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Effect of Nifedipine on the Elimination of Theophylline in the Rabbit Subjected to Hypoxia or to an Inflammatory Reaction

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Abstract

This study was performed to assess whether nifedipine could prevent the decrease in hepatic cytochrome P450 induced by acute moderate hypoxia or an inflammatory reaction. Rabbits were subjected to acute moderate hypoxia ($PaO_2 \gg 37$ mmHg), with or without pretreatment with nifedipine (0·5 mg kg^{-1} subcutaneously every 8 h, for 48 h). Another group received 5 mL of turpentine subcutaneously with or without pretreatment with nifedipine (0·5 mg kg^{-1} s.c. every 8 h, for 72 h). The kinetics of 2·5 mg kg^{-1} of theophylline were studied in all rabbits up to 8 h, at which time total cytochrome P450 and malondialdehyde were assessed in the liver.

Compared with control rabbits, hypoxia and an inflammatory reaction increased theophylline plasma concentrations, as a result of a decrease in theophylline systemic clearance. Both experimental conditions reduced hepatic cytochrome P450 by 40 to 50% and increased hepatic malondialdehyde by approximately 50% ($P < 0.05$). In control animals, pretreatment with nifedipine did not influence theophylline kinetics, the liver content in cytochrome P450 or malondialdehyde. Pretreatment with nifedipine partially prevented the hypoxia- and the inflammation-induced decrease in liver cytochrome P450; however, nifedipine did not prevent the decrease in theophylline clearance or the increase in liver malondialdehyde.

It is concluded that nifedipine affords a partial protection against hypoxia- or inflammation-induced hepatic cellular injury.

In patients with obstructive lung disease, the clearance of theophylline is decreased by as much as 80% (Hendeles et al 1986), and in patients with infectious complications the clearance of theophylline may also be reduced. In animals, hypoxia and inflammatory reactions reduce the activity of selected isoforms of cytochrome P450 (Letarte & du Souich 1984; Parent et al 1992). Concomitant to the decrease in amount of hepatic cytochrome P450, there is an increase in the hepatic production of reactive species (Proulx & du Souich 1995a,b). It has been shown that the hypoxia-induced reduction in ATP impairs the function of Na^+ K^+-ATPase and Ca^{2+}-ATPase, leading to the intracellular accumulation of calcium and sodium, and to the decrease in potassium (de Groot et al 1988). It has been proposed that calcium-channel blockers have an antioxidant activity (Mak & Weglicki 1994) since they protect endothelial cells against free radicals (Mak et al 1992), and inhibit the production of reactive species during an ischaemic insult (Oyanagui & Sato 1991). We hypothesized that calcium-channel blockers could prevent the cellular damage induced by acute moderate hypoxia or by an acute inflammatory reaction by reducing the entry of Ca^{2+} into the cell, and by having an antioxidant activity. The objective of the present study was to investigate in-vivo whether nifedipine could prevent the decrease in hepatic cytochrome P450 induced by acute moderate hypoxia or by an acute inflammatory reaction, and so impede the decrease in theophylline clearance in conscious rabbits.

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Materials and Methods

Male New Zealand rabbits (Ferme Cunicole, Les Lapins Léonard, Mirabel, Canada), 2·0–2·2 kg were acclimatized for eight days in well-ventilated cages and had free access to dry food and water.

To assess theophylline kinetics in-vivo and thereafter, ex-vivo hepatic amount of total cytochrome P450 and hepatic malondialdehyde, the rabbits were segregated into six groups. The rabbits of the first group (n = 6) were used as controls. The second group (n = 4) was used to assess the effect of nifedipine on theophylline clearance. Groups 3 (n = 6) and 4 (n = 6) were used to document the effect of hypoxia on theophylline kinetics in the absence and in the presence of nifedipine. Groups 5 (n = 6) and 6 (n = 6) were used to assess the effect of an inflammatory reaction on theophylline kinetics in the absence and in the presence of nifedipine.

Experimental protocol

At time 0, rabbits of the 6 experimental groups received in a lateral vein of an ear 2·5 mg kg^{-1} theophylline dissolved in 0·9% NaCl. Blood samples were drawn before and at 5, 10, 15, 20, 30, 60, 120, 180, 240, 300, 360, 420 and 480 min after the injection of theophylline through a catheter (Butterfly-21, Abbot Ireland, Sligo, Ireland) inserted in the central artery of an ear.

