid and 1,3-dimethyluric acid, respectively. H_{CONT} and H_{INFLA} are hepatocytes of control and of rabbits with an inflammatory reaction ($n=5$). HS_{CONT} and HS_{INFLA} are serum of healthy and diseased volunteers ($n=5$). ¶$P<0.05$ compared with $H_{CONT}+HS_{CONT}$. *$P<0.05$ compared with $H_{INFLA}+HS_{CONT}$.

patocytes of rabbits with an AIR may indicate that hepatocytes primed by the AIR present more surface receptors for the serum mediator(s) than do control hepatocytes. This explanation is plausible if it is taken into account that surface receptors of AIR mediators, such as cytokines, are increased by the inflammatory reaction itself or by other cytokines (Akahoshi *et al*., 1988; Koch *et al*., 1992; Ye *et al*., 1992; Geisterfer *et al*., 1993; Dinarello, 1994).

The serum of rabbits with AIR depressed the P450 of hepatocytes primed by an AIR in 4 h. These results are in agreement with those from a study in mice receiving daily injections of *Corynebacterium parvum* for 14 days, showing that the administration of endotoxin caused a decrease in hepatic P450 in 90 min (Ghezzi *et al*., 1986). On the other hand, it has been found that the induction of an AIR by the injection of turpentine or LPS induces a measurable depression of total P450 by 24 h (Mahu & Feldmann, 1984; Morgan, 1989). These apparent contradictions may in fact suggest that the mechanism underlying the down-regulation of the P450 in primed hepatocytes imply differences in the nature and activity of the serum mediator(s) and/or in the mode of action. It is accepted that P450 down-regulation during infection and inflammation occurs at a pre-translational level (Williams *et al*., 1991; Muntané-Relat *et al*., 1995), and therefore, the decay in P450 activity and amount must occur according to the half-life of the apoenzyme. Taking into account that the half-life of the main apoenzymes catalysing theophylline, i.e. CYP1A1, 1A2 and 3A4, range from 15 to 36 h (Shiraki & Guengerich, 1984; Muntané-Relat *et al*., 1995), we may speculate that in the presence of 100% depression of their mRNA, the concentration of the apoenzymes will decrease by about 6–13% in 4 h. In the present study, in 4 h the activity as well as the amount of P450 decreased by more than 30%, suggesting that a pre-translational mechanism cannot account for all the effect measured. Therefore, we may postulate that RS_{INFLA} and HS_{INFLA} down-regulate the P450 of H_{INFLA} mainly by a post-translational mechanism, without excluding a pre-translational effect.

The production of theophylline metabolites in the hepatocytes of rabbits with AIR was directly associated with the volume of serum of rabbits with AIR added to the incubation medium. The inhibitory ability of the serum was rather specific to each metabolite of theophylline. The maximal ability of the serum (E_{max}) to inhibit the formation of 3MX, 1MU and 1,3DMU varied substantially between the metabolites. On the other hand, the volume of serum required to reduce the E_{max} by 50% (IC_{50}) for 3MX and for 1MU was very similar, and tended ($P>0.05$) to be greater for 1,3DMU. These differences in response may be explained by the presence in the rabbit of several isoforms contributing to the formation of each metabolite, as has been described in man, i.e. the formation of 3MX is performed by CYP1A2, that of 1MU by CYP1A1 and 1A2, and that of 1,3DMU by CYP1A2, 2D6, 2E1, and 3A4 (Sarkar & Jackson, 1994; Ha *et al*., 1995; Zhang & Kaminsky, 1995). Alternatively, and not excluding the former explanation, the serum of rabbits with an AIR may contain several mediators showing different ability to inhibit each specific isoform.

Serum of healthy volunteers did not reduce the ability of the hepatocytes of control rabbits or rabbits with an AIR to generate the metabolites of theophylline. On the other hand, serum of subjects with an upper respiratory tract viral infection was able to reduce significantly the formation of 3MX and of 1,3DMU in hepatocytes of control rabbits. These results contrast with those obtained with the rabbits, in the sense that serum of rabbits with AIR did not diminish the production of theophylline metabolites by hepatocytes of control rabbits. The addition of serum of subjects with an upper respiratory tract viral infection to the incubation medium containing hepatocytes of rabbits with an AIR induced a substantial reduction in the production of theophylline metabolites. The differences observed between the serum of rabbits and the serum of individuals with a viral infection might be explained on the basis of the nature of the inflammatory reaction, i.e. aseptic chemical abscess versus viral infection, or on the nature of the mediator(s), i.e. variety, amount and efficacy. The efficacy, as reflected by the E_{max}, to diminish 3MX by serum of humans with a viral infection was greater than the efficacy of the serum of rabbits with an AIR, but was identical for 1MU and 1,3DMU. The potency of the mediator(s) was rather variable and no differences were apparent between human serum and rabbit serum. The differences observed between human and rabbit serum could be explained assuming that (1) some of the mediators found in human serum differ from those in the rabbit and (2) the concentration of the mediators found in human serum are greater than those in rabbit serum. Indeed, further studies are required to unravel the nature of the mediator(s) in both human and rabbit serum.

