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THE RELATIVE HYPOLIPIDAEMIC ACTIVITY AND HEPATIC EFFECTS OF CIPROFIBRATE ENANTIOMERS IN THE RAT*

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Abstract—The aim of this study was to establish whether the individual enantiomers of racemic ciprofibrate, a potent hypolipidaemic agent and peroxisome proliferator, differ significantly in either pharmacological potency or toxic potential. After a single oral dose to male Fischer F344 rats at dosages below 10 mg/kg, *S*(−) ciprofibrate produced slightly, but statistically significantly, greater reductions in plasma concentrations of cholesterol than *R*(+) ciprofibrate. Similarly, at low concentrations in F344 rat hepatocyte cultures, *S*(−) ciprofibrate produced slightly, but statistically significantly, greater inductions of peroxisomal β-oxidation activity than *R*(+) ciprofibrate. However, after seven daily doses, the differences in pharmacological effects of the two enantiomers were no longer apparent. Furthermore, in contrast to its effects *in vitro*, *R*(+) ciprofibrate produced slightly, but statistically significantly, greater inductions of peroxisomal β-oxidation activity *in vivo* than *S*(−) ciprofibrate. These observations may be possibly explained on the basis that following multiple dosing, plasma concentrations of *R*(+) ciprofibrate 24 hr post-dose were greater than those of its optical antipode. Thus the slightly greater potency of the *S*(−) enantiomer after a single dose may have been overcome by the greater plasma concentrations of the less potent enantiomer. Both enantiomers produced similar reductions in plasma concentrations of thyroxine. The data indicate that at low dosages *S*(−) ciprofibrate is a slightly more potent hypolipidaemic agent after a single dose in rats and a slightly more potent peroxisome proliferator at low concentrations *in vitro*. However, following multiple dosing, both enantiomers produced changes in plasma concentrations of lipids, hepatic enzyme activities and plasma concentrations of thyroxine which were of comparable magnitude to those produced by the racemate. Since these early changes have been linked mechanistically to the chronic toxicity of the racemate in the rat, it could be predicted that the individual enantiomers of ciprofibrate under conditions employed in chronic safety studies, would produce the same spectrum of rodent toxicity as the racemate.

Key words: hepatocytes; peroxisomes; liver; hypocholesterolaemia; hypotriglyceridaemia; thyroxine

Ciprofibrate (2-[4-(2,2-dichlorocyclopropyl)phenoxy]isobutyric acid) is a phenoxyisobutyrate hypolipidaemic agent which contains a single chiral centre (in the dichlorocyclopropyl moiety) (Fig. 1) and is administered as a racemic mixture. In common with other members of this class of compounds, ciprofibrate produces a pleiotropic response in the livers of rats and mice. The initial response, which is maximal following daily administration for 10–14 days, is characterized by hepatomegaly (consisting of hypertrophy and hyperplasia), proliferation of smooth endoplasmic reticulum, some alterations in mitochondrial number and structure and, most strikingly of all, extensive proliferation of peroxisomes with concomitant induction of associated enzymes [1]. In addition, as with many other peroxisome proliferators, chronic administration of ciprofibrate to rats and mice produces hepatocellular adenomas and carcinomas [1]. However, in spite of their carcinogenic activity in rats and mice, peroxisome proliferators have proved negative in a range of tests for genotoxicity [2–4]. This had led to the hypothesis that maintenance of the early extensive change in peroxisomal numbers and induction of associated enzymes is mechanistically related to the carcinogenic activity of such

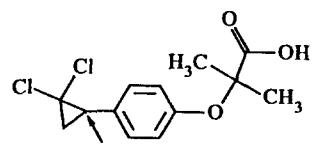

Fig. 1. Molecular structure of ciprofibrate. The position of the chiral centre is indicated by the arrow.

* All research involving animals described in this publication was performed in accord with the Sterling Winthrop Pharmaceuticals Research Division Policy on Animal Use and all national and federal legislation. All Sterling Winthrop Pharmaceuticals Research Division animal facilities and programs are accredited by the American Association for Accreditation of Laboratory Animal Care.

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compounds. Furthermore, it has been suggested that peroxisome proliferators represent a novel class of non-genotoxic carcinogens [5].

In addition to its effects on rodent liver, ciprofibrate produces thyroid follicular cell hyperplasia in rats following chronic treatment. This is believed to occur as a consequence of increased hepatic clearance of thyroxine (T_4)* which is reflected by decreased serum concentrations of T_4, a change which occurs following short term treatment [6, 7].

The ability of biological systems, e.g. receptors and enzymes, to distinguish between enantiomers of pharmaceutical agents has, in some instances, led to the observation that the pharmacological activity of a particular compound is entirely attributable to a single enantiomer. For example, the prostaglandin synthase inhibitory action of Indoprofen is associated almost entirely with the *S*(−) enantiomer, the *R*(+) antipode being virtually inactive [8]. However, a more common observation is that of enantiomers exhibiting different degrees of efficacy when administered *in vivo*. This is usually due to stereoselective metabolism and/or excretion leading to lower plasma concentrations of one enantiomer relative to its antipode. Thus, *S*-hexobarbital is a more potent hypnotic than *R*-hexobarbital; the latter being more extensively metabolized than the former [9].

The phenomenon of stereoisomerism may lead to other therapeutically important possibilities, of which the ability to dissociate efficacy and toxicity of a racemate is of particular importance. Examples of this are rare, but known. For example, the myotoxicity of structural analogues of clofibrate has been shown to be attributable to the *R*(+) enantiomer only, while both the *R*(+) and the *S*(−) enantiomers possess hypolipidaemic activity [10, 11]. Thus, the aim of this study was to investigate the relative pharmacological and toxicological effects of racemic ciprofibrate and its *R*(+) and *S*(−) enantiomers in male Fischer rats following daily oral dosing. Additionally, in order to assess intrinsic potencies of the enantiomers of ciprofibrate to produce peroxisome proliferation without the complicating influence of the processes of absorption, distribution or metabolism, induction of peroxisomal β-oxidation activity was determined in primary rat hepatocyte cultures following exposure to racemic, *R*(+) or *S*(−) ciprofibrate for 72 hr.

MATERIALS AND METHODS

Resolution of ciprofibrate enantiomers. Individual enantiomers of ciprofibrate were synthesised from racemic 2,2-dichlorocyclopropylbenzene involving resolution of the intermediate 4-(2,2-dichlorocyclopropyl)aniline [12]. Optical purity of the resolved enantiomers was 99% [12].

In vivo *studies.* Male Fischer F344 rats (six per group, 6 weeks old, Charles River, U.K.) were dosed by oral gavage once every 24 hr for 7 days with racemic, *R*(+) or *S*(−) ciprofibrate at dosages of 1, 3 or 10 mg/kg. Control animals received an equivalent volume (5 mL) of vehicle only (0.25% aqueous gum tragacanth). Dosages were selected on the basis of data from a previous study (data not shown) with the racemate which indicated they would produce dose-related pharmacological effects after a single dose and that after multiple dosing they would adequately represent the dose–response for induction of peroxisomal enzymes and reductions in plasma concentrations of thyroxine. In a subsequent single dose study, a lower dosage of 0.3 mg/kg was added to this range of dosages to characterise more fully the dose–response relationship for hypolipidaemia.

Blood samples (250 μL into lithium heparin) were taken via the tail vein 24 hr after a single dose and 24 hr after the seventh dose for determination of plasma concentrations of cholesterol and triglycerides using commercially available kits (Roche Diagnostics) and a Cobas Fara centrifugal analyser. In addition, a further 250 μL of blood (into lithium heparin) was taken from the tail vein for determination of plasma concentrations of racemic, *R*(+) or *S*(−) ciprofibrate by HPLC using a Waters fatty acid analysis column (3.9 mm × 30 cm) following solid phase extraction (AASP C_{18} cassette). The mobile phase was 0.01 M phosphate buffer, pH 5.0 : acetonitrile : tetrohydrofuran (13 : 6 : 1 by vol.) containing tetra-*n*-butylammonium hydroxide (5×10^{-3} M) delivered at a flow rate of 2 mL/min. Detection was by UV absorption at 232 nm. Enantiomeric stability was confirmed by chiral HPLC analysis [13] of ciprofibrate in plasma.

Following the administration of ciprofibrate enantiomers for 7 days, 2 mL of blood was obtained (under pentobarbitone anaesthesia) from the orbital sinus for determination of serum concentrations of thyroxine (T_4), triiodothyronine (T_3) and thyroid stimulating hormone (TSH) by radioimmunoassay using commercially available kits (T_4 and T_3: Amersham; TSH: Pituitary Hormones and Antisera Centre, UCLA Medical Centre, CA). Animals were then killed by an intraperitoneal dose of pentobarbitone. Livers were removed, weighed, perfused with ice-cold saline (0.9% w/v) and homogenized (33% w/w) in ice-cold 0.25 M sucrose using a polytron. Aliquots of homogenate were used to determine the activities of the following enzymes: catalase [14], carnitine acetyl transferase [15] and peroxisomal β-oxidation [16]. Excess homogenate was centrifuged at 9000 *g* for 20 min followed by centrifugation of the resulting supernatant at 105,000 *g* for 60 min. Aliquots of the resulting supernatant were used to determine the activities of glutathione transferase [17] and glutathione peroxidase [18]. These were included since they are antioxidant enzymes known to be affected by peroxisome proliferators [19] and consequently are of relevance to the proposed oxidative stress mechanism of carcinogenicity of such compounds [15]. Homogenate and cytosolic protein concentrations were determined according to Lowry *et al.* [20].

In vitro *studies.* Primary rat hepatocytes were isolated from male Fischer F344 rats by a recirculating collagenase perfusion method. The isolated cells

* Abbreviations: DMSO, dimethylsulphoxide; T_3, triiodothyronine; T_4, thyroxine; TSH, thyroid stimulating hormone; CAT, carnitine acetyl transferase.

Table 1. Effect of a single oral dose of racemic, *R*(+) or *S*(−) ciprofibrate on plasma concentrations of cholesterol and triglycerides in male Fischer rats 24 hr post-dose—multiple dose study

Dosage (mg/kg)	Cholesterol (control = 1.95 ± 0.19)			Triglycerides (control = 1.36 ± 0.20)		
	Racemic	*R*(+)	*S*(−)	Racemic	*R*(+)	*S*(−)
1	1.54 ± 0.04	1.66 ± 0.09	1.52 ± 0.08*	0.96 ± 0.13	1.31 ± 0.15	1.07 ± 0.12‡
3	1.46 ± 0.09	1.56 ± 0.08	1.38 ± 0.10†	0.89 ± 0.10	0.89 ± 0.11	0.88 ± 0.21
10	1.25 ± 0.08	1.29 ± 0.05	1.18 ± 0.05*	0.91 ± 0.10	0.83 ± 0.96	0.77 ± 0.13

The values given are the means ± SD for six animals in each group. Units = mmol/L. Racemate, *R*(+) and *S*(−) represent racemic, *R*(+) and *S*(−) ciprofibrate, respectively. All treatments significantly reduced plasma concentrations of cholesterol and triglycerides ($P < 0.001$).

* = $P < 0.05$, † = $P < 0.01$, ‡ = $P < 0.001$ for comparison with *R*(+) ciprofibrate.

Table 2. Effect of a single oral dose of racemic, *R*(+) or *S*(−) ciprofibrate on plasma concentrations of cholesterol and triglycerides in male Fischer rats 24 hr post-dose—repeat single dose study

Dosage (mg/kg)	Cholesterol (control = 1.75 ± 0.08)			Triglycerides (control = 1.09 ± 0.18)		
	Racemic	*R*(+)	*S*(−)	Racemic	*R*(+)	*S*(−)
0.3	1.54 ± 0.07	1.71 ± 0.07NS	1.57 ± 0.04†	1.08 ± 0.14NS	1.23 ± 0.23NS	1.01 ± 0.11NS
1	1.43 ± 0.13	1.60 ± 0.12	1.41 ± 0.13†	1.10 ± 0.17NS	1.20 ± 0.25NS	0.93 ± 0.16NS
3	1.21 ± 0.11	1.35 ± 0.08	1.21 ± 0.10†	0.85 ± 0.10	0.81 ± 0.13	0.73 ± 0.10
10	1.04 ± 0.01	1.10 ± 0.09	1.06 ± 0.09	0.75 ± 0.12	0.61 ± 0.11	0.79 ± 0.21

The values given are the means ± SD for six animals in each group. Units = mmol/L. Racemate, *R*(+) and *S*(−) represent racemic, *R*(+) and *S*(−) ciprofibrate, respectively. All treatments significantly reduced plasma concentrations of cholesterol and triglycerides ($P < 0.01$) except where indicated (NS = not significant).

† = $P < 0.01$ for comparison with *R*(+) ciprofibrate.

were washed three times by centrifugation at 50 *g* and resuspended in culture medium [RPMI 1640 medium containing 5% (v/v) foetal calf serum, 50 μg/mL gentamicin, 0.1 mM hydrocortisone-21-hemisuccinate and 1.0 μM insulin]. Cells were seeded at a density of 1×10^6 viable cells/mL into 60 mm petri dishes in a total volume of 3 mL. Cell viability, determined by trypan blue exclusion, was always greater than 85%. Following a 2 hr attachment period, non-adherent cells were removed by changing the culture medium. Cells were then exposed to racemic, *R*(+) or *S*(−) ciprofibrate (5, 25, 100 or 250 μM), dissolved in dimethylsulphoxide [DMSO, final concentration of 0.4% (v/v)] for 72 hr with culture medium changed every 24 hr (4 dishes/concentration). Control cultures were exposed to an equivalent volume of DMSO only.

Following exposure to ciprofibrate or DMSO, cells were washed with ice-cold buffer (0.154 M KCl/50 mM Tris, pH 7.4), harvested by scraping into 1 mL of the same buffer, lysed by sonication and the lysate assayed for peroxisomal β-oxidation activity [16]. Protein concentrations of the lysates were determined according to Lowry *et al.* [19].

Statistical analysis. Analysis of variance techniques were used in the statistical analysis of all parameters. The model included a factor for treatment group.

RESULTS

In vivo *studies*

Twenty-four hours after a single dose of 1, 3 or 10 mg/kg, both enantiomers of ciprofibrate produced dosage-related reductions in plasma concentrations of cholesterol and triglycerides comparable in magnitude to those produced by the racemate (Table 1). However, *S*(−) ciprofibrate produced slightly, but statistically significantly, greater reductions in plasma concentrations of cholesterol than its optical antipode at all dosages except 10 mg/kg (Table 1). This slight difference in hypocholesterolaemic activity after a single dose was confirmed in the subsequent single dose study (Table 2) in which a lower dosage was included. However, at the lowest dosage used (0.3 mg/kg), the *R*(+) enantiomer failed to produce a statistically significant reduction in plasma concentrations of cholesterol and neither enantiomer or the racemate produced statistically significant reductions in plasma concentrations of triglycerides (Table 2). After multiple dosing, the slight differences in potency and the relationship to dosage of the pharmacological effects of ciprofibrate enantiomers observed after a single dose, were no longer apparent (Table 3).

Both enantiomers and the racemate produced statistically significant, dosage-related reductions (12–54% relative to controls) in plasma concentrations of T_4 when measured 24 hr after the seventh dose (Table 4). The effects of the individual enantiomers and the racemate were not significantly different. Plasma concentrations of triiodothyronine (T_3) and TSH were unaffected at this time by treatment with either enantiomer or the racemate (Table 4).

The racemate and its individual enantiomers

Table 3. Effect of daily oral administration of racemic, $R(+)$ or $S(-)$ ciprofibrate on plasma concentrations of cholesterol and triglycerides in male Fischer rats 24 hr after the seventh dose

Dosage (mg/kg)	Cholesterol (control = 1.65 ± 0.12)			Triglycerides (control = 1.28 ± 0.30)		
	Racemic	$R(+)$	$S(-)$	Racemic	$R(+)$	$S(-)$
1	1.10 ± 0.05	1.10 ± 0.15	1.15 ± 0.09	0.54 ± 0.06	0.68 ± 0.10	0.62 ± 0.11
3	1.05 ± 0.08	1.05 ± 0.05	1.06 ± 0.09	0.59 ± 0.07	0.57 ± 0.10	0.66 ± 0.10
10	1.05 ± 0.14	1.00 ± 0.06	1.10 ± 0.08	0.58 ± 0.20	0.53 ± 0.06	0.66 ± 0.10

The values given are the means ± SD for six animals in each group. Units = mmol/L. Racemate, $R(+)$ and $S(-)$ represent racemic, $R(+)$ and $S(-)$ ciprofibrate, respectively. All treatments significantly reduced plasma concentrations of cholesterol and triglycerides ($P < 0.001$ for all treatments). There were no statistically significant differences between the effects of each enantiomer.

Table 4. Effect of daily oral administration of racemic, $R(+)$ or $S(-)$ ciprofibrate on plasma concentrations of thyroid hormones in the male Fischer rat 24 hr after the final (seventh) dose

Treatment	T_3	T_4	TSH
Control	0.42 ± 0.11	29.2 ± 6.30	2.37 ± 1.46
1 mg/kg racemic	0.37 ± 0.08	22.0 ± 3.20†	1.95 ± 1.13
1 mg/kg $R(+)$	0.41 ± 0.12	23.7 ± 3.71*	2.02 ± 0.51
1 mg/kg $(S-)$	0.49 ± 0.15	25.8 ± 1.00	2.93 ± 0.91
3 mg/kg racemic	0.42 ± 0.06	19.4 ± 3.46‡	2.63 ± 0.77
3 mg/kg $(R(+)$	0.33 ± 0.05	21.4 ± 4.92†	2.05 ± 0.53
3 mg/kg $(S-)$	0.46 ± 0.13	20.8 ± 2.33‡	2.78 ± 1.10
10 mg/kg racemic	0.42 ± 0.05	13.4 ± 1.84†	2.17 ± 0.42
10 mg/kg $R(+)$	0.45 ± 0.10	15.6 ± 2.90‡	2.60 ± 0.86
10 mg/kg $(S-)$	0.42 ± 0.07	15.7 ± 1.95‡	2.72 ± 1.29

The values given are the means ± SD for six animals in each group. Units = ng/mL.

* = $P < 0.05$, † = $P < 0.01$, ‡ = $P < 0.001$ for comparison with control.

produced dosage-related increases in hepatic peroxisomal β-oxidation activity *in vivo* (Fig. 2a). However, while both enantiomers were clearly active at increasing peroxisomal β-oxidation activity, there was a small but statistically significant difference in effect, particularly at the highest a dosage of 10 mg/kg where $R(+)$ ciprofibrate increased peroxisomal β-oxidation activity 25-fold (similar to the racemate) whereas its optical antipode produced a 19-fold increase. Both enantiomers also produced dosage-related increases in hepatic carnitine acetyl transferase (CAT) activity (Fig. 2b). However, in contrast to the effect on peroxisomal β-oxidation activity, at a dosage of 10 mg/kg, the $S(-)$ enantiomer produced a slightly greater increase in CAT activity (87-fold) than the $R(+)$ enantiomer (74-fold; $P < 0.01$) with the effect of the racemate being intermediate (77-fold increase). Both enantiomers and the racemate produced small, comparable increases in catalase activity (1.1–1.53-fold, Fig. 2c). Glutathione-*S*-transferase activity was inhibited to a comparable degree by both enantiomers and racemic ciprofibrate in a dosage-related manner (Fig. 3a). Glutathione peroxidase activity was slightly, but not statistically significantly, inhibited by both enantiomers and the racemate at the top dosage (Fig. 3b).

Racemic, $R(+)$ and $S(-)$ ciprofibrate produced comparable, statistically significant, dosage-related increases (1.17–1.84-fold increase relative to controls) in relative liver weight (Fig. 4). There were no statistically significant differences between the effects of the enantiomers.

Plasma concentrations of racemic, $R(+)$ or $S(-)$ ciprofibrate measured 24 hr after the final dose, increased with dosage (Table 5). However, at all dosages, plasma concentrations of $R(+)$ ciprofibrate were greater (1.75–2.58-fold) than those of $S(-)$ ciprofibrate with concentrations of the racemate being intermediate. Analysis of plasma concentrations $R(+)$ or $S(-)$ ciprofibrate by chiral HPLC indicated that only the administered enantiomer was detected.

In vitro *studies*

Racemic ciprofibrate and its composite enantiomers all produced concentration-related increases in peroxisomal β-oxidation activity determined in hepatocytes following exposure for 72 hr in primary monolayer culture (Table 6). However, at concentrations of 5 and 25 μM, the increases produced by $S(-)$ ciprofibrate were statistically significantly greater than those produced by $R(+)$

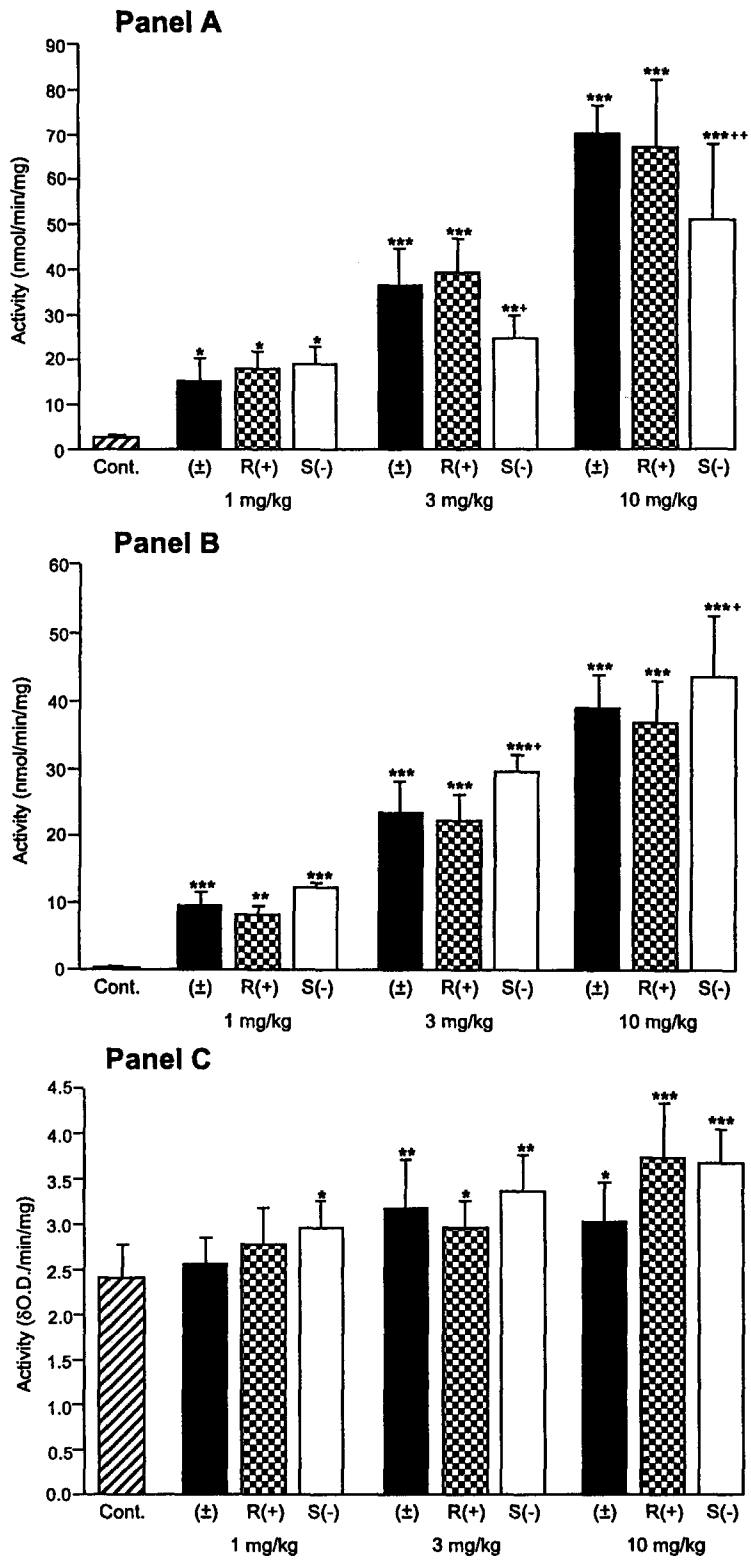

Fig. 2. Effects of racemic (±), *R*(+) and *S*(−) ciprofibrate on hepatic peroxisomal β-oxidation (panel A), carnitine acetyl transferase (panel B) and catalase (panel C) activities following daily oral administration to male Fischer rats for 7 days. Peroxisomal β-oxidation was assessed as cyanide insensitive oxidation of palmitoyl CoA, results are expressed as nmol NAD reduced/min/mg. Catalase activity is expressed as ΔOD/min/mg. Carnitine acetyl transferase activity is expressed as nmol NAD reduced/min/mg. All data shown represent the means ± SD for six animals in each group. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ for comparison with control. +$P < 0.05$, ++ $P < 0.01$ for comparison with optical antipode.

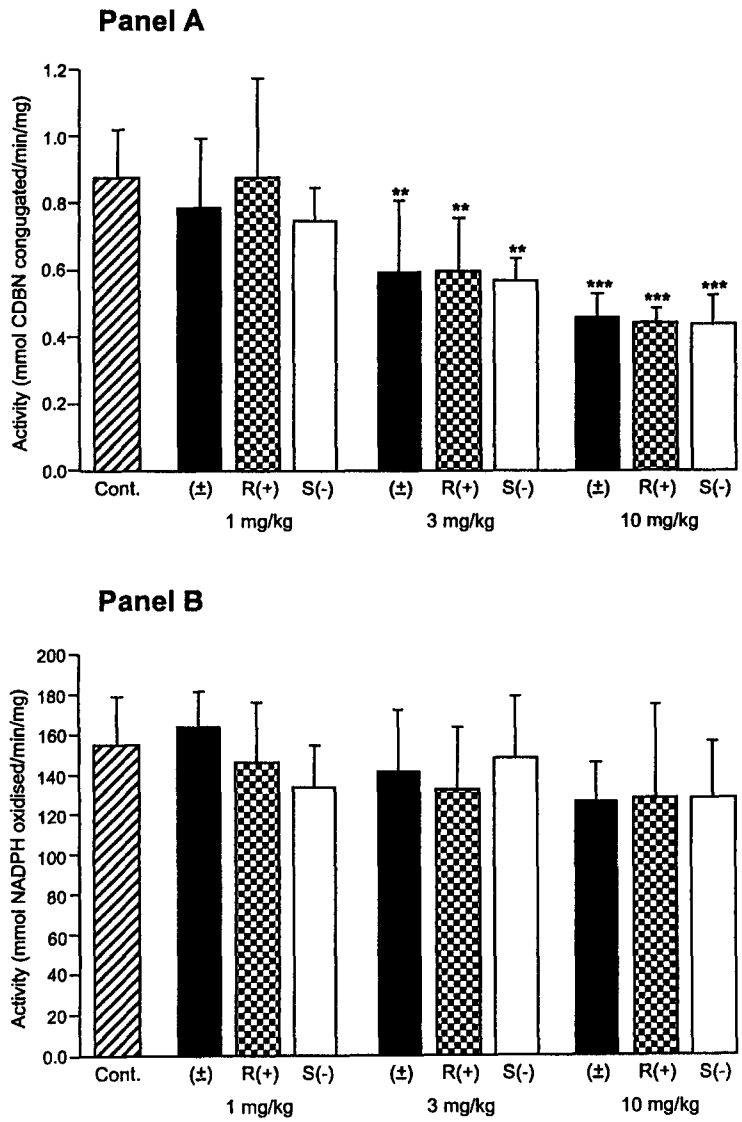

Fig. 3. Effects of racemic (±), $R(+)$ and $S(-)$ ciprofibrate on hepatic glutathione-*S*-transferase (panel A) and glutathione peroxidase (panel B) activities following daily oral administration to male Fischer rats for 7 days. Glutathione-*S*-transferase activity is expressed as mmol CDNB conjugated/min/mg. Units of glutathione peroxidase activity are nmol NADPH oxidised/min/mg. The data presented represent the means ± SD for six animals in each group. $^{**}P < 0.01$, $^{***}P < 0.001$ for comparison with control.

ciprofibrate with the effect of the racemate being intermediate.

DISCUSSION

Previous data describing the hypolipidaemic and hepatic effects of ciprofibrate were generated using a racemic mixture of $R(+)$ and $S(-)$ enantiomers. The relative effects of the composite enantiomers and therefore their contribution to the effects observed with the racemate, have until now not been investigated. In the absence of any evidence of *in vivo* chiral inversion, the results of the present studies demonstrate that both enantiomers of ciprofibrate share the biological actions of the racemate.

Hypolipidaemia after a single dose *in vivo* and induction of peroxisomal β-oxidation activity *in vitro* were considered to be the most appropriate indices of intrinsic pharmacological and peroxisomal activities, respectively. Since $S(-)$ ciprofibrate, after a single dose at low dosages *in vivo* and low concentrations *in vitro*, produced slightly, but statistically significantly, greater reductions in plasma concentrations of cholesterol and a greater induction of peroxisomal β-oxidation than $R(+)$ ciprofibrate, it may be concluded from this data that the former

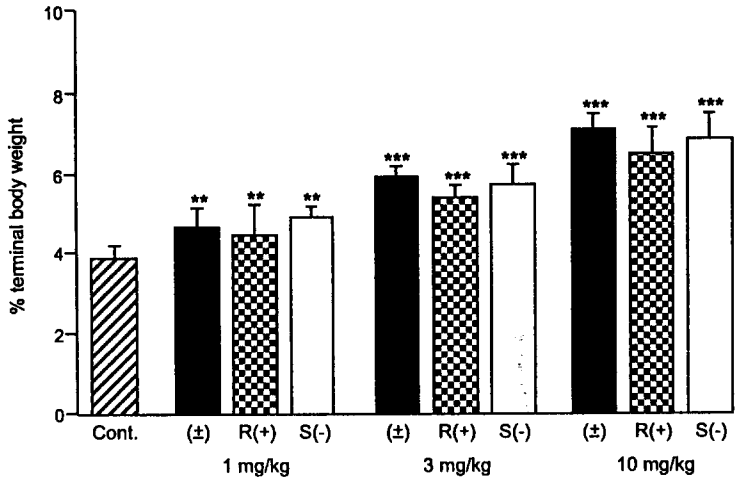

Fig. 4. Effects of racemic (±), $R(+)$ and $S(-)$ ciprofibrate on relative liver weight (% terminal body weight) following daily oral administration to male Fischer rats for 7 days. Data shown are the mean ± SD for six animals in each group. **P < 0.01, ***P < 0.001 for comparison with control.

Table 5. Plasma concentrations of racemic, $R(+)$ or $S(-)$ ciprofibrate 24 hr post-dose following daily oral administration to male Fischer rats

Dosage (mg/kg)	Plasma concentration (μg/mL)		
	Racemic	$R(+)$	$S(-)$
1	12.5 ± 1.2	14.6 ± 1.3	5.7 ± 0.6
3	27.0 ± 2.5	39.0 ± 5.7	15.9 ± 1.2
10	84.7 ± 3.8	94.8 ± 10.7	54.1 ± 7.7

The values given are the means ± SD for six animals in each group and are expressed as μg/mL. Plasma concentrations of ciprofibrate were determined by HPLC using a Waters fatty acid analysis column (3.9 mm × 30 cm) following solid phase extraction (C_{18}). Enantiomeric stability was confirmed by chiral HPLC analysis of ciprofibrate in plasma (stationary phase consisted of beta cyclodextrin derivatised with *S*-1-naphthylethylisocyanate).

enantiomer is a slightly more potent hypolipidaemic agent and peroxisome proliferator than its optical antipode. However, the slight difference in hypolipidaemic activity between $R(+)$ and $S(-)$ ciprofibrate and the relationship to dosage after a single dose were lost upon multiple dosing. Furthermore, after multiple dosing, the $R(+)$ enantiomer produced a statistically significantly greater induction of peroxisomal β-oxidation than $S(-)$ ciprofibrate, in spite of the slightly greater potency of the latter as a peroxisome proliferator *in vitro*. These observations may be explained on the basis of the differences in plasma concentrations of $R(+)$ and $S(-)$ ciprofibrate observed 24 hr after the seventh dose; concentrations of the former, less potent enantiomer, were higher than those of its optical antipode, thus overcoming any differences in intrinsic potency.

In the 7 day study, the lowest dosage chosen was in excess of that required to fully investigate the relative pharmacological effects of ciprofibrate enantiomers in rats after multiple dosing (but not to investigate relative peroxisomal effects and effects

Table 6. Peroxisomal β-oxidation activity of primary rat hepatocytes following exposure to racemic, $R(+)$ or $S(-)$ ciprofibrate for 72 hr

Concentration (μM)	Activity (nmol NAD reduced/min/mg)		
	Racemic	$R(+)$ ciprofibrate	$S(-)$ ciprofibrate
5	2.2 ± 0.3 (2.4)	1.6 ± 0.2 (1.7)NS	3.4 ± 0.5 (3.7)†
25	9.5 ± 0.6 (10.5)	7.8 ± 2.0 (8.6)	10.7 ± 0.9 (11.7)‡
100	12.0 ± 0.8 (13.4)	13.6 ± 0.8 (15.0)	12.3 ± 1.1 (13.5)
250	12.4 ± 1.2 (13.6)	12.9 ± 0.6 (14.2)	14.0 ± 0.8 (15.4)

Peroxisomal β-oxidation activity was assessed by measuring cyanide insensitive oxidation of palmitoyl CoA in cell lysates. The data represent the mean ± SD of the activity determined in cells from four plates. All exposures produced a statistically significant increase in activity relative to control (P < 0.01), except where indicated (NS = not significant). Numbers in parentheses represent fold increase over mean control activity (0.91 ± 0.26).

† = P < 0.01, ‡ = P < 0.001 for comparison with optical antipode.

on plasma thyroxine concentrations). Thus, on the basis of the slightly greater hypolipidaemic potency of $S(-)$ ciprofibrate at low single dosages, it may be possible, by using lower dosages in a multiple dose regime, to produce a pharmacological effect in the rat in the absence of a significant induction of peroxisomal β-oxidation activity. However, the dosages used in the present 7 day study are clinically relevant since the mid dosage of 3 mg/kg (racemic ciprofibrate) in rats produces an AUC which approximates to that observed in man at the clinical dosage of 100 mg/day (unpublished data). Thus, after multiple dosing, at dosages which produced clinically relevant exposure, both $R(+)$ and $S(-)$ ciprofibrate produced comparable biological effects.

Previous studies examining the effect of stereochemistry on hepatic peroxisome proliferation, using stereoisomers of clofibrate analogues [20, 21] and enantiomers of 2-ethylhexanoic acid [22], have shown that the induction of peroxisome associated enzyme activities is markedly influenced by stereochemical configuration. However, the lack of stereochemical selectivity for similar effects of $R(+)$ and $S(-)$ ciprofibrate may be explained on the basis that the chiral centre in the molecular structure of ciprofibrate is within the dichlorocyclopropyl moiety whereas the chiral centre in the racemic molecules used in previous studies is at the α-carbon of the carboxylic acid [21–23]. However, since ciprofibrate is one of the most potent peroxisome proliferators so far identified, the dichlorocyclopropyl moiety, being that part of the molecular structure of ciprofibrate unique among fibrates, must influence the potency of the molecule. Thus, our data indicate that whatever the molecular mechanisms of peroxisome proliferation, the processes involved are not extensively influenced by the stereochemical configuration of the ciprofibrate dichlorocyclopropyl group. Such data may be of use in determining structure–activity requirements for peroxisome proliferation.

In conclusion, the results of these studies indicate that after a single dose at low dosages *in vivo* and low concentrations *in vitro*, $S(-)$ ciprofibrate is slightly more potent than its optical antipode. However, after multiple administration at dosages and dose regimes which produce clinically relevant exposure, both enantiomers produced changes in hepatic enzyme activities and reductions in plasma concentrations of thyroxine which were comparable to those produced by the racemate. Since these early changes have been linked mechanistically to the chronic toxicity of the racemate in the rat, it could be predicted that the individual enantiomers of ciprofibrate under conditions employed in chronic safety studies, would produce the same spectrum of rodent toxicity as the racemate.

REFERENCES

1. Reddy JK and Lalwani ND, Carcinogenesis by hepatic peroxisome proliferators: evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit Rev Toxicol* **12**: 1–58, 1983.
2. Warren JR, Simon VF and Reddy JK, Properties of hypolipidemic peroxisome proliferators in the lymphocyte [^{3}H]-thymidine and salmonella mutagenesis assays. *Cancer Res* **40**: 36–41, 1980.
3. Glauert HP, Reddy JK, Kennam WS, Salter GL, Subbarao V and Pitot HC, Effects of hypolipidaemic drugs on unscheduled DNA synthesis in cultured hepatocytes and mutagenesis in salmonella. *Cancer Lett* **24**: 147–156, 1984.
4. Agarwal DK, Lawrence WH, Nunezi LT and Aution J, Mutagenicity evaluation of phthalic acid esters and metabolites. *J Toxicol Environ Health* **16**: 61–69, 1985.
5. Reddy JK, Azarnoff DL and Hignite CE, Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* **283**: 397–398, 1980.
6. Visser TJ, Kaptein E and Harpur ES, Differential expression and ciprofibrate induction of hepatic UDP-glucuronyltransferases for thyroxine and triiodothyronine in Fischer rats. *Biochem Pharmacol* **42**: 444–446, 1991.
7. Deavy L, Astley N, Harpur ES and Bonner FW, An investigation of the mechanism of ciprofibrate-induced thyroid hyperplasia in the male Fischer 344 rat. *Hum Exp Toxicol* **10**: 76–77, 1991.
8. Buttinoni A, Ferrari M, Colombo M and Ceserani R, Biological activity of Indoprofen and its optical isomers. *J Pharm Pharmacol* **35**: 603–604, 1983.
9. Briemer DD and Van Rossum JM, Pharmacokinetics of (+)- and (±)-hexobarbitone in man after oral administration. *J Pharm Pharmacol* **25**: 762–763, 1973.
10. Feller DR, Kammana VS, Newman HAI, Romstedt KJ, Witiak DT, Bettoni G, Bryant SH, Conte-Camarino D, Loiodice F and Torteralla V, Dissociation of hypolipidaemic and antiplatelet actions from adverse myotonic effects of clofibric acid related enantiomers. *J Med Chem* **30**: 125–1257, 1987.
11. Bettoni G, Loiodice F, Torterella V, Conte-Camarino D, Mambrini M, Ferranini E and Bryant SH, Stereospecificity of the chloride ion channel: the action of chiral clofibric acid. *J Med Chem* **30**: 1267–1270, 1987.
12. Baxter MP, Carr G, Ellames GJ, Herbert JM, Mansour GA, Nazir MA, Saindane MT, Smith DI and Vojvodic PR, Synthesis of the enantiomers of the hypolipidaemic agent 2-[4-(2,2-dichlorocyclopropyl) phenoxy]-2-methylpropionic acid (ciprofibrate). *J Chem Res* (*S*), 47, 1993.
13. Armstrong DE, Stalcup AM, Hilton ML, Duncan JD, Faulkner JR and Chang S-C, Derivatized cyclodextrins for normal-phase liquid chromatographic separation of enantiomers. *Anal Chem* **50**: 1610–1615, 1990.
14. Bock P, Kramer R and Parelka M, Peroxisomes and related particles in animal tissues. In: *Cell Biol Monographs*, Vol. 1. Springer-Verlag, Berlin, 1980.
15. Bieber LL, Abraham T and Helmrath T, A rapid spectrophotometric assay for carnitine palmitoyl transferase. *Anal Biochem* **50**: 509–518, 1972.
16. Bronfman M, Inestrosa NC and Leighton F, Fatty acid oxidation by human liver peroxisomes. *Biochem Biophys Res Commun* **83**: 952–958, 1979.
17. Habig WH, Pabst MJ and Jakoby W, Glutathione-*S*-transferase. The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
18. Lawrence RA and Burke RF, Glutathione peroxidase activity in selenium deficient rat liver. *Biochem Biophys Res Commun* **71**: 952–958, 1976.
19. Furakawa K, Numoto S, Furuya K, Furakwa NT and Williams GM, Effects of the hepatocarcinogen Nafenopin, a peroxisome proliferator, on the activities of rat liver glutathione-requiring enzymes and catalase in comparison to the action of phenobarbital. *Cancer Res* **45**: 5011–5019.
20. Lowry OH, Roseborough NJ, Farr AL and Randall RJ, Protein measurement with Folin phenol reagent. *J Biol Chem* **193**: 265–275, 1951.

21. Chinje E and Gibson GG, Stereochemical selectivity in the induction of cytochrome P450IVA1 (P452)-dependent fatty acid hydroxylation and peroxisome proliferation. *Biochem Pharmacol* **41**: 769–774, 1991.
22. Esbenshade TA, Kammana VS, Newman HAI, Torterella V, Witiak DT and Feller DR, *In vivo* and *in vitro* peroxisome proliferation properties of selected clofibrate analogues in the rat. *Biochem Pharmacol* **40**: 1263–1274, 1990.
23. Macherey AC, Gregoire S, Tainturier G and Lhugenot JC, Enantioselectivity in the induction of peroxisome proliferation by 2-ethylhexanoic acid. *Chirality* **4**: 478–483, 1992.