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Hypocholesterolemic Activity of Racemic Dichlorophenoxypropionic Acid or Its Enantiomers in Rats

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This work is dedicated to the memory of J. Deniau.

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Abundant circumstantial evidence has shown that chronic hyperlipidemia is one of the major clinical manifestations of ischemic heart disease (IHD) and many drugs have been developed which reduce serum lipids. Clofibrate has been one of the most effective drugs used for lowering serum cholesterol and reducing the morbidity of patients suffering from IHD. However, prolonged treatment with clofibrate has been reported to cause higher incidences of gastrointestinal disorders and to induce cholesterol oversaturation of bile (Bateson et al., 1978). These deleterious side effects have resulted in a restriction of the use of clofibrate and new drugs exerting selective and non toxic hypolipidemic actions have been intensively researched. Mukhopadhyaz et al. (1983) have systematically investigated the antilipidemic and antiaggregatory activities of chroman analogs which partly share a common chemical structure with clofibrate and suggested that it is possible to develop clofibrate-related analogs which are highly tissue selective and/or equally as active as clofibrate as antilipidemic or antiplatelet agents.

(Dichloro-2,4 phenoxy)-2 propionic acid, structurally-related to fibrates with an asymetric carbon (Fig. 1), is well known for its herbicide effectiveness (Lanini and Radosevich, 1982). To our knowledge, however, nobody has investigated its possible effect on cholesterol metabolism. The purpose of this study is to explore the effects of this compound on cholesterol metabolism in the rat and to compare them with those of clofibrate. It is the first time that a low concentration (0.04 mmole) of this well-known pesticide has been shown to lower plasma cholesterol when added to the diet.

MATERIALS AND METHODS

(Dichloro-2,4 phenoxy)-2 propionic acid was prepared by reacting dichloro-2,4 phenol with (±) bromo-2 propionic acid in the presence of NaOH. The D-enantiomer (α_D = +25°, ethanol) was obtained by resolution of the racemate in the presence of dehydroabiethylamine. To obtain the L-enantiomer, D-alanine was converted by nitrosation followed by methylation to D-chloro-2 propionic acid methyl ester which by reaction with dichloro-2,4 phenol in 2,4 phenol in the presence of NaOH in DMF gave the desired product (α_D = -27°, ethanol). The NMR spectra of the two enantiomers measured in $CDCl_3$ in the presence of (S)-methyl-2 benzylamine revealed that their optical purity was over 95%. Clofibrate was kindly provided by Scherrer (67930 Beinheim, France).

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Male and female Wistar rats, 5 weeks old, were obtained from JANVIER (St Berthevin, France). They were housed in an air-conditioned room at 24 ± 1°C with an alternating 12 hr dark-light cycle (8-20 and 20-8). They were fed a semi-synthetic diet (Mathé et al., 1977) ad libitum until used for experiments. The animals were allowed free access to diet and water.

Three experiments were conducted.
In experiment 1, the hypocholesterolemic efficiency of the racemate 2-4D (2-4D) was examined at the two dose levels of 0.04 mmol and 0.2 mmol/kg body weight/day for 28 days. Male rats weighing an average of 315 g were divided into 3 groups of 6 rats each. One group served as control. Two groups were given rac-2-4 D supplemented to the semi-synthetic diet so that each rat could ingest the amount defined according to its body weight. The diet containing different levels of the test substance was prepared once a week. Blood was drawn from the orbital plexus of rats without overnight fasting, under light ether anesthesia, one day before (day - 1) and 7 and 14 days (day + 7 and + 14) after drug treatment. On the final day (day + 28), the control and 0.04 mmol rac 2-4-D group of rats received a subcutaneous injection of ^{14}C acetate (100 µCi). They were sacrificed 70 minutes later (at 10 a.m.) by aortic exsanguination (M. Perrodin and Lutton, 1985).