In the present study, the incubation of hepatocytes of rabbits with an AIR with serum of rabbits with AIR or with serum of individuals with a viral infection reduce the amount of total P450, reduction that is closely associated with the increase in hepatic lipid peroxidation ($r^2=0.8870$) (Figure 2). These *in vitro* results confirm other *in vivo* studies where it was shown that a turpentine-induced AIR increased hepatic lipid peroxidation and hepatic xanthine oxidase activity, and decreased the activity of the enzymatic scavengers as well as reduced glutathione (Proulx & du Souich, 1995; Proulx *et al*., 1995; Barakat & du Souich, 1996). Activation of the immune system by viruses, bacteria, parasites, vaccines, antigens or adjuvants (Renton, 1986), by agents causing a localized inflammatory reaction (Mahu & Feldmann, 1984; Morgan, 1989; Proulx & du Souich, 1995) or by LPS (Morgan, 1989) depress the hepatic P450. The down-regulation of hepatic P450 has been associated with an increased production of reactive oxygen intermediates. Supporting such a contention, the P450 depression induced by the administration of endotoxin is prevented by the administration of N-acetylcysteine (Ghezzi *et al*., 1985). Interferon increases the activity of xanthine oxidase as well as hepatic lipid peroxidation and decreases hepatic P450 (Deloria *et al*., 1985; Koizumi *et al*., 1986; Moochhala & Renton, 1991), effects that are reversed by the administration of N-acetylcysteine (Ghezzi *et al*., 1985), α-tocopherol and allopurinol (Moochhala & Renton, 1991). Therefore, we may postulate that during an inflammatory reaction, rabbit and human serum mediators induce the formation of reactive oxygen intermediates in the hepatocyte which act as cellular mediators for the down-regulation of the P450. Further studies are required to confirm such a hypothesis.

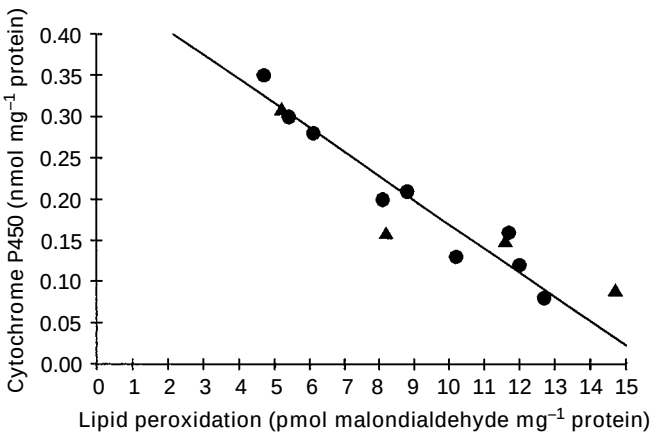

Figure 2 Changes in the amount of total cytochrome P450 in hepatocytes of rabbits with a turpentine-induced acute inflammatory reaction as a function of the lipid peroxidation ($r^2=0.8870$) induced by the 4 h incubation of 100 μl of (●) serum from rabbits with a turpentine-induced acute inflammatory reaction ($n=9$) or (▲) serum from humans with an upper respiratory tract viral infection ($n=4$) with the hepatocytes.

In conclusion, the serum of rabbits with an acute inflammatory reaction was shown to contain mediator(s), present already 6 h after the induction of the inflammatory reaction, that down-regulated in 4 h the P450 of hepatocytes primed *in vivo* by the inflammatory reaction. The serum mediator(s) diminished the formation of the three metabolites of theophylline by the hepatocytes, although the efficacy to inhibit the formation of the metabolites differed, i.e. 1,3DMU > 1-MU > 3MX. However, the potency of the serum mediator(s) was similar for 3MX and 1MU, but lower for 1,3DMU. These results suggest that several mediators inhibit noncompetitively several isoenzymes of the hepatic P450. The serum of humans with an upper respiratory tract viral infection also down-regulated the P450 of primed hepatocytes. The depression of P450 induced by human serum was similar to that produced by the mediators in serum of rabbits, with the exception that the efficacy of human serum to inhibit the formation of 3MX was greater than that of the rabbit serum. The decrease in P450 induced by rabbit or human serum was closely associated with the increase in lipid peroxidation suggesting that lipid peroxidation could directly or indirectly be involved in the P450 down-regulation.

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