Acute moderate hypoxia was induced by placing the rabbits in a Plexiglass chamber (0·75 × 1·20 × 1·25 m^3) where the FiO_2 was 10%, regulated with an oxygen monitor (OM-15, Sensor Medics Corporation, CA) connected to an electrovalve (Asco Valves, Brantford, Ontario) that allowed the access of nitrogen. An FiO_2 of 10% was chosen to obtain an arterial partial pressure of O_2 (PaO_2) of 35 mmHg. Humidity in the

chamber was maintained at 50% by the recirculation of the air through a refrigerating system. The temperature was kept at 22–24°C. The rabbits were placed in the chamber 24 h before and for the 8 h kinetic experiment. Arterial blood samples were drawn at different times to control blood gases and pH (Model II Micro 13-03/213-05, Instrumentation Laboratory, Lexington, MA).

An inflammatory reaction was induced locally by injecting turpentine (5 mL) subcutaneously at two distinct sites on the back of the rabbits. The kinetics of theophylline were assessed 48 h later. The rectal temperature was measured before and at the peak of the inflammatory reaction. To discard an effect of nifedipine on theophylline kinetics, nifedipine dissolved in 0·9% NaCl (saline) was injected subcutaneously (0·5 mg kg^{-1}), every 8 h for 48 h, before the kinetics of theophylline were assessed. In hypoxic rabbits, nifedipine was administered 24 h before the hypoxia, and during the 24 h period of hypoxia. Rabbits with an inflammatory reaction received nifedipine for 72 h, starting 24 h before the administration of turpentine. The selection of the mode of administration of nifedipine was done to replicate the doses and intervals of administration of nifedipine to humans, i.e. 30–40 mg (≫0·5 mg kg^{-1}), three times a day. Theophylline in plasma was quantified by high pressure liquid chromatography (HPLC) as described elsewhere (Letarte & du Souich 1984).

Rabbits of all groups were killed 8 h after the administration of theophylline, and the liver was removed to assess total cytochrome P450 and malondialdehyde as a marker of lipid peroxidation. A portion of the liver was used to obtain a 17% (w/v) homogenate in 0·25 M sucrose, which was centrifuged at 600 *g* for 8 min, and the resulting supernatant at 12 000 *g* for 10 min. The supernatant was recentrifuged with 8 mM $CaCl_2$ at 27 000 *g* for 15 min. The ensuing supernatant was collected and stored at −80°C, and the pellet was resuspended in 0·15 M KCl solution and recentrifuged at 27 000 *g* for 15 min. The pellet was isolated and covered with ice-cold 0·25 M sucrose solution, and stored at −80°C. The amount of malondialdehyde formed during hypoxia or the inflammatory reaction was assessed in the supernatant by means of the thiobarbituric acid reaction. The amount of total hepatic cytochrome P450 was measured in the pellet (Omura & Sato 1964). Protein content in the hepatic supernatant and microsomal fractions (pellet) was measured using the method of Lowry et al (1951).

Analysis of data

Standard methods were used for the determination of the area under theophylline plasma concentration curve as a function of time ($AUC_{0-\infty}$), systemic clearance (CL), terminal half-life (t½), and predicted volume of distribution at steady state (Vd_{ss}).

All results are presented as mean ± standard error (s.e.). The comparison of the results from the various experimental groups and control group was carried out using a one-way analysis of variance for parallel groups. Statistical difference was determined using Dunnett's distribution table (Winer 1971). The significance criterion was established at $P < 0.05$.

Results

In control rabbits, breathing air, the administration of nifedipine for two days did not modify the kinetics of theophylline (Table 1). In control rabbits, the amount of total hepatic cytochrome P450 was 1·087 ± 0·034 nmol (mg protein)$^{-1}$, which was not altered by the administration of nifedipine (1·017 ± 0·081 nmol (mg protein)$^{-1}$). Similarly, compared with controls, the administration of nifedipine did not modify hepatic malondialdehyde (0·177 ± 0·005 compared with 0·165 ± 0·003 nmol (mg protein)$^{-1}$).

Effect of nifedipine on hypoxia-induced decrease in hepatic cytochrome P450

In rabbits breathing room air, mean arterial PaO_2 was 90 ± 3 mmHg, and in those exposed to a 10% FiO_2, average PaO_2 was decreased to 35 ± 2 mmHg ($P < 0.05$). Arterial $PaCO_2$ and pH were not affected by the experimental conditions (24 ± 1 mm Hg and 7·50 ± 0·01 in hypoxic rabbits compared with 25 ± 1 mm Hg and 7·49 ± 0·02 in control rabbits).