In experiment 2, the hypolipidemic efficiency of the rac or enantiomers 2-4-D was compared to that of clofibrate, at the dose level of 0.04 mmol/kg body weight/day for 8 days. Male rats weighing 350 ± 10 g were divided into 4 groups (n = 5) and fed with semisynthetic diet with clofibrate, RAC-2-4-D, D-2-4-D or L-2-4-D. Blood was collected as in experiment 1, on day + 1, + 4 and + 8 after drug treatment.

In experiment 3, the long-term effect of rac 2-4-D on plasma cholesterol was followed in young male and female rats (6 weeks old) at the average dose level of 0.04 mmol/kg body weight/day for 5 months. Blood samples were drawn from the orbital plexus at day-1 et thereafter every two weeks. Feces were individually collected during the second, sixth and tenth weeks following 2-4-D administration.

The methods used for extracting and analyzing sterols, assaying cholesterol and determining the radioactivity have been previously described (Mathé et al., 1977). Chemical techniques for the acetate experiments have also already been described (Perrodin and Lutton, 1985). Plasma cholesterol was measured according to Bhandaru et al. (1977).

RESULTS AND DISCUSSION

The effect of RAC-2-4-D (at 0.04 and 0.2 mmol/kg body weight/day) on body weight and plasma cholesterol is shown in Fig. 2. There is some evidence that RAC-2-4-D is somewhat toxic, at least at the high level usually used for clofibrate (0.2 mmol/kg b.w./day). For this dose, the body weight promptly decreased from the 4th day of drug treatment. We also observed a liver weight increase (Table I) and urinary obstruction. Moreover, previous preliminary trials showed that rats fed 0.8 mmol/kg/day of RAC-2-4-D in 0.25 % methylcellulose for 3 days were also seriously affected with leg paralysis; these symptoms diseappeared several

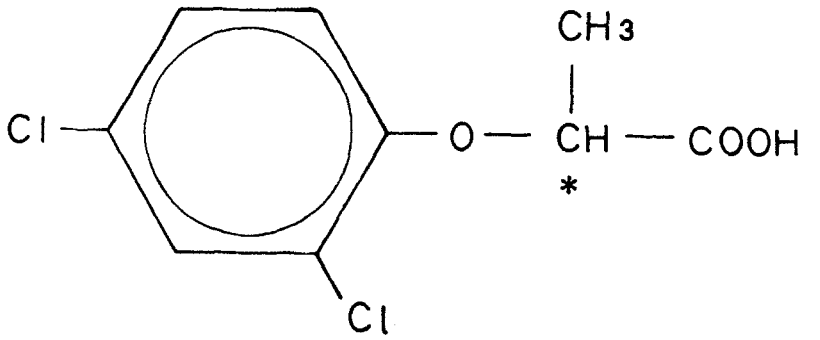

Figure 1. Structural formula of 2-4 Dichlorophenoxypropionic acid (2-4-D).

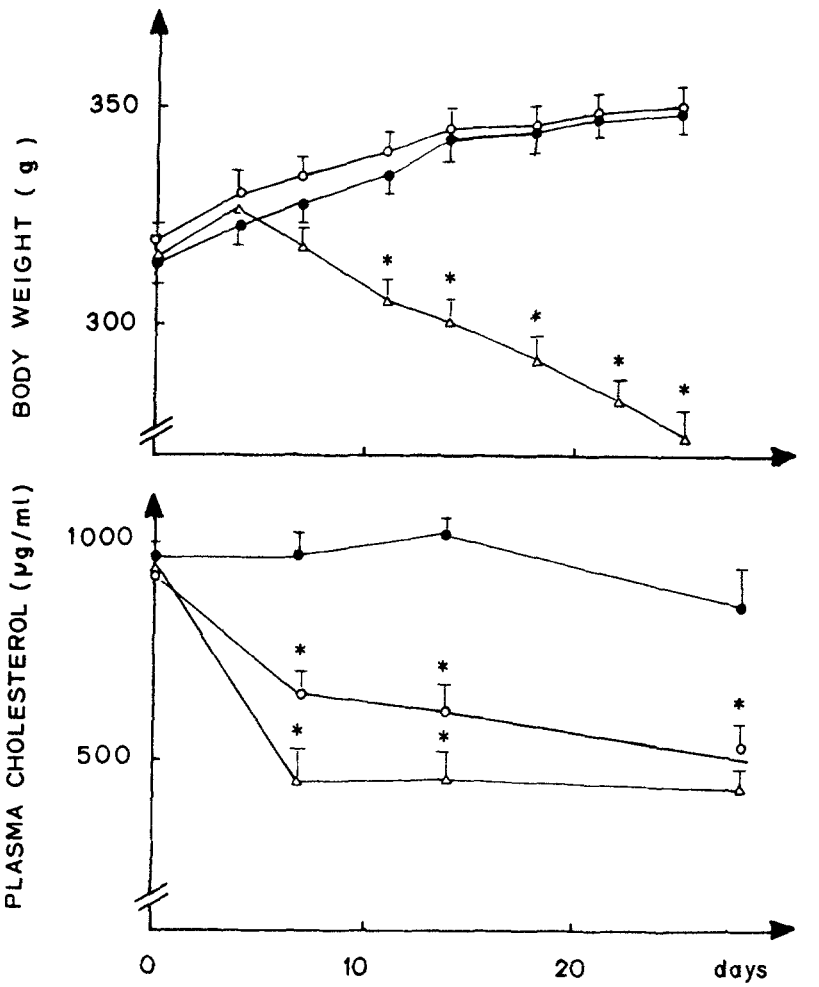

Figure 2. Evolution of body weight and plasma cholesterol in rats receiving a semisynthetic diet (●) with (o) or without 0.04 or 0.2 mmol/kg b.w. 2-4-D for 28 days (Δ).

Table 1. Weight and relative weight of the liver and small intestine in rats receiving 0.04 or 0.2 mmol/kg w/day of RAC-DPP for 28 days or in the controls.

	Liver (g)	(% b.w.)	Small intestine (g)	(% b.w.)
Controls	11.6 ± 1.0*	(3.2 ± 0.1)	7.4 ± 0.7	(2.0 ± 0.1)
0.04 RAC 2-4D	13.3 ± 1.1	(3.8 ± 0.2)	6.8 ± 0.7	(1.9 ± 0.2)
0.2 RAC 2-4D	13.0 ± 1.4	(4.7 ± 0.2[a])	5.8 ± 0.7	(2.1 ± 0.2)

*Mean ± SEM (n = 6) ; a : $P \leq 0.05$ versus controls

Table 2. Sterol radioactivity in the plasma, liver and small intestine of rats 70 min after a subcutaneous injection of ^{14}C acetate (100 µCi)

	Plasma (dpm/ml)	Liver (dpm/whole organ)	Small intestine (dpm/whole organ)
Controls	1293 ± 286*	55 593 ± 23 375	228 906 ± 17 650
RAC-2-4D	463 ± 130[a]	28 020 + 11 070[a]	163 849 ± 27 230

*Mean ± SEM (n = 5) ; a : $P \leq 0.05$ versus controls
RAC-2-4D = 0.04 mmol/kg body weight/day for 8 days

Table 3. Neutral fecal sterols in RAC-DPP rats or in the controls

	Fecal sterol (mg/day)			
	2nd week	6th week	10th week	all data
Controls	7.8 ± 0.4*	8.4 ± 0.4	8.1 ±0.3	8.1 ± 0.2
RAC-2-4D	6.9 ± 0.4	8.3 ± 0.4	7.3 ± 0.5	7.5 ± 0.3[a]

*Mean ± SEM (n=6; all data n=18) ; a : $P < 0.05$ versus controls
RAC 2-4D = 0.04 mmol/kg body weight/day for 5 months

