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HEPATIC MICROSOMAL INDUCTION PROFILE OF CARBAMIC ACID [[2,6-BIS(1-METHYLETHYL)PHENOXY] SULFONYL]-2,6-BIS(1-METHYLETHYL) PHENYL ESTER, MONOSODIUM SALT (PD138142-15), A NOVEL LIPID REGULATING AGENT

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Abstract—Induction of hepatic microsomal cytochrome P450 produced by carbamic acid [[2,6-bis(1-methylethyl)phenoxy]sulfonyl]-2,6-bis(1-methylethyl) phenyl ester, monosodium salt (PD138142-15), a novel water-soluble inhibitor of acyl-CoA : cholesterol acyltransferase, was examined in male and female rats, dogs, and monkeys, and in male guinea pigs. Relative to control, PD138142-15 increased hepatic microsomal total spectral P450 in all species examined. Hepatic microsomal ethoxyresorufin-*O*-deethylase, pentoxyresorufin-*O*-dealkylase, and peroxisomal carnitine acetyltransferase activities and cyanide-insensitive β-oxidation were affected only marginally. Erythromycin-*N*-demethylase activity was increased (2- to 6-fold) in all three species in which it was examined (rat, dog and pig). Marked increases in immunoreactive P450 3A were noted in rats and dogs, while slight increases were seen in monkeys. Pharmacokinetic studies of PD138142-15 in rats and dogs revealed pronounced decreases (80–90%) in plasma C_{max} and AUC within 2 weeks of initiation of daily dosing. In spite of the marked decline in plasma drug levels, efficacy in dogs, as determined by serum cholesterol levels, was maintained for up to 6 weeks with continued dosing. Potential acid (gastric) breakdown products of PD138142-15 were examined for their hepatic cytochrome P450 induction profiles in rats and were found to differ both quantitatively and qualitatively from profiles produced by the parent compound. This suggested that induction observed in rats was due to parent PD138142-15 and not to any of the known potential acid breakdown products. The cumulative data establish that PD138142-15 is an inducer of P450 3A in rats and dogs. The results also suggest that P450 3A is induced in monkeys and pigs as well, although the data are less definitive. Decreases in plasma drug levels imply that the compound may be an autoinducer in dogs and rats. The maintenance of efficacy in spite of decreased drug levels in dogs suggests that the effects on serum cholesterol are due to a metabolite or that cholesterol lowering effects occur before the compound is metabolized by the liver.

Key words: PD138142-15; ACAT inhibition; microsomal induction; cytochrome P450; dog; monkey

PD138142-15, chemically known as carbamic acid [[2,6 - bis(1 - methylethyl)phenoxy]sulfonyl] - 2,6 - bis(1-methylethyl) phenyl ester, monosodium salt, is a novel lipid regulating agent. The compound is being developed as an inhibitor of ACAT‖ (EC 2.3.1.26) and is unusual in that it has a comparatively high aqueous solubility (21 mg/mL). The compound is relatively weak as an inhibitor of ACAT *in vitro* compared with lipophilic inhibitors (IC_{50} = 5.3 μM, rat liver microsome model) but retains excellent lipid regulating activity *in vivo* [1]. During the preclinical development of the compound, enlarged livers with proliferation of SER were noted in dogs¶, suggesting possible induction of hepatic microsomal enzymes.

Induction of hepatic cytochrome P450(s) is not an uncommon finding during the preclinical development of therapeutic agents.** The significance of the finding in any one species can cause considerable consternation, but the actual relevance to human exposure generally remains unknown until clinical trials are conducted. However, induction of

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‖ Abbreviations: ACAT, acyl-CoA:cholesterol acyltransferase; SER, smooth endoplasmic reticulum; PB, phenobarbital; β-NF, β-naphthoflavone; ERY, erythromycin; EROD, ethoxyresorufin-*O*-deethylase; PROD, pentoxyresorufin-*O*-dealkylase; END, erythromycin-*N*-demethylase; CAT, carnitine acetyltransferase; β-OX, cyanide-insensitive β-oxidation; CIPRO, ciprofibrate; LDL, low density lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; and RI, HMG-CoA reductase inhibitor.

¶ Unpublished data.

** Pritchard JF, Approaches in assessing enzyme induction potential of new drug candidates: Overview of approaches taken by PMA companies. Survey for the Pharmaceutical Manufacturers Association, 1988.

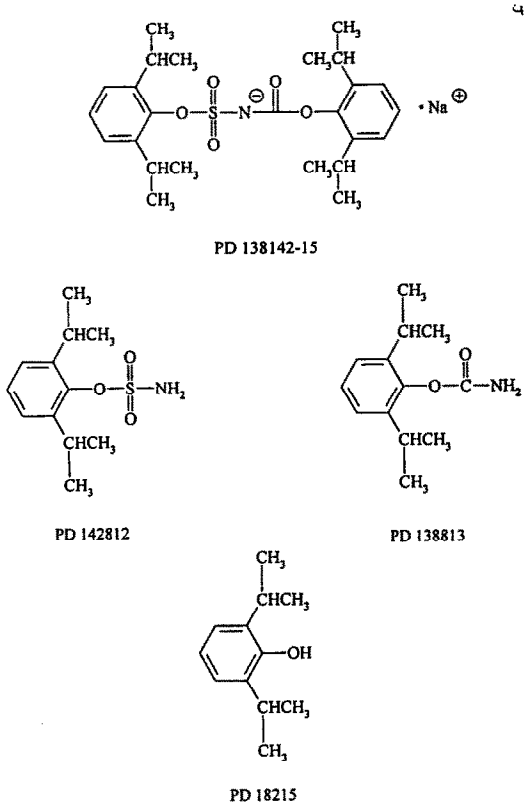

Fig. 1. Structures of compounds used in these investigations.