Table 1. Pharmacokinetic parameters of theophylline.

	$AUC_{0-\infty}$ (μg min mL^{-1})	CL (mL min^{-1} kg^{-1})	Vd_{ss} (mL kg^{-1})	t½ (min)
Control (n = 6)	1110 ± 42	2·27 ± 0·09	736 ± 30	234 ± 8
Control + nifedipine (n = 4)	1117 ± 103	2·29 ± 0·19	618 ± 26	192 ± 26
Hypoxia (n = 6)	1854 ± 120*	1·21 ± 0·07*	619 ± 23	346 ± 14
Hypoxia + nifedipine (n = 6)	2254 ± 121*	1·12 ± 0·05*	510 ± 40*	320 ± 29
Inflammation (n = 6)	2147 ± 259*	1·25 ± 0·15*	766 ± 34	461 ± 59*
Inflammation + nifedipine (n = 6)	2365 ± 248*	1·13 ± 0·14*	622 ± 78	385 ± 31*

Values are means ± s.e. *$P < 0.05$ compared with control rabbits; $AUC_{0-\infty}$ is the area under theophylline plasma concentration-time curve from time 0 to ∞; CL is theophylline total clearance; Vd_{ss} is theophylline apparent volume of distribution at steady state and t½ is theophylline terminal half life.

Hypoxia induced an increase in theophylline plasma concentrations (Fig. 1). As a consequence, in hypoxic rabbits, theophylline $AUC_{0-\infty}$ was almost 70% greater than that in control rabbits; the increase in $AUC_{0-\infty}$ was secondary to a reduction in theophylline clearance (Table 1). Theophylline volume of distribution was not affected by hypoxia. After 24 h of hypoxia, the hepatic cytochrome P450 was 40% less than in control animals. In hypoxic rabbits, hepatic malondialdehyde was almost 50% greater than in control rabbits (Table 2).

Pretreatment with nifedipine did not impede the hypoxia-induced decrease in theophylline clearance, and as a consequence, theophylline plasma concentrations were as elevated as observed in animals with hypoxia not receiving the calcium-channel blocker (Fig. 1). In hypoxic animals pretreated with nifedipine, hepatic cytochrome P450 was 25% greater than in hypoxic rabbits without nifedipine, but remained 21% lower than the values estimated in the control group. The administration of nifedipine did not hinder the hypoxia-induced increase in hepatic malondialdehyde (Table 2).

Effect of nifedipine on the decrease in hepatic cytochrome P450 induced by an inflammatory reaction

Rectal temperature increased from baseline values of $39{\cdot}1 \pm 0{\cdot}1$ to $41{\cdot}0 \pm 0{\cdot}1$°C ($P < 0{\cdot}05$) 48 h after the subcutaneous administration of turpentine. In rabbits with the inflammatory reaction, the rate of decline of theophylline plasma concentrations decreased (Fig. 2), and as a consequence, the $AUC_{0-\infty}$ of theophylline was almost twice that estimated in control rabbits. The $AUC_{0-\infty}$ was enhanced because the systemic clearance of theophylline was diminished (Table 1). In the rabbits with the inflammatory reaction, the amount of hepatic cytochrome P450 was almost 50% less than in controls, and the amount of malondialdehyde in the liver was enhanced by 44% (Table 3).

Pretreatment of rabbits with an inflammatory reaction with nifedipine failed to restore theophylline systemic clearance (Table 1). Therefore, theophylline plasma concentrations remained higher than those observed in controls (Fig. 2).

Table 2. Effect of nifedipine (0·5 mg kg^{-1} s.c. every 8 h for 48 h) on the amount of total hepatic cytochrome P450, and malondialdehyde administered to rabbits with hypoxia ($PaO_2 = 35$ mmHg).

	Concn (nmol (mg protein)$^{-1}$)	
	Cytochrome P450	Malondialdehyde
Control	1·08 ± 0·04	0·18 ± 0·01
Hypoxia	0·64 ± 0·05*	0·29 ± 0·01**
Hypoxia + nifedipine	0·86 ± 0·05*	0·25 ± 0·01*

Mean ± s.e. (n = 6). *$P < 0{\cdot}05$ compared with the corresponding control; **$P < 0{\cdot}05$ compared with corresponding groups of hypoxia and control.

Nifedipine increased total hepatic cytochrome P450 by 30%; however this value was still 24% less than the amount measured in control animals. Pretreatment with nifedipine did not prevent the increase in malondialdehyde in the liver induced by the inflammatory reaction (Table 3).

Table 3. Effect of nifedipine (0·5 mg kg^{-1} s.c. every 8 h, for 72 h) on the amount of total hepatic cytochrome P450 and malondialdehyde administered to rabbits with an inflammatory reaction (5 mL turpentine s.c.).

	Concn (nmol (mg protein)$^{-1}$)	
	Cytochrome P450	Malondialdehyde
Control	1·08 ± 0·03	0·18 ± 0·01
Inflammation	0·58 ± 0·05*	0·28 ± 0·02*
Inflammation + nifedipine	0·89 ± 0·09**	0·23 ± 0·01*

The results are mean ± s.e. (n = 6). *$P < 0{\cdot}05$ compared with corresponding control; **$P < 0{\cdot}05$ compared with corresponding groups of inflammation.

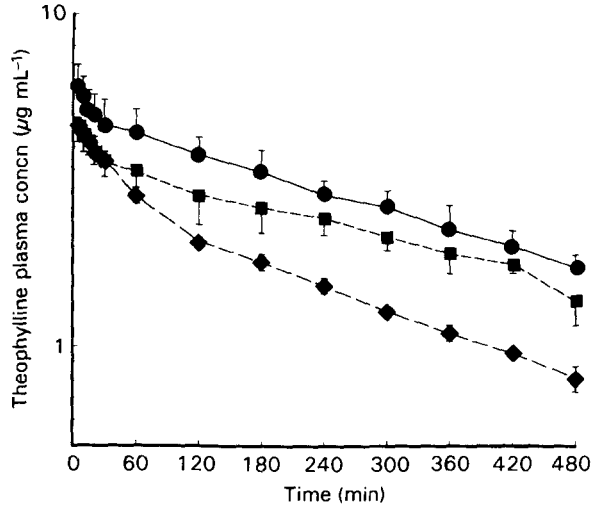

FIG. 1. Theophylline plasma concentrations in control rabbits (◆) (n = 6), rabbits with hypoxia ($PaO_2 = 35$ mmHg) (■) (n = 6) and hypoxic rabbits (●) (n = 6) receiving nifedipine (0·5 mg kg^{-1} s.c. every 8 h for 48 h). The results are mean ± s.e.

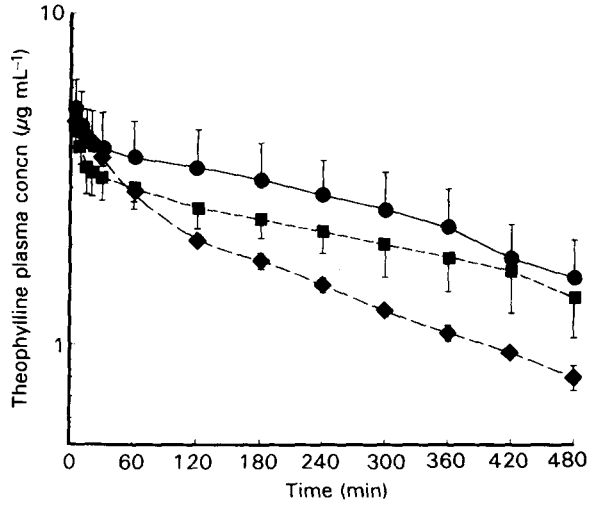

FIG. 2. Theophylline plasma concentrations in control rabbits (◆) (n = 6), rabbits with an inflammatory reaction (5 mL turpentine s.c.) (■) (n = 6) and rabbits with an inflammatory reaction receiving nifedipine (0·5 mg kg^{-1} s.c. every 8 h, for 72 h) (●) (n = 6). The results are mean ± s.e.

Discussion

The present results confirm that in-vivo acute moderate hypoxia, as well as an inflammatory reaction reduce the clearance of theophylline. Concurrently, in the same rabbits, both experimental conditions decrease the amount of total hepatic cytochrome P450 and increase the concentration of malondialdehyde. Nifedipine does not prevent the reduction of theophylline clearance, even if the amount of total hepatic cytochrome P450 appears to be partially protected by the pretreatment with the calcium-channel blocker. Nifedipine does not prevent the hypoxia- and inflammation-induced increase in hepatic malondialdehyde.

The partial protection conferred by nifedipine appears to be specific to some cytochrome P450 isoforms since, in-vivo, the clearance of theophylline was not raised to baseline values, despite total hepatic cytochrome P450 being increased by the calcium-channel blocker. Theophylline metabolism is mediated by several isoforms (Zhang & Kaminsky 1995); nifedipine is a substrate for the CYP 3A gene subfamily (Guengerich et al 1991), and as such, is able to inhibit the biotransformation of other substrates metabolized by the same isoform. As nifedipine did not modify the kinetics of theophylline or the amount of total cytochrome P450 in control animals, we believe that in hypoxic rabbits or in those with an inflammatory reaction, nifedipine does not induce the isoforms of hepatic cytochrome P450 but rather prevents the decrease of selected isoforms, other than CYP 1A and CYP 3A.

In this laboratory, we have shown that acute moderate hypoxia and acute inflammatory reactions increase hepatic malondialdehyde, decrease the amount of hepatic reduced glutathione, and decrease the activity of enzymatic scavengers in the liver (Proulx & du Souich 1995a,b). Thus we postulate that the decrease in hepatic cytochrome P450 induced by acute hypoxia or by an acute inflammatory reaction, is closely associated with the production of free radicals. This hypothesis is supported by the demonstration that the production of oxygen free radicals is involved in hypoxic liver injury (Videla 1991).

The partial protection conferred by nifedipine against the deleterious effects of acute moderate hypoxia or an acute inflammatory reaction, may be secondary to a direct scavenging effect of nifedipine, or to an indirect effect on intracellular calcium homeostasis. In-vitro at high concentrations, 2000 to 8000 ng mL^{-1}, nifedipine has a lipid antiperoxidative activity (Mak et al 1992) and high doses (60 mg kg^{-1}) given orally to mice suppress the ischaemia/reflow-induced paw oedema, while only very high concentrations (30–200 mg mL^{-1}) inhibit the production of superoxide radical from neutrophils and xanthine oxidase (Oyanagui & Sato 1991). However, Arouma et al (1991) postulated that in subjects receiving usual therapeutic doses, calcium-channel blockers will elicit a rather limited antioxidant activity. Since in the present study, low doses of nifedipine (0·5 mg kg^{-1}) did not prevent the hypoxia- or inflammation-induced increase in hepatic malondialdehyde, we suggest that nifedipine did not elicit an antioxidant effect.

Hypoxia induces an intracellular accumulation of Ca^{2+}. In cultured pulmonary arterial myocytes, hypoxia induces a rise in intracellular Ca^{2+} characterized by two components, an early release of Ca^{2+} from the sarcoplasmic reticulum, and a later influx of extracellular Ca^{2+} (Salvaterra & Goldman 1993). In rat liver, mitochondria may also release Ca^{2+} under hypoxic conditions (Guidoux et al 1993). At steady state, nifedipine attenuates the hypoxia-induced increase in intracellular Ca^{2+} by only 35% (Salvaterra & Goldman 1993). Thus nifedipine may have conferred a partial protection against hypoxia-induced hepatic injury essentially by preventing the influx of extracellular calcium into the hepatocyte, and not by impeding the mobilization of calcium from intracellular sources.

It is highly probable that the mechanisms underlying the effect of nifedipine in animals subjected to the inflammatory reaction are similar to those proposed for hypoxia. This hypothesis is supported in the present study; the nifedipine response in hypoxic rabbits was identical to that in rabbits with an inflammatory reaction. Furthermore, in previous studies (Proulx & du Souich 1995a,b) the effect of hypoxia on the amount and activity of hepatic cytochrome P450, as well as on scavenger enzymatic systems, was similar to that observed in animals with an inflammatory reaction.

At the present time it is not possible to determine whether hepatic injury is initiated by the uncontrolled entry of Ca^{2+} into the cell, or whether lipid peroxidation is triggering the entry of Ca^{2+}. Kimura et al (1992) showed that exposure of endothelial cells to hydrogen peroxide increased intracellular Ca^{2+}, whereas an increase in intracellular oxygen free radicals facilitates the entry of Ca^{2+} into the cell (Arouma et al 1991). As nifedipine afforded a partial protection, without preventing the increase in malondialdehyde, we suggest that the increase in intracellular calcium is secondary to the increase in oxygen free radicals, and that cellular damage is only partially mediated by the increase in intracellular Ca^{2+}.

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