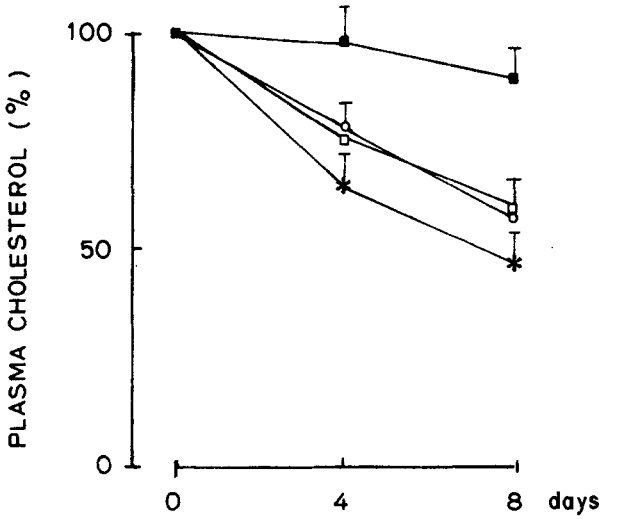

Figure 3. Evolution of plasma cholesterol concentration (expressed as a percent of the initial value) in rats receiving 0.04 mmol/kg b.w. of clofibrate (■) of RAC (□), D (*) or L (o) 2-4-D during 8 days.

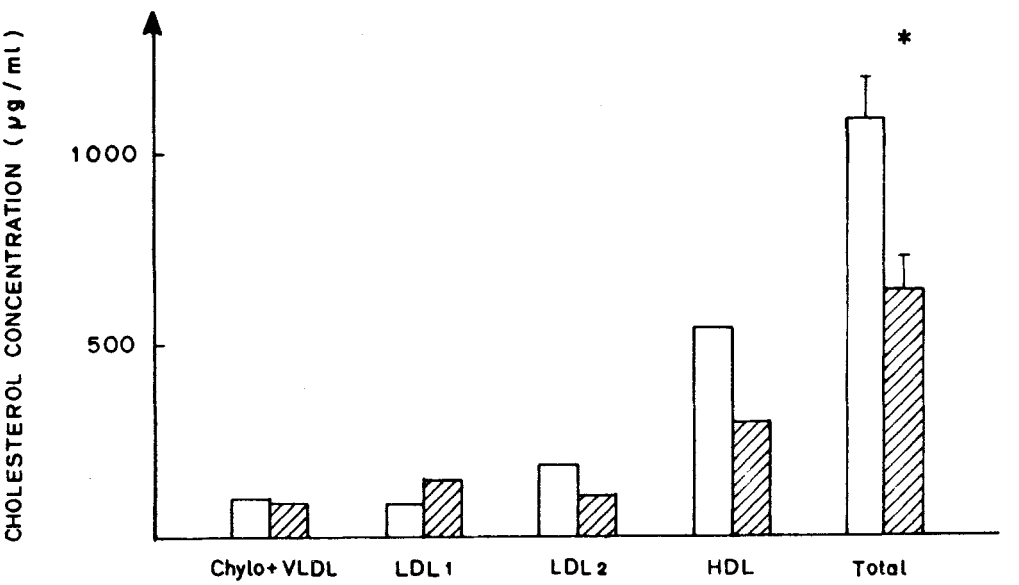

Figure 4. Mean cholesterol concentration in the plasma lipoproteins of rats fed a semi-synthetic diet containing or not RAC-2-4-D for 5 months.

□ controls

▨ RAC-2-4-D 0.04 mmol/kg b.w.

*$P \leq 0.05$ versus controls (n = 6).

days after cessation of drug administration. At the lower dose level of 0.04 mmol/kg body weight/day, the grossly pathological problems did not occur.

It is clear that RAC-2-4-D induces a pronounded hypocholesterolemia even at the lower dose level of 0.04 mmol/kg b.w./day. The serum cholesterol concentration in the control rats was virtually unchanged throughout the experimental period (28 days) whereas the plasma cholesterol of RAC-2-4-D treated rats was reduced to 69% and 47% of the initial value for the dose levels of 0.04 and 0.2 mmol respectively, 7 days after the treatment. These lowered plasma cholesterol levels persisted for at least another 3 weeks. RAC-2-4-D did not significantly decrease the concentration of cholesterol in the red blood cells, small intestine and liver. To check if 2-4-D induced hypocholesterolemia was related to a modified cholesterogenesis, in vitro and in vivo ^{14}C acetate incorporation was measured in liver and intestinal sterols. In vitro, sterol radioactivity was reduced 3 and 2 fold respectively by adding 1.5 mM RAC-2-4-D to slice incubations of liver and intestine (Data not shown). Seventy minutes after a subcutaneous injection of ^{14}C acetate, in vivo sterol radioactivity in the plasma, liver and small intestine was measured in the control and 0.04 mmol RAC-2-4-D treated rats from experiment 1 (Table II). A significantly lower sterol radioactivity was found in the plasma and liver from 2-4-D treated rats versus controls. The mean value for sterol radioactivity in the intestine of 2-4-D treated rats was 30% lower than that of the controls, although not significant. However sterol specific activity in the plasma, liver and intestine was significantly lowered in RAC-2-4-D treated rats versus controls. These findings suggest that 2-4-D can inhibit sterol synthesis, particularly in the liver as observed with 0.3% clofibrate (Berndt et al. 1978).

The short term effect (8 days) of 0.04 mmol/kg b.w./day of clofibrate, RAC-2-4-D, L-2-4-D or D-2-4-D on plasma cholesterol (expressed as a percentage of the initial value 1000 µg/ml $\pm$ 22, n=20) in male adult rats (359 $\pm$ 4 g) is illustrated in Figure 3. It clearly shows that while clofibrate was practically without effect at this dose, both enantiomers of 2-4-D are very effective in lowering plasma cholesterol (-34 $\pm$ 2% and -52 $\pm$ 2%, n=5, after 4 and 8 days respectively for D-2-4-D treated rats and -17 $\pm$ 5% and -41 $\pm$ 6% for L-2-4-D treated rats). Although other data are necessary, D-24D seems to be a more potent drug than L or RAC-2-4-D for lowering plasma cholesterol. RAC, D or L 2-4-D is significantly more hypolipidemic than is the reference clofibrate but their efficiency is comparable to that of ciprofibrate (Arnold, 1979, Edelson, 1979). During this experiment, the body weights, food intakes or daily fecal sterol eliminations were similar throughout the four groups of rats and did not vary during drug administration. This observation suggests that RAC-2-4-D does not grossly modify the absorption of luminal cholesterol (Lutton et Chevallier, 1976, Lutton, 1976).

The above results needed to be verified during a long term experiment. Fecal sterol excretion was measured during the second, sixth and tenth weeks following the beginning of RAC-2-4-D administration (Table III). No statistical differences were found for sterol excretion from RAC-2-4-D treated rats versus controls for each period (n=6). When all the data were analyzed (n=18), the daily fecal sterol elimination of RAC-2-4-D treated animals was slightly lower than that in the controls (7.5 $\pm$ 0.3 versus 8.1 $\pm$

0.2 P $\leq$ 0.05). Throughout the experimental period, the body weight increase of RAC-2-4-D treated animals was similar to that of controls. In the female rat, body weight increase and fecal sterol elimination were unchanged after 0.4 mmol/b.w./day RAC-2-4-D administration throughout a similar 5 months experiment. Lipoprotein cholesterol and total plasma cholesterol was measured when the animals were 6 1/2 months old (Fig. 4). After 5 months of drug administration, plasma cholesterol was still lowered (0.65 $\pm$ 0.09 mg/ml, n = 6) in RAC-2-4-D rats compared to the controls (1.09 $\pm$ 0.11 mg/ml, n = 6). This was essentially due to the decrease of LDL_2 (1.040 < d < 1.069) and HDL (1.069 < d < 1.21) cholesterol. Similar findings were obtained with LDL_2 and HDL protein suggesting that there is a strong decrease in the number of LDL_2 and HDL particles in the male 2-4-D treated rat probably as a consequence of liver peroxisome proliferations as shown with numerous hypolipidemic compounds (M. Arakawa, 1978, Sirtori, 1978).

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