cytochrome P450 in animal models can and has been used as a reason to discontinue preclinical development of a compound in favor of a suitable non-inducing analog that is frequently either further behind in development or has other flaws that lead to its back-up status in the first place [2]. The work described here combines data from preclinical pharmacology and toxicology studies relevant to the induction of hepatic microsomal P450s and details biochemical, pharmacological and pharmacokinetic information used to characterize induction by PD138142-15 and the significance of these findings in the evaluation of this novel ACAT inhibitor.

MATERIALS AND METHODS

Compounds

PD138142-15 was synthesized in-house as previously described [1]. PD18215, PD138813 and PD142812 were also synthesized in-house. The structures of these compounds are indicated in Fig. 1. The nuclear magnetic resonance, infrared, and mass spectra for all compounds were consistent with the structures of the compounds, and the purity of the compounds used was > 99% as determined by HPLC and elemental analysis. PB, β-NF, and ERY were obtained from the Sigma Chemical Co. (St. Louis, MO).

Animals

Adult feral cynomolgus monkeys (2.0 to 7.0 kg) were obtained from Charles River Research Primates (Port Washington, NY); adult beagle dogs (7.6 to 15.4 kg) were obtained from Marshall Research Animals (North Rose, NY); and Sprague–Dawley rats (200 to 225 g) and male Hartley guinea pigs (450 to 500 g) were obtained from Charles River (Portage, MI). Wistar rats (Crl:(WI)BR), weighing from 150 to 225 g, were obtained from Charles River (Kingston, NY). All animals were housed in stainless steel cages in clean, climate-controlled quarters with a 12-hr light cycle. Water and food (normal chow) were available *ad lib*. High cholesterol diets used for dog efficacy studies were prepared as described by Krause *et al.* [3].

Drug administration

PD138142-15 was administered orally in single daily doses as bulk drug in capsules to dogs and as a suspension in 0.5% methylcellulose to monkeys, rats and guinea pigs.

Experimental designs

Rat. Data represent work combined from four separate rat studies as follows: (1) Microsomal analyses: total spectral P450 (P450), EROD, PROD, and END were performed on samples from male and female Sprague–Dawley rats dosed with either 0 (control) or 100 mg/kg of PD138142-15, PD18215, PD138813 or PD142812 (females only for the last three compounds) for a period of 7 days. (2) Immunoblots were performed on separate groups of Sprague–Dawley rats, similarly treated. (3) CAT and β-OX assays were conducted on crude peroxisomal fractions from Wistar rats (5/sex/dose) dosed with 0, 50, 250 or 750 mg/kg PD138142-15 for a period of 14 days. (4) A pharmacokinetic study was conducted in which male and female Wistar rats (3/sex/dose) were administered 50, 100, 250 or 500 mg/kg for a period of 14 days. Plasma PD138142-15 concentrations were determined predose, and 0.5, 3.0, 5.0 and 8.0 hr after dose on days 1 and 14. From these data, noncompartmental pharmacokinetic parameters (C_{max}, T_{max}, and AUC) for each dosing interval (0–24 hr) were calculated by standard methods.

Dog. Microsomal (except for END) and peroxisomal assays were conducted with tissue from dogs (2/sex/dose) that received 0, 30, 100 and 300 mg/kg daily for 2 weeks. Plasma drug concentrations were determined predose, and 1, 3, 6, 12 and 24 hr after dose on days 1 and 10, and pharmacokinetic parameters were calculated. A major concern with autoinducing compounds is whether or not efficacy is maintained after the dramatic decreases in plasma drug levels. To assess this, female dogs (6/group) maintained on a high-cholesterol diet were administered PD138142-15 for a period of 47 days at doses of 0, 1 and 30 mg/kg. Serum cholesterol levels were monitored on days 1–7, 10, 12, 14, 21, 28, 35, 42, and 46, and serum lipoproteins were profiled in control and 30 mg/kg treated animals at study termination.

Microsomal parameters including total P450, EROD, PROD and END were determined at study termination (the END values from this study are included in Table 2).

Monkey. Monkeys (2/sex/dose) were dosed daily for 2 weeks with 0, 10, 50 or 100 mg/kg PD138142-15. Plasma drug concentrations were determined 0.5, 1, 2, 3, 4, 8 and 24 hr after dose on day 10, and pharmacokinetic parameters were calculated. Measured microsomal parameters included total P450, EROD, and PROD.

Guinea pig. Male guinea pigs (2/dose) were dosed for 14 days with 0, 30 or 100 mg/kg PD138142-15. Measured microsomal parameters included total P450, EROD, and PROD.

Tissue preparation

At the conclusion of dosing, rats and guinea pigs were killed by CO_2 or ether inhalation. Dogs and monkeys were anesthetized with intravenous 4% Surital and subsequently exsanguinated. Livers, or portions thereof, were rapidly removed, weighed and placed on ice. Homogenates, crude peroxisomal fractions [4], and/or microsomal fractions [5] were prepared immediately and then stored at −70° until used.

Tissue assays

Microsomal protein content was determined using the method of Lowry *et al.* [6]. Total spectral P450 was determined as described by Guengerich [5]. EROD and PROD were determined as described by Burke *et al.* [7], and END was assayed as described by Ciaccio and Halpert [8]. Immunochemical assessments of P450 1A, 2B and 3A isozymes were performed using Western blot kits supplied by Oxygene (Dallas), which utilize rabbit anti-rat P450 protein antibodies. Microsomes from dogs and rats treated with β-NF, PB or ERY (rats only) served as positive controls for the biochemical and/or immunochemical assessment of P450 1A, P450 2B/3A and P450 3A, respectively. Positive control dosing regimens were as previously described [9–12]. Positive control standards were not available for monkey or pig microsomes. CAT and β-OX were conducted using the procedures of Bieber and Fiol [13] and Lazarow [14], respectively. CAT and β-OX were measured as an indirect determination of P450 4A, since induction of the 4A isozyme is usually accompanied by peroxisome proliferation [15, 16]. Crude peroxisomal fractions from CIPRO-treated rats served as the positive control for both assays [17].

Serum assays

Serum cholesterol levels and high performance gel filtration chromatography (HPGC) lipoprotein profiles were determined as previously described [3, 18].

Plasma drug level determination

Plasma PD138142-15 concentrations were determined by HPLC. Briefly, to 200 μL of plasma sample was added 10 μL of water containing 50 μg/mL internal standard [2,6-bis(1,1,-dimethylethyl)-phenyl [[2,6-bis(1-methylethyl)phenoxy]sulfonyl]-carbamate (PD140511), followed by 400 μL acetonitrile. The components were mixed and centrifuged (2000 *g*). The supernatant was combined with 2.5 mL water, and this mixture was then applied to an Icc C-18 cartridge (Bond-Elut, Analytichem, Harbor City, CA). The column was washed with 2 mL of water, and analytes were eluted with 0.5 mL of 65% acetonitrile in 0.02% ammonium phosphate. A 65-μL aliquot was injected into the HPLC (655A-40, Hitachi, Tokyo, Japan). Analytes were separated on a C-8 column (VYDAC, 4.6 × 100 mm, The Separations Group, Hesparia, CA) and eluted isocratically with acetonitrile:0.02 M ammonium phosphate:8.5% phosphoric acid (650:350:0.15) at a flow rate of 0.9 mL/min (SP8810, Spectra-Physics, San Jose, CA). Eluate was monitored at 215 nm, and drug concentration was quantitated by peak height ratios (model V-4, ISCO, Lincoln, NE). The method was linear from 0.100 to 5.000 μg/mL. Noncompartmental pharmacokinetic parameters, maximum plasma concentration (C_{max}), time for C_{max} (T_{max}), and area under the plasma concentration time curve from time zero to the last detectable concentration (AUC) were determined by standard methods.

Table 1. Control values for biochemical determinations

Assay	Units	Rat (N = 9)*		Dog (N = 2)†		Monkey (N = 2)		Guinea pig (N = 2)
		♂	♀	♂	♀	♂	♀	♂
P450‡	nmol/mg§	0.36 ± 0.014‖	0.31 ± 0.01	0.39	0.22	0.65	0.83	0.78
EROD	pmol/min/mg	68.4 ± 5.5	64.0 ± 4.7	58.5	41.4	75.8	131.3	35.0
PROD	pmol/min/mg	11.4 ± 3.4	3.25 ± 0.2	16.5	10.5	0.78	1.10	0.90
END	nmol/min/mg	0.38 ± 0.04	ND¶	ND	0.52 ± 0.13	ND	ND	2.07
CAT	nmol/min/mg	0.46 ± 0.09	1.51 ± 0.03	2.52	1.9	4.74	5.00	ND
β-OX	nmol/min/mg	5.59 ± 0.51	5.32 ± 0.91	5.47	6.09	1.98	1.31	ND

* N = 5 for CAT and β-OX assays.
† N = 6 for END.
‡ Total spectral P450.
§ mg = mg of microsomal protein.
‖ Mean ± SEM.
¶ ND = not determined.

Table 2. Species comparison of microsomal assays*†

Assay	PD-138142-15 dose‡	Rat M	Rat F	Dog M	Dog F	Monkey M	Monkey F	Guinea pig M
P450	L			177	327	278	182	
	M			179	305	231	193	253
	H	144	123	185	300	248	195	236
	PB§	363			609			
EROD	L			66	89	195	109	
	M			51	82	176	117	318
	H	170	140	42	51	207	101	369
	β-NF§	3333			5108			
	PB§	498			145			
PROD	L			185	450	54	75	
	M			264	407	59	80	89
	H	282	145	271	221	22	74	100
	β-NF§	38			151			
	PB§	9160			3087			
END	L				275			
	M				558			210
	H	226						206
	PB§	681			792			
CAT	L	180	105	68	109	99	109	73
	M	200	128	99	117	74	117	85
	H	174	114	96	127	114	127	88
	CIPRO§	2983						
β-OX	L	106	111	88	117	78	93	
	M	89	127	93	127	80	140	
	H	98	96	69	96	74	110	
	CIPRO§	2404						

* Data are reported as percent of control (mean).

† The numbers of animals are the same as in Table 1. Rat positive control represents a single determination of pooled microsomes from five rats. Dog positive control represents the result from a single animal.

‡ Relative dose levels (L = Low, M = Mid, H = High). Dose levels for each species are described under experimental design.

§ Positive control.

RESULTS

Microsomal assays

Control values for biochemical assays conducted on microsomes from the species examined in this report are indicated in Table 1. Table 2 summarizes P450-related data collected from various species. PD138142-15 increased hepatic microsomal total cytochrome P450 in all species tested. Although doses varied slightly, male and female rats appeared to be the least sensitive (as determined by total P450) and male guinea pigs, male monkeys and female dogs the most sensitive. Functional assays designed to differentiate contributions of several different isozymes were used to identify the relevant contribution of each isozyme. EROD, PROD, CAT and β-OX activities varied with treatment in all species, but in all cases the changes were slight when compared with increases in activities noted with positive control compounds in rat or dogs (< 15% change relative to increase produced by positive control—Table 2). END activities were increased relative to control in the three species in which it was tested (rat, dog and pig). Although END activity was increased by no more than 5.6-fold in any species, these increases (26–70% relative to increases produced by the positive control) were substantial. While the positive controls produced the greatest increase in their respective P450 isozymes, there was clear evidence of increased immunodetectable P450 3A protein in dogs and rats. The density of the P450 3A immunoreactive band was greater in PD138142-15-treated monkeys than in controls; however, the difference was slight when compared with the increased density noted in the dog or rat. Immunoblots of rat microsomal P450 2B revealed that the rat with the highest P450 level and PROD activity had increased levels of immunochemically identified P450 2B (Fig. 2). However, analyses of the rest of the animals revealed that this animal was an exception, with no other animal demonstrating increased P450 2B (see Fig. 3). Immunochemical assessment of P450 1A was unaffected by PD138142-15 treatment in any species.

Induction profile of potential breakdown products

The potential for PD138142-15 to break down in gastric acid was identified during preclinical evaluation. These breakdown products (PD18215, PD138813 and PD142812) did not inhibit ACAT *in vitro* and were inactive in the acute cholesterol-fed

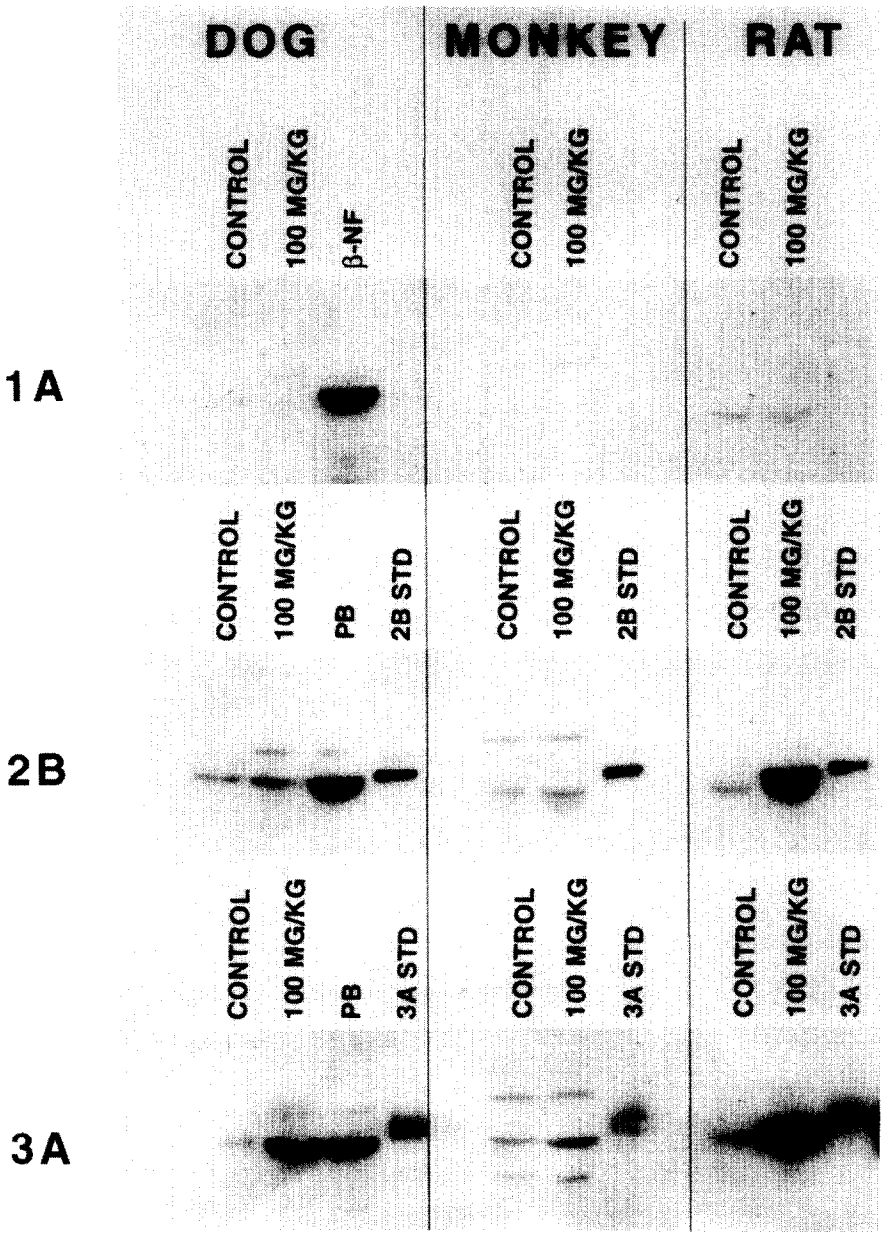

Fig. 2. Species comparison of immunodetectable P450 1A, 2B and 3A using rabbit anti-rat primary antibodies. Results from control microsomes and microsomes from dogs, monkeys and rats treated with 100 mg/kg PD138142-15 are presented. Samples (75 μg of microsomal protein) from each group having the highest total spectral P450 levels were selected for the species comparison. PB = phenobarbital, and β-NF = β-naphthoflavone; both were used as positive controls. Note that rat P450 2B was increased in the sample presented here, which was an exception (see Fig. 4 and text for explanation). Twenty microliters of reconstituted purified rat protein standards (STD) supplied by the manufacturer was used in Fig. 3 and in Fig. 4. The standards supplied by the manufacturer were reportedly obtained from PB-treated rats (the strain was not specified); however, both the 2B and 3A standards ran heavier than microsomes from PB-treated rats in our experiments. The reason for this discrepancy is not known.

rat model.* However, it was unknown if these products, if formed, contributed to the induction noted in animal studies. Therefore, induction profiles of the three potential breakdown products were compared in female rats (Table 3). Total hepatic microsomal cytochrome P450 was increased by PD138142-15, PD138813 and PD142812 (23, 19 and 43%, respectively) but decreased by PD18215 (26%). EROD was elevated slightly by PD142812 (≈2-fold) while unaffected by PD18215 and PD138813. The

* Unpublished data.

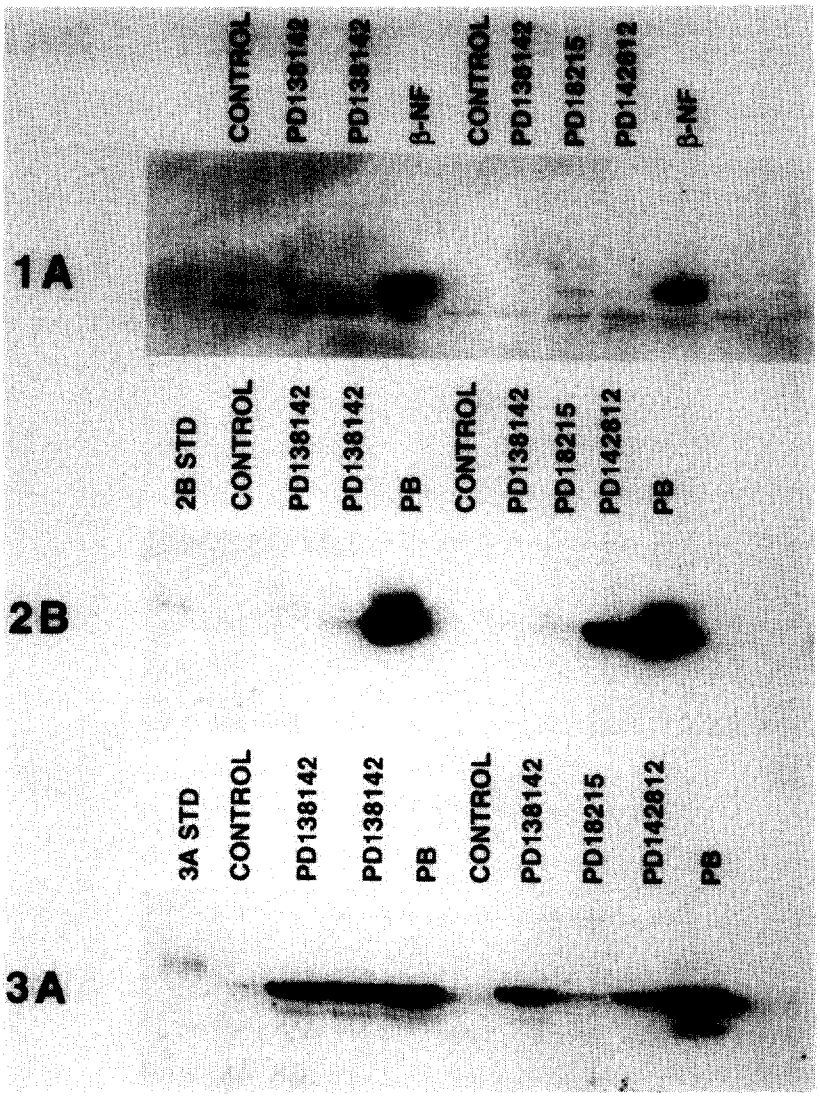

Fig. 3. Effect of acidic breakdown products of PD138142-15 (PD138142) on rat immunodetectable P450 1A, 2B and 3A. Microsomal protein (75 μg) was added to each lane. Note that PD138142-15 had little effect on P450 2B from three different animals (lanes 3, 4 and 7).

Table 3. Induction profiles of PD138142-15 and potential breakdown products in female rats*

Treatment†	N	P450 (nmol/mg)	EROD (pmol/min/mg)	PROD (pmol/min/mg)
Control	9	0.31 ± 0.01	64.0 ± 4.7	3.25 ± 0.2
PD138142-15	3	0.38 ± 0.04	89.4 ± 5.6	4.83 ± 0.8
PD18215	6	0.23 ± 0.05	60.2 ± 4.5	4.26 ± 0.3
PD138813	6	0.37 ± 0.02	67.79 ± 5.5	31.5 ± 2.4‡
PD142812	6	0.44 ± 0.04‡	132.0 ± 10.6‡	78.2 ± 11.5‡

* Values are mean ± SEM.
† All drugs were administered as daily 100 mg/kg doses for 7 consecutive days.
‡ Significantly different from control by Dunnett's test ($P < 0.05$).

Table 4. Pharmacokinetic parameters for rat, dog and monkey combined sexes*

Species	PD-138142-15 dose (mg/kg)	Day	C_{max} (μg/mL)	AUC (0–24) (μg·hr/mL)	T_{max} (hr)
Rat	50	1	17.2 ± 7.6	92.1 ± 45.7	3.0 ± 1.8
		14	2.48 ± 1.0	10.3 ± 3.4	1.3 ± 1.0
	100	1	38.4 ± 17.9	195.0 ± 106.1	6.2 ± 2.2
		14	10.3 ± 5.6	13.6 ± 1.4	3.0 ± 4.4
	250	1	59.5 ± 30.8	611.0 ± 85.3	7.0 ± 1.6
		14	11.2 ± 10.9	63.6 ± 43.6	3.4 ± 2.2
	500	1	90.1 ± 15.6	1190 ± 178.5	7.5 ± 1.2
		14	14.4	137.0	4.0
Dog	30	1	37.8 ± 37.4	262 ± 217	3.8 ± 1.5
		10	4.98 ± 4.53	14.5 ± 12.0	3.8 ± 1.5
	100	1	158 ± 1.09	1266 ± 924	3.8 ± 1.5
		10	39.0 ± 44.5	189 ± 185	4.5 ± 1.7
	300	1	376 ± 97.8	3206 ± 834	7.5 ± 3.0
		10	10.7 ± 7.30	66.4 ± 33.9	6.0 ± 4.2
Monkey	10	10	0.60 ± 0.37	1.30 ± 0.70	1.1 ± 0.6
	50	10	1.68 ± 0.44	7.30 ± 2.63	1.9 ± 1.6
	100	10	2.83 ± 0.59	20.90 ± 13.79	1.6 ± 1.1

* Values are means ± SEM. N = 6, 4 and 4 for rat, dog and monkey, respectively.

most remarkable findings were significant increases in PROD activity produced by PD142812 (24-fold) and PD138813 (10-fold) relative to either control or PD138142-15-treated animals. To ascertain if the increase in PROD activity was due to an increase in P450 2B protein, immunoblots using 1A, 2B and 3A antibodies were performed (Fig. 3). Microsomes from PD138813-treated animals were not analyzed. None of the tested compounds had any effect on P450 1A. As expected, PD138142-15 increased P450 3A but had no effect on P450 2B. PD18215 slightly increased 3A protein relative to control but had little effect on P450 1A or 2B. Although PD142812 increased P450 3A protein, the increase was less than noted with PD138142-15. In addition, PD142812 produced a marked increase in P450 2B protein, correlating to the increase in PROD activity.

Pharmacokinetic analyses

Rat plasma drug level data (combined sex) are summarized in Table 4. Dose-related increases in both C_{max} and AUC occurred on both days 1 and 14. However, PD138142-15 concentrations at each dose dropped substantially (approximately 90% at each dose) between days 1 and 14. T_{max} values at each dose also declined between days 1 and 14. Dogs treated with PD138142-15 exhibited similar reductions in plasma drug levels within 10 days with a greater than 85% decrease in C_{max} and AUC at all dose levels. Day 10 pharmacokinetic data for monkeys are given in Table 4. Both AUC and C_{max} increased in a dose-related fashion; however, both AUC and C_{max} values were less than those noted at similar doses in rats and dogs.

Dog efficacy study

PD138142-15, at doses of 1 and 30 mg/kg, reduced mean serum cholesterol in cholesterol-fed dogs in a progressive fashion through the first 14 days of dosing. After 14 days, mean serum cholesterol levels appeared to stabilize for both the 1 and 30 mg/kg dose groups at levels approximately 20 and 35% less than cholesterol-fed controls, respectively (Fig. 4). The decreases, relative to pretest (day 0), were statistically significant for all time points at the 30 mg/kg dose, whereas the decreases were statistically significant only on days 5 through 14 for the 1 mg/kg dose group. At 30 mg/kg, mean serum cholesterol appeared to rebound slightly between days 15 and 35 but subsequently declined. Compared with cholesterol-fed controls, relative and absolute

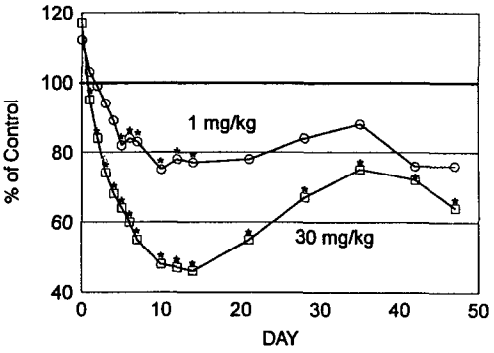

Fig. 4. Serum cholesterol levels in dogs administered either 1 or 30 mg/kg PD138142-15 for 6 weeks. Each data point represents the mean of six animals plotted as a percentage of mean serum cholesterol determined for six corresponding control animals. Day 0 cholesterol levels were 362 ± 25, 404 ± 53 and 424 ± 65 mg/dL for the high cholesterol control, 1 mg/kg and 30 mg/kg groups, respectively. Key: * = significantly different from pretest—day 0 (Dunnett's test, $P < 0.05$).

Table 5. Effect of PD138142-15 (30 mg/kg) on female dog lipoprotein distribution after 47 days of treatment*

Treatment		VLDL	LDL	HDL
Control	Absolute (mg/dL)	11 ± 1	159 ± 17	200 ± 3
	Relative (%)†	3.0 ± 0.3	42.3 ± 2.6	54.7 ± 2.7
PD138142-15	Absolute (mg/dL)	6 ± 1‡	59 ± 8†	216 ± 18
	Relative (%)	2.2 ± 0.5	20.9 ± 2.0‡	76.9 ± 2.1‡

* Values are means ± SEM (N = 6).
† Relative = percent of total cholesterol.
‡ Significantly different from control by Student's *t*-test ($P < 0.05$).

serum LDL and absolute VLDL were decreased significantly (63, 51 and 45%, respectively), whereas relative HDL was increased 40% (Table 5).

DISCUSSION

It is evident from the accumulated data that PD138142-15 is an inducer of hepatic microsomal P450. In particular, the data are consistent with induction of the 3A isozyme, although the possibility exists that other, not yet quantified, isozymes were also induced. The induction of canine and rodent P450, accompanied by marked decreases in plasma drug levels within 2 weeks, suggests that the compound is an autoinducer in these two species. The fact that induction was noted in all four species tested, and that induction occurred at doses as low as 1 mg/kg in dogs* suggest that induction in humans will be a likely consequence of PD138142-15 treatment.

Although a number of ACAT inhibitors are currently under development [19], information on hepatic microsomal induction by other potential therapeutic agents of this class is limited. CI-976, another ACAT inhibitor developed in our laboratories, was found to be a mild "phenobarbital-like" inducer of rat hepatic microsomal P450, inducing both the 2B and 3A isozymes [20]. The ACAT inhibitor CI-984 was found to cause canine hepatocellular hypertrophy and hypereosinophilia consistent with SER proliferation, although further characterization of microsomal induction was not performed [21]. While experience with other ACAT inhibitors in our laboratories suggests that induction of hepatic P450s by this class of compounds is not uncommon, the paucity of data on P450 effects by ACAT inhibitors of divergent chemical structure prevents definitive conclusions.

The relationship between P450 induction (in its broadest sense), lipoprotein regulation and heart disease has been well documented previously [22–25]. Therefore, the finding of microsomal induction by a putative lipid regulating compound is of more than just anecdotal interest. In addition to the ACAT inhibitors mentioned here, lovastatin, an HMG-CoA reductase inhibitor (RI), has been demonstrated to be an inducer of microsomal P450 2B1/2 in an *in vitro* model [16], and pravastatin (another RI) increased urinary 6β-hydroxycortisol (a marker of P450 3A) in humans [26]. These findings, along with the well described effect of fibrates on P450 4A [27], suggest that P450 induction by lipid regulating/anti-atherosclerotic activity is not unusual. Although a correlation between induction and anti-atherosclerotic activity has been observed, there has been little mechanistic work explaining the nature of such a link. Recently, Kocarek *et al.* [16] suggested that lovastatin modulated P450 2B1/2 induction in adult rat hepatocytes via alterations in oxysterol intermediates. Whether or not this idea can be expanded to other types of lipid regulators and whether this idea holds true for induction of other isozymes remain to be seen. Our work does not support a consistent link between induction and lipid regulation. PD142812 was clearly an inducer of P450 2B but was not efficacious as a plasma lipid regulator. In addition, the induction of different isozymes noted between PD142812 and PD138142-15 would be difficult to explain by a common intermediate such as an oxysterol. However, the studies described here were not designed to examine this relationship, and the role of the P450s in blood lipid regulation and heart disease will remain an area of active research.

The efficacy of PD138142-15 in cholesterol-fed dogs was demonstrated by both decreased total serum cholesterol and serum LDL, while absolute HDL remained unchanged and relative HDL actually increased over the course of the study. The effects are consistent with the reported efficacy of PD138142-15 in rats [1]. An interesting facet of our investigation was that the efficacy of PD138142-15 was maintained despite markedly reduced plasma drug levels. Several possibilities exist that may explain this phenomenon. Efficacy may be related to a metabolite of the parent compound. Another possibility is that the efficacious actions of PD138142-15 occur pre-metabolism, perhaps at the level of the gut. It could also be argued that the initial high plasma levels of PD138142-15 produce a long-term effect on plasma lipid regulation. However, efficacy was rapidly lost after cessation of drug treatment.† If the toxicity noted with PD138142-15‡

* Wolfgang GHI, Robertson DG and Metz A, Manuscript submitted for publication.

† Unpublished data.

‡ Wolfgang GHI, Robertson DG and Metz A, Manuscript submitted for publication.

is due to the parent molecule, this might be an unusual situation where induction increases the safety margin, by lowering plasma levels, while efficacy is maintained. Future work identifying metabolites produced in the various species should help clarify the situation.

Breakdown of PD138142-15 in the gut could not have been extensive in that efficacy was achieved and maintained. Induction profiles in rats were consistent with the parent but not with any of the breakdown products. Therefore, if the breakdown products were formed, they either did not occur in any great quantity, were not absorbed, or were themselves rapidly metabolized. One possible exception was the single rat noted to have a "phenobarbital-like" induction profile consistent with PD142812. The distinct difference in isozyme profiles induced by PD142812 vs PD138142-15 provides some interesting structure–activity data for mechanistic studies of P450 2B and 3A induction.

Although EROD, PROD, and END activities were used to assess induction in all species, a legitimate concern is the appropriateness of using these compounds as isozyme-specific probes in species other than rodents. Although EROD has been used as a specific probe in both dog and monkey studies [28, 29], the use of PROD as a probe in these species has been much less studied. We found that β-NF and PB induced dog hepatic microsomal EROD and PROD activities, respectively, correlating with increases in immunoreactive 1A and 2B proteins, which supports the appropriateness of these probes for use in the dog. In addition, the rabbit anti-rat P450 2B antibody supplied in the Western blot kit inhibited PB-induced canine PROD activity by over 70%. END activity in dog microsomes has been suggested to be due partly to the 3A isozyme (PBD-1) but also to other isozymes as well [8]. Therefore, although EROD, PROD and END appear to be appropriate markers of P450 1A, 2B and 3A activity in the dog, interpretations as to their specificity should be made with caution. The appropriateness of these biochemical assays in the monkey and guinea pig is even less clear. Although EROD appears to be a suitable marker for P450 1A in the cynomolgus monkey [30], work by Bullock *et al.* [31] and Weaver *et al.* [30] demonstrated that PROD was not a suitable marker for P450 2B in the monkey, and until adequate validation of other probes in these species is conducted, the utility of functional probes for the determination of isozyme profiles, in the absence of immunochemical analyses, is questionable.

It was evident that the rat was the least sensitive (at least by the methods employed in our laboratory) to induction produced by PD138142-15. Given the small increases in total P450 (44%) and only slight increases in functional activity (<3-fold) seen in the rat studies, investigators conducting routine screening for P450 induction in the rat may have dismissed these slight increases. Only the immunochemical analyses definitively identified PD138142-15 as an inducer in rats, and immunoblots, in the absence of other changes, are not always included in routine screening procedures. This work, as well as other work in our laboratory [32], suggests that the rat may not be the best species for routine screening (total P450 and functional assays) of hepatic microsomal P450 induction. The important question for the pharmaceutical industry to address is which animal model serves as the best predictor of human response. As another primate, the monkey might be assumed to be the best model for induction in humans, but recent work [30] suggests that this may not be the case. The monkey and dog data reported here were, by necessity, obtained from a very limited number of animals, and the use of dogs and monkeys in routine screening would raise a number of practical and ethical considerations making the choice of these species unlikely. Unfortunately, the question of the best model still has not been answered definitively and may vary with chemical or therapeutic class.

In summary, PD138142-15 was found to be an inducer of hepatic cytochrome P450 3A in rats and dogs. Hepatic cytochrome P450(s), as determined by total spectral P450, was also induced in monkeys and male guinea pigs. Like the dog and rat, data from the monkey and guinea pig suggest induction of P450 3A; however, the extent of response and the specificity of the probes employed make this conclusion tentative. Potential acid breakdown products of PD138142-15 have induction profiles distinctly different from that of PD138142-15, and probably are not involved in induction produced by the parent compound. Marked decreases in plasma drug levels over 2 weeks suggested that the compound is an autoinducer in dogs and rats. Interestingly, efficacy was maintained for up to 6 weeks in spite of the apparent autoinduction.

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