Clofibrate Prevents and Reverses the Hemodynamic Manifestations of Hyperthyroidism in Rats

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BACKGROUND

This study analyzed the effects of the chronic administration of clofibrate, a peroxisome proliferator-activated receptor-α (PPARα) agonist, on the development and established hemodynamic, morphologic, metabolic, and renal manifestations of hyperthyroidism in rats.

METHODS

The prevention study used four groups of male Wistar rats: control, clofibrate (240 mg/kg/day by gavage), T_4 (75 μg thyroxine/rat/day s.c.), and T_4+clofibrate. All treatments were maintained for 3 weeks. Body weight (BW), tail systolic blood pressure (SBP), and heart rate (HR) were recorded weekly. Finally, temperature, SBP, pulse pressure (PP) and HR were recorded in conscious rats, and morphologic, metabolic, plasma, and renal variables were measured. The reversion study used two groups of rats, T_4 (treated for 6 weeks) and T_4+clofibrate, measuring their hemodynamic variables and temperature for 3 weeks.

RESULTS

T_4 increased BP, HR, PP, and temperature when compared with control rats. Clofibrate prevented and reversed the increase in SBP, HR, PP, and temperature produced by T_4 administration, reduced plasma thyroid hormone levels, and increased plasma thyroid-stimulating hormone values and phenol-uridine diphosphate-glucuronosyl-transferase (UGT) activity. However, clofibrate did not modify the cardiac or renal hypertrophy, polyphagia, polydipsia, or proteinuria of hyperthyroid rats. In normal rats, clofibrate treatment did not significantly change thyroid hormone levels, phenol-UGT activity, or any hemodynamic, morphologic, or renal variables.

CONCLUSIONS

Chronic clofibrate treatment suppressed the hemodynamic manifestations and increased temperature of hyperthyroidism, an effect that can be produced by direct antithyroid effects. However, clofibrate administration did not modify the morphologic, metabolic, or renal alterations of hyperthyroid rats, indicating specificity in the antithyroid actions of clofibrate.

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Fibrates are synthetic agonists of peroxisome proliferator-activated receptor-α (PPARα), a subfamily of the nuclear receptor super-family naturally activated by ligands such as free fatty acids and eicosanoids.[1] Fibrates have been in clinical use as hypolipidemic agents for several decades, and their beneficial effects on cardiovascular function[1–3] and increased blood pressure (BP) have more recently been reported.[4]

Thyroid hormone receptors (TRs) belong to a large superfamily of nuclear hormone receptors that include steroid, vitamin D, retinoic acid (RXR), and PPARs.[5] Cross-talk between the thyroid hormone and peroxisome proliferator activated nuclear receptors has been observed.[6] TRs and other nuclear hormone receptors can modulate each other's transcriptional activities. This cross-talk can occur via several mechanisms. *In vitro* and *in vivo* studies have provided evidence for interaction between TR and PPARα signaling pathways by mutual competition for RXR.[7] PPARs sequest TRs from a TR–RXR complex by forming PPAR–RXR complexes.[7] Competition also occurs for DNA binding between TRs and PPARs at their respective binding sites and for cofactors shared among these receptors.[5,6,8]

Several studies have shown that fibrates induce uridine diphosphate-glucuronosyl-transferases (UGTs).[9–11] Thyroid hormones, thyroxine (T_4), and triiodothyronine (T_3) are substrates of UGTs, and glucuronidation of these hormones is the main metabolic pathway for their deactivation.[10] Fibrates also interfere with the transport, activation, and action mechanisms of thyroid hormones. Thus, clofibrate reduces the gene expression of their transporters,[12–14] the activity of deiodinases[12] and also reduces the gene expression of $TR\alpha_1$ in the liver of rats[15] and pigs.[12]

The mechanism for the interaction between thyroid hormones and PPARα activation by clofibrate has been studied using various experimental models and all of the above indicates that PPARα activation has various antithyroid effects. Nevertheless, no studies have tested this interaction in hyperthyroidism. Therefore, the aim of this study was to analyze the effects of clofibrate on the main cardiovascular and renal manifestations of hyperthyroidism in rats.

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METHODS

Animals. Thirty-two male Wistar rats born and raised in the experimental animal service of the University of Granada were used. The experiment was performed according to European Union guidelines for the ethical care of animals. Rats initially weighing 280 ± 4 g were randomly assigned to the different groups. Each experimental group comprised eight animals. All rats had free access to food and tap water. Clofibrate (240 mg/kg/day) was given by gavage because of the low solubility of this compound. T_4 (Merck) was dissolved in isotonic saline plus 0.5 N NaOH (1/100 v/v), buffered to pH 7, and s.c. injected. Doses of T_4 and of clofibrate were in accordance with previously published protocols used at our laboratory.[16]

Experimental protocol

Effects of clofibrate on the development of hyperthyroidism. The groups were: control, clofibrate-treated, T_4-treated (75 µg/rat/day), and T_4+clofibrate–treated rats. Treatments were administered for 3 weeks. Body weight (BW), tail systolic BP (SBP), and heart rate (HR) were measured once a week. Tail SBP and HR were measured with the use of tail-cuff plethysmography in conscious rats (LE 5001-Pressure Meter, Letica SA, Barcelona, Spain).

When the experimental period was completed, rectal temperature was measured (Regulador RTM1, Cibertec, Spain), and all rats were then housed in metabolic cages (Panlab, Barcelona, Spain) with free access to food and water for a 4-day period (2 days for adaptation + 2 experimental days), during which food and water intakes were measured and urine samples were collected. Twenty-four-hour urine volume, proteinuria, creatinine, and total sodium and potassium excretion were measured. Mean values of all intake and urinary variables obtained during the 2 experimental days were used for statistical analyses among groups.

After completion of the metabolic study, the rats were anesthetized with ethyl ether. A polyethylene catheter (PE-50) containing 100 U of heparin in isotonic sterile NaCl solution was inserted into the femoral artery to measure intra-arterial BP and HR and pulse pressure (PP) measurements in conscious rats and to extract blood samples. The catheter was tunneled s.c. and brought out through the skin at the dorsal side of the neck. Intra-arterial BP was measured 24 h after implantation of the femoral catheter. Direct BP and HR were recorded continuously for 60 min with a sampling frequency of 400/s (McLab, AD Instruments, Hastings, UK). BP and HR values obtained during the last 30 min were averaged for inter-group comparisons. Subsequently, blood samples taken with the femoral catheter were used to determine plasma variables. Finally, the rats were killed by exsanguination, and kidneys and ventricles were removed and weighed. The heart was divided into right ventricle and left ventricle plus septum.

Effects of clofibrate in the established phase of hyperthyroidism. Rats treated with T4 for 4 weeks were randomly assigned to two groups: control T_4-treated rats and T_4+clofibrate–treated rats. In this experiment, BW, tail SBP, HR, and rectal temperature were measured once a week for 3 weeks.

Analytical procedures. Proteinuria was measured by the method of Bradford.[17] Plasma and urinary electrolytes, plasma lipids, and creatinine were measured in an autoanalyzer (Hitachi-912, Roche, Spain). Plasma levels of thyroid hormones (total circulating T_3 and T_4) were determined by using rat radioimmunoassay kits according to the manufacturer's instructions (Diagnostic Products Corporation, Los Angeles, CA). Rat plasma thyroid-stimulating hormone was measured by a solid phase competitive chemiluminescent enzyme immunoassay using the IMMULITE 2000 Analyzer (EURO/DPC, Llanberis, Gwynedd, UK).

UGT measurement. Because UGTs are the main enzymes responsible for thyroid hormone deactivation, we measured the activity of phenol-UGT to analyze its possible role in the antithyroid effects of clofibrate. This UGT isoform was chosen because its activity is relevant in the liver, kidney, and heart of control, hyper-, and hypothyroid rats.[18] Phenol-UGT activity was measured in the liver, kidney, and heart of four groups of rats treated in the same way as those used in the main experiment of the paper. Each experimental group comprised six animals.

Organs were quickly dissected and collected in ice-cold 0·9% NaCl. Organs were homogenized with the aid of a tissue grinder (Omni International, NJ) at 3,000 rpm in ice-cold homogenization buffer (250 mmol/l sucrose, 10 mmol/l HEPES–Tris at pH 8·0 and 1 mmol/l DTT). The homogenized tissues were placed in 1 ml of the same buffer and sonicated (10 s × 6). The crude homogenate was centrifuged for 5 min at 3,000 g and aliquots of the supernatant were either stored at −20 °C for total protein determination[17] or used immediately for phenol-UGT activity measurement.

Phenol-UGT enzyme assay was performed as reported by Beetstra *et al.*[9] In brief, homogenates (100 µl with a protein concentration of 1 mg/ml) were incubated with 1 mmol/l *p*-nitrophenol in a total volume of 200-µl incubation medium (in a final concentration of 100 mmol/l Tris–HCl, 5 mmol/l $mgCl_2$, 0·05% Brij56 at pH 7·4) in the presence or absence of 5 mmol/l uridine 5′-diphophoglucuronic acid (30 min at 37 °C). The reaction was quenched by adding 3·8 ml 0·1 M ice-cold NaOH, and absorbance of the unconjugated *p*-nitrophenol was measured at 405 nm. Specific phenol-UGT activity is expressed in mol *p*-nitrophenol/µg per min.

Statistical analysis. Changes in SBP and HR over time were compared with the use of a nested design, with groups and days as fixed factors and rat as random factor. When the overall difference was significant, Bonferroni's method with an appropriate error was used. Comparisons of each variable at the end of the experiment were done by performing the Mann–Whitney non-parametric test. Differences were considered significant when $P < 0.05$.

RESULTS

Effects of clofibrate on the development of hyperthyroidism

BP and HR. The values of these variables are summarized in Figures 1 and 2. The upper graphs in Figures 1a and 2a show the time course of the tail SBP and HR and the lower graphs in Figures 1b and 2b show the final SBP and HR measured by direct recording in the experimental groups. T_4 administration produced an increase in SBP and HR and in final SBP, HR, and PP when compared with control rats. Clofibrate administration to normal rats at the dose used in this experiment did not modify the time course of BP, HR, final SBP, HR, or PP. However, clofibrate administration to hyperthyroid rats reduced the values of all hemodynamic variables. Thus, the T_4-clofibrate group showed a similar SBP and HR time course and final SBP, HR, and PP to those of control rats. PP values in the groups were: control, 36.2 ± 2.2; clofibrate, 34.2 ± 2.2; T_4 55.3 ± 1.0*; T_4+clofibrate, 44.3 ± 1.5***,*; *P < 0.01 when compared with controls (***P < 0.01 compared with T_4-treated group).

Body temperature. Rectal temperature was significantly higher in the T_4 group than in controls. Clofibrate treatment produced a slight non-significant reduction in temperature vs. controls and a marked temperature reduction in T_4-treated rats, whereas rectal temperature was similar between the T_4-clofibrate group and controls (Figure 3).

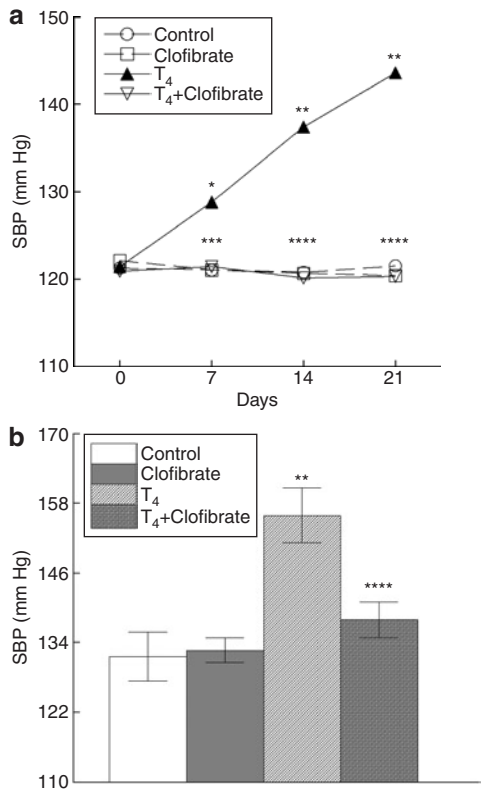

Figure 1 | Time course of systolic blood pressure (SBP) measured by (**a**) tail-cuff method, and final SBP measured by (**b**) direct recording (femoral artery) in conscious rats. Data are means ± s.e.m. *P < 0.05; **P < 0.01, compared with controls. ***P < 0.05; ****P < 0.01, compared with the thyroxine (T_4) group.

Morphological variables. BW at the end of the 3-week study period was significantly lower in the T_4 group than in controls. Kidney-to-BW ratio and left ventricular-to-BW ratio were significantly higher in the T_4 group than in controls. Left ventricular-to-heart weight ratio did not significantly differ between T_4-treated groups and controls. Clofibrate treatment had no effect on any of these morphologic variables in either normal or T_4-treated rats (Table 1).

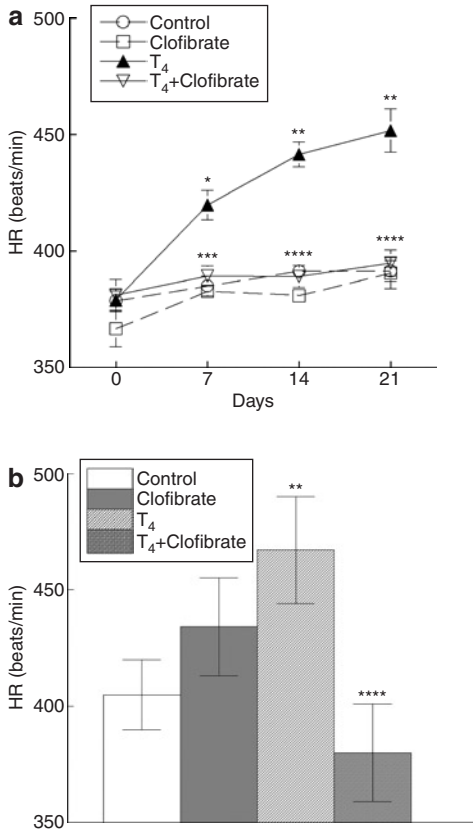

Figure 2 | Time course of heart rate (HR) measured by (**a**) tail-cuff method, and final HR measured by (**b**) direct recording (femoral artery) in conscious rats. Data are means ± s.e.m. *P < 0.05; **P < 0.01, compared with controls. ***P < 0.05; ****P < 0.01, compared with the thyroxine (T_4) group.

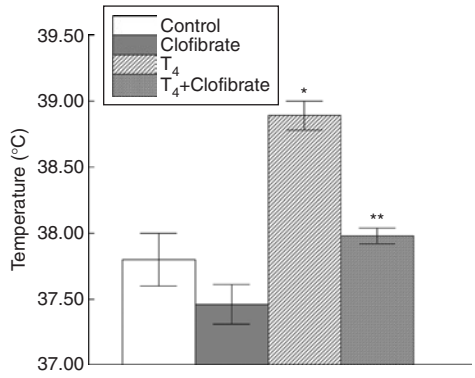

Figure 3 | Rectal temperature in the experimental groups at the end of the experiment. Data are means ± s.e.m. *P < 0.01, compared with controls. **P < 0.01, compared with the thyroxine (T_4) group.

Thyroid hormone levels and plasma, metabolic, and urinary variables

The increased thyroid hormone (T_4 and T_3) levels in T_4-treated rats were markedly reduced by clofibrate treatment, remaining higher than in control rats. However, plasma thyroid hormones in normal rats were not significantly affected by clofibrate (Table 2). Plasma thyroid-stimulating hormone values were reduced in both T_4-treated groups, but this decrease was attenuated in the T_4+clofibrate group, finding significant differences between both T_4-treated groups (Table 2).

Table 1 | Morphologic variables in the experimental groups

Groups	FBW (g)	KW/BW (mg/g)	LVW/BW (mg/g)	LVW/HVW
Control	325.1 ± 12.4	2.32 ± 0.08	1.86 ± 0.06	0.80 ± 0.01
Clofibrate	313.7 ± 13.2	2.48 ± 0.12	1.99 ± 0.03	0.81 ± 0.01
T_4	286.7 ± 4.3*	3.09 ± 0.09**	2.60 ± 0.07**	0.80 ± 0.01
T_4+Clofibrate	295.6 ± 7.7	3.24 ± 0.12**	2.43 ± 0.06**	0.80 ± 0.01

Data are expressed as means ± s.e.m.
FBW, final body weight; KW/BW, ratio kidney weight vs. body weight; LVW/BW, ratio left ventricular weight vs. body weight; LVW/HVW, ratio left ventricular weight vs. heart ventricular weight; T4, thyroxine.
*$P < 0.05$, **$P < 0.01$ vs. the control group.

Table 2 | Plasma variables in the experimental groups

Groups	Control	Clofibrate	T_4	T_4+Clofibrate
Na (mEq/l)	143.3 ± 1.29	146.1 ± 0.52	144.9 ± 0.74	147.4 ± 1.21
K (mEq/l)	5.02 ± 0.42	5.44 ± 0.24	4.83 ± 0.10	4.89 ± 0.14
Creatinine (mg/dl)	0.34 ± 0.05	0.46 ± 0.02*	0.37 ± 0.02	0.32 ± 0.02
LDL (mg/dl)	7.36 ± 0.31	5.35 ± 0.48*	3.91 ± 1.06**	4.03 ± 1.03*
HDL (mg/dl)	56.01 ± 2.44	40.54 ± 4.33*	41.23 ± 3.75*	33.55 ± 3.38**
Cholesterol (mg/dl)	68.80 ± 2.38	53.19 ± 1.12*	51.28 ± 5.33*	42.74 ± 3.57**
Triglycerides (mg/dl)	27.23 ± 4.90	35.24 ± 1.35	37.89 ± 4.03	35.88 ± 3.04
TSH (ng/dl)	3.0 ± 0.2	3.5 ± 0.6	0.2 ± 0.04**	1.1 ± 0.01*,***
T_4 (µg/dl)	4.6 ± 0.3	4.0 ± 0.5	45 ± 6**	10 ± 1.5**,***
T_3 (ng/dl)	78 ± 3.2	69 ± 2.5	230 ± 10**	115 ± 7*,***

Data are expressed as means ± s.e.m.
HDL, high-density lipoprotein; LDL, low-density lipoprotein; T3, triiodothyronine; TSH, thyroid-stimulating hormone.
*$P < 0.05$, **$P < 0.01$ vs. the control group, ***$P < 0.05$ vs. the thyroxine (T4) group.

There were no significant differences in plasma sodium or potassium among the groups. Plasma creatinine was increased in the clofibrate group but did not differ among the other groups (Table 2). T_4 administration produced a significant reduction in low-density lipoprotein, high-density lipoprotein, cholesterol, and triglycerides. Clofibrate produced a significant reduction in all of these variables except for triglycerides in normal rats but produced no further reduction in these variables in hyperthyroid rats.

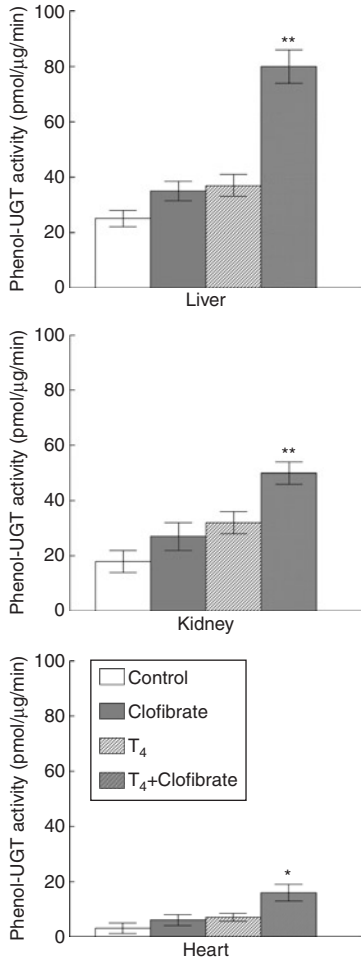

Figure 4 | Hepatic, renal, and cardiac phenol-uridine diphosphate-glucuronosyl-transferase (UGT) activity in the experimental groups at the end of the experiment. Data are means ± s.e.m. *$P < 0.01$, compared with controls. **$P < 0.001$, compared with controls.

Table 3 | Food and water intake, water and sodium balances, proteinuria and creatinine clearance in the experimental groups

Groups	Food intake (g/100 g·24 h)	Water intake (ml/100 g·24 h)	Water balance (ml/100 g·24 h)	Sodium balance (mmol/100 g·24 h)	Protein excretion rate (mg/mg creatinine)	Creatinine clearance (ml/min·g kidney)
Control	6.08 ± 0.37	6.86 ± 0.71	3.11 ± 0.36	0.19 ± 0.06	6.35 ± 0.67	0.78 ± 0.07
Clofibrate	5.30 ± 0.38	7.05 ± 0.66	2.70 ± 0.40	0.16 ± 0.02	7.54 ± 0.95	0.67 ± 0.06
T_4	9.13 ± 0.61*	12.51 ± 1.77*	9.15 ± 1.43*	0.30 ± 0.04*	19.06 ± 0.89*	0.64 ± 0.04
T_4+Clofibrate	8.62 ± 0.32*	11.91 ± 1.24*	9.35 ± 1.04*	0.25 ± 0.03*	17.19 ± 1.18*	0.54 ± 0.06*

Data are expressed as means ± s.e.m.
T_4, thyroxine.
*$P < 0.05$ vs. the control group.

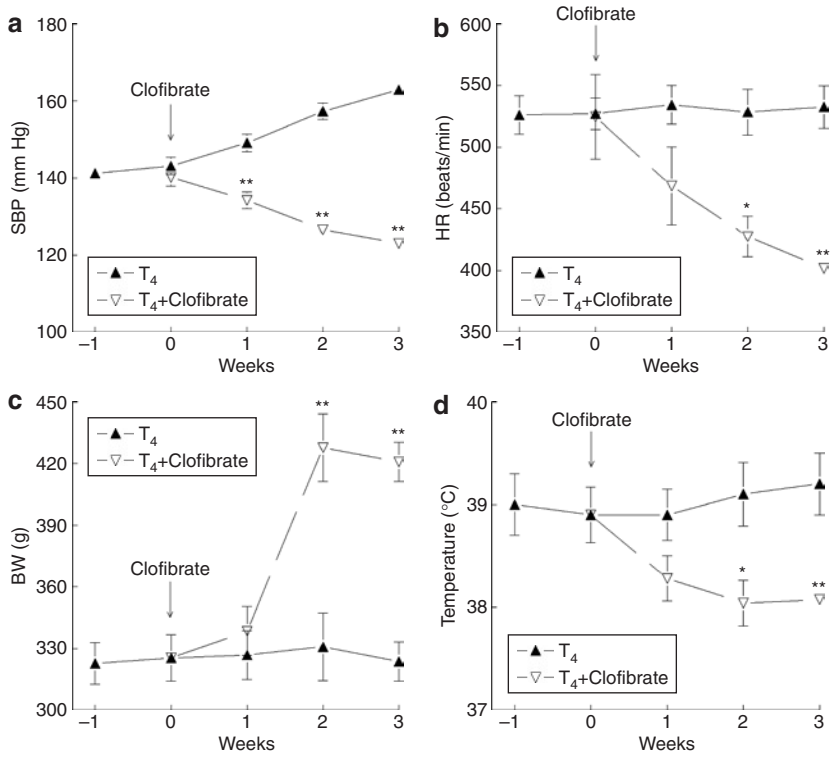

Figure 5 | Time course of (**a**) systolic blood pressure (SBP), (**b**) heart rate (HR), (**c**) body weight (BW), and (**d**) temperature measured by tail-cuff method in hyperthyroid rats treated with thyroxine (T_4) for 4 weeks before clofibrate administration. Data are means ± s.e.m. *$P < 0.05$; **$P < 0.01$, compared with the T_4 group.

Metabolic studies at the end of treatment showed increased food and fluid intake (g/100 g BW) in the T_4-treated group in comparison with controls. Clofibrate treatment did not affect food and fluid intake in normal or T_4-treated rats (Table 3). Water and sodium balances and proteinuria were significantly increased in the T_4-treated group. Creatinine clearance (normalized per g of kidney weight) was significantly decreased in the T_4-clofibrate group when compared with controls, with no significant changes in the other groups. None of the other variables were significantly modified by clofibrate in either normal or hyperthyroid rats (Table 3).

UGT activity

Phenol-UGT activity was markedly increased in the organs (liver, kidney, and heart) obtained from the T_4-clofibrate group. This variable was slightly but not significantly increased in the tissues from the T_4 and clofibrate groups (Figure 4).

Effects of clofibrate during the established phase of hyperthyroidism

Clofibrate administration, 4 weeks after hyperthyroidism induction, produced a progressive increase in BW and significant reductions in tail SBP, HR, and rectal temperature (Figure 5).

DISCUSSION

The main finding of this study was that chronic clofibrate administration to T_4-treated rats prevented and reversed the hemodynamic manifestations and increased temperature of hyperthyroidism. These effects can be mediated by the antithyroid actions of fibrates reported in the Introduction section, which include increasing thyroid hormone deactivation[10] and reducing hormone transport and action.[5,6,8,12–15] Thus, in this study, clofibrate treatment markedly reduced plasma thyroid hormone levels and increased phenol-UGT tissular activity in hyperthyroid rats.

UGTs are the enzymes responsible for thyroid hormone glucuronidation. Three UGT isoforms are known to be involved in the glucuronidation of iodothyronines: phenol-UGT (or UGT1A9) and bilirubin-UGT (or UGT1A1) and androsteron-UGT (or UGT2B7).[19] Among these isoenzymes, the measurement of phenol-UGT activity was chosen for this paper because this isoform is the main enzyme for thyroid hormone deactivation in rats.[18] The present results indicate that clofibrate shows a greater ability to stimulate this enzyme in hyperthyroid rats, which may contribute to its antihypertensive effect.

PPARα activation also has important protective effects on cardiovascular function that can interfere with the prohypertensive effects of thyroid hormones. PPARα is expressed both in the liver and in tissues with very active fatty acid metabolism, such as the heart, kidney, endothelium, and vascular smooth muscle, all primarily related to BP control. The antihypertensive effects of clofibrate-induced PPARα activation may include: higher production of endothelial[20] and renal[21] nitric oxide, which plays an important homeostatic role in BP control in hyperthyroidism;[22] lower production of reactive oxygen species and reduced NAD(P)H oxidase activity,[3]

which are increased in hyperthyroid rats;[23] and lower production of ET-1,[3] also elevated in hyperthyroid rats.[24] Moreover, fibrates also act as inducers of cytochrome P-450 enzymes.[25] Arachidonic acid can be metabolized by a family of cytochrome P-450 enzymes that catalyze the formation of epoxyeicosatrienoic acid (5,6-EET) and 20-hydroxyeicosatrienoic acid (20-HETE), among others. 20-HETE and EETs act as second messengers in vascular and renal function[25,26] and might therefore participate in the antihypertensive effect of clofibrate. However, the mechanism responsible for the protective effects of clofibrate on the cardiovascular manifestations of hyperthyroidism appears to be linked to its antithyroid actions, as reported above, with the contribution of the beneficial extrathyroid activities of clofibrate remaining to be evaluated in further investigations.

Despite the above-reported BP-lowering properties of clofibrate, this study found that treatment with this compound had no significant effect on any hemodynamic or morphological variable in euthyroid rats, and this absence of effect was consistent with the insignificant changes in phenol-UGT activity or thyroid hormone levels in these animals, suggesting that the effects of clofibrate on UGTs are related to the level of thyroid hormones. In consonance with this finding, some investigators have shown that interaction or competition between TR and PPARα occurs in a T_3 dependent manner,[7] and it has been reported that the effects of 9-cis-RXR on the expression of UGT isoforms is affected by thyroid status.[27]

Our observation, however, contrasts with the BP reduction produced by a 1-week treatment with bezafibrate (30 mg/kg i.p.) in Sprague-Dawley rats.[3] Moreover, previous reports showed that fibrates increase UGT activities and reduce thyroid hormone levels in several species. In Wistar rats, a dose of 800 mg clofibrate/kg BW/day reduced plasma T_3 concentration by 27% but did not reduce plasma concentrations of total or free T_4.[28] In mice, a dose of 300 mg clofibrate/kg BW/day reduced plasma free T_4 concentrations by 13% but did not reduce plasma free T_3 levels.[29] In pigs treated with clofibrate (220 mg/kg/day for 28 days), the reduction in plasma concentrations of total T_3 (by 47% vs. control), free T_4 (by 32% vs. control), and total T_4 (by 35%) is even greater than that observed in rats.[12] The reason for these discrepancies is not clear, although a longer period than 3 weeks may be required for the appearance of the antithyroid effects of clofibrate in normal euthyroid rats at the dose used in our study.

Despite reducing thyroid hormone levels, clofibrate treatment did not prevent the cardiac or renal hypertrophy, increased food or fluid intake, or proteinuria of hyperthyroid rats. Although the mechanisms underlying these observations have not been investigated, clofibrate may have distinct effects on deiodinases or thyroid receptor isoforms that vary in the different tissues.[5] In this context, it has been reported that when T_3 is specifically bound to the rat thyroid β-receptor it is not displaced by the PPARα agonists ciprofibrate or benzafibrate,[30] allowing thyroid hormone activity via thyroid β-receptor. Hence, characterization of the thyroid receptor responsible for distinct thyroid effects might explain the specificity of the effects of clofibrate in this study and would support the future elucidation of its biological function. In this sense, $TR\alpha_1^{-/-}$ knockout mice show decreased HR, prolonged Q–T interval on electrocardiogram, and decreased temperature;[31] manifestations that resemble our findings with clofibrate in hyperthyroid rats. Moreover, it has been reported that the thyroid hormone induces cardiac myocyte hypertrophy as a direct result of binding to the $TR\alpha_1$ isoform via a non-genomic pathway that is dependent on p38 and requires activation of the transforming growth factor β-activated kinase, transforming growth factor β-activated kinase 1,[32] a mechanism that could differ from that used to affect HR and contractility. Finally, the inability of clofibrate to prevent cardiac hypertrophy and proteinuria in T_4-treated rats, despite preventing hypertension, is in agreement with previous reports from our laboratory showing that both variables are unrelated to the BP level in hyperthyroid rats.[16]

In conclusion, this study shows that chronic clofibrate treatment suppresses the increased temperature and characteristic hemodynamic manifestations of hyperthyroidism. These effects were associated with a marked reduction in plasma thyroid hormone levels. However, clofibrate administration did not modify the cardiac and renal hypertrophy, proteinuria, polyphagia, or polydipsia of hyperthyroid rats.

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