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Effects of F2833 on cholesterol metabolism in the genetically hyperlipidemic rat

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Abstract

The effects of the new hypolipidemic agent, F2833 or (chloro 2′ (1-1′) biphenyl-4)-2 propanol-2, on cholesterol metabolism were studied in genetically hyperlipidemic rats (RICO). Cholesterolemia decreased after 2 days of treatment to 60% of its initial value (1.20 ± 0.10 g/l vs. 1.99 ± 0.08, $P < 0.001$) and then stabilised within 10 days. This hypocholesterolemic action was effective for as long as 3 months. Concerning the different classes of lipoproteins, a significant drop was observed in HDL (high density lipoproteins) (25%, 0.49 ± 0.02 g/l vs. 0.66 ± 0.007, $P < 0.01$) and particularly in LDL (low density lipoproteins) (70%, 0.30 ± 0.04 g/l vs. 0.92 ± 0.05, $P < 0.001$). Whole body cholesterol showed a higher fractional catabolic rate (0.25 ± 0.02 vs. 0.17 ± 0.005 day^{-1}, $P < 0.01$) together with an increased cholesterol synthesis (60 ± 5 vs. 36 ± 4 mg/day, $P < 0.01$). LDL kinetics showed that the decrease in these lipoproteins is essentially caused by an increase in the fractional catabolic rate ($10.6 \pm 0.1\%$/h vs. $5.2 \pm 0.1\%$/h, $P < 0.001$) and by a lesser decrease in the LDL production rate. This cholesterol metabolic profile created by treatment suggests an effect through stimulation of cholesterol output (biliary cholesterol elimination or cholesterol transformation into bile acids). © 2001 Published by Elsevier Science B.V.

Keywords: Hypocholesterolemic drug; Cholesterol turnover; Low density lipoprotein kinetics

1. Introduction

Among animal models for hyperlipidemia, RICO (genetically hyperlipidemic rats) is particularly useful for its genetic hypercholesterolemia and to a much lesser extent for its hypertriglyceridemia (Muller et al., 1979; Cardona-Sanclemente et al., 1988; Ouguerram et al., 1990). The lipoprotein profile of this strain is essentially characterised by an increase in the concentrations of low density lipoproteins (Ouguerram et al., 1992). This situation is the result of a decrease in LDL (low density lipoproteins) catabolism, together with an increase in LDL production (Ouguerram et al., 1996). A new molecule, F2833 or (chloro 2′ (1-1′) biphenyl-4)-2 propanol-2, has been described as presenting hypocholesterolemic effects in the diet-induced hyperlipidemic hamster but not in hamsters fed a standard diet (Oms et al., 1992). The purpose of the present study was to evaluate the effect of F2833 on cholesterol metabolism in the genetically hypercholesterolemic RICO rat. Whole body cholesterol turnover and LDL turnover were studied during F2833 treatment in this strain of rats. For the studies on lipoprotein kinetics, an intracellularly trapped tracer (Pittman and Taylor, 1986) was used as much as possible to minimise tracer recycling.

2. Materials and methods

2.1. Chemicals and isotopes

F2833 (batch no. L3028) was synthesised by the Chemistry Department, Centre de Recherche Pierre Fabre (France). Iodogen (1,3,4,6-tetrachloro-3α, 6α-diphenylglycouril) was obtained from Pierce Chemical. [^{125}I] and [^{3}H]cholesterol (40 to 60 Ci/mmol) were obtained, respectively, from Amersham and CEA (Saclay, France).

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2.2. Animals

The animals were handled and cared for in accordance with the guide for the care and use of laboratory animals (NRC, 1996) and the European directive 86/609, and the protocols were carried out in compliance with French regulations and with local ethical committee guidelines for animal research. Genetically hypercholesterolemic (RICO) male rats were purchased from Ciba-Geigy (Basel, Switzerland). They were fed a semi-purified diet of sucrose (53%), casein (23%), lard (9.2%), mineral mix (5%), skim milk (4%), yeast (2.3%), vitamin mix (5%), walnut oil (0.8%), and cystine (0.2%) as previously described (Perrodin and Lutton, 1985). The rats were fed the diet for 2 months before the experiments, at which time their body weight was 400–450 g.

2.3. Experimental procedures

Treated RICO rats were intubated daily with 1 ml of methyl cellulose (1%) containing F2833, 150 mg/kg/day. Control RICO rats received 1 ml of methyl cellulose.

Two groups, 15 treated rats and 15 control rats, were used to study cholesterolemia evolution during treatment. Blood samples were collected every day to determine cholesterol concentration. These samples were taken from the tail vein for 15 days after the treatment began. At the end of the experiment, the samples were also used for determination of triglyceridemia, phospholipidemia and total apolipoprotein concentrations.

Two groups, five treated rats and five control rats, were used to determine the plasma lipoprotein profile. The rats were killed by aortic puncture under ether anaesthesia after 15 days of treatment.

Two groups, seven treated rats and seven control rats, were used for whole body cholesterol study. The rats were intubated daily for 1 month before the kinetic study. Blood samples (0.5 ml) were collected from the caudal vein at different time intervals for 2 months following the radioactive labelling. At the end of experiment, the rats were killed by aortic puncture under anaesthesia and the blood was analysed for cholesterolemia.

In order to study LDL kinetics, the rats were intubated for 15 days before the experiments. Two groups, five treated rats and five control rats, were used as recipient rats. Donor rats (four treated rats and four control rats) were killed by aortic puncture. LDL isolated from the donor rats were injected via the dorsal vein of the penis (0.5 ml, about 10^6 dpm). Blood samples (50 μl) were collected from the caudal vein at various times over 48 h. At the end of the experiment, the rats were anaesthetised with diethyl ether and exsanguinated. Blood was collected on 4% EDTA (ethylene diamine tetraacetic acid) and 3.7% MIA (monoiodoacetamide) and centrifuged ($2000 \times g$, 15 min, 4°C) to obtain plasma for lipoprotein separation by density gradient. These lipoproteins were assayed to determine radioactivity distribution. In order to differentiate the role played by the treatment in catabolic mechanisms and in particle modifications, the rats received LDL from the same group (autologous LDL) and from the other group (heterologous LDL).

2.4. Plasma lipoprotein profile

The blood from each rat was collected on 0.3 ml of 4% Na_2EDTA, 4% monoiodoacetamide, 0.02% NaN_3 and was centrifuged at $2200 \times g$ for 20 min at 4°C. After overlaying 5 ml of plasma with 2 ml of NaCl solution with a density of 1.006 and centrifuging at 15 000 rpm for 30 min at 15°C using a 40 Ti rotor in a Beckman L8-70 centrifuge, the chylomicrons were isolated from the meniscus of the tube. The density of the chylomicron-free plasma from the rats was adjusted to density 1.21 g/ml with solid Kbr and ultracentrifuged to obtain the lipoproteins by flotation (Terpstra et al., 1981). A density gradient was then prepared in 11-ml polyallomer tubes, successively filled from the bottom up with 1.5 ml of density 1.006 g/ml, 1.5 ml of density 1.020 g/ml, 2 ml of density 1.040 g/ml, 3 ml of density 1.063 g/ml, 2 ml of the plasma sample with density 1.21 g/ml and 1 ml of density 1.25 g/ml. The tubes were centrifuged at 40 000 rpm for 24 h at 15°C in a Beckman SW41 rotor. Twenty two fractions of 0.5 ml each were successively removed from the meniscus with a syringe. Correspondence with lipoprotein classes was assessed from a control tube, prepared using a saline solution with density of 1.21 g/ml and by measuring 22 fractions of 0.5 ml each, which had been successively collected from the top of the tube after ultracentrifugation under the same conditions: VLDL (very low density lipoproteins) fractions 1 to 3 (density < 1.006), IDL (intermediate density lipoproteins) fractions 4 to 7 (density 1.006–1.020), LDL fractions 8 to 14 (density 1.020–1.063), HDL (high density lipoproteins) fractions 15 to 22 (density 1.063–1.25).

2.5. Whole-body cholesterol turnover

The rats received 0.5 ml Tween 20 containing [^{3}H]cholesterol (54 μCi) intravenously. Blood samples (0.5 ml) were collected from the caudal vein at various time intervals for 2 months following the radioactive labelling. Specific radio activity of total plasma cholesterol was measured. The experimental curve was fitted to a pluriexponential equation, using the SAAMII program (Barrett et al., 1998). The coefficients obtained were used to calculate the total input flux of cholesterol into the system and the fractional catabolic rate of body cholesterol (Perl and Samuel, 1969; Magot, 1990).

2.6. LDL turnover

LDL isolation: LDL (density 1.020–1.050) were isolated by ultracentrifugation as described above. The separation densities were chosen to minimise contamination of LDL by HDL. An aliquot of LDL fraction was used for cholesterol and apolipoprotein characterisation. The rest was used for apolipoprotein labelling.

Apolipoprotein characterisation: samples were dialysed against 0.05% EDTA, 0.9% NaCl, 0.02% NaN_3, pH 7.4 at 4°C and lyophilised, then dissolved in Tris 0.02 M, urea 6 M, dodecyl sulfate 0.01 M, pH 8.4. Apolipoproteins were separated on SDS (sodium dodecyl sulfate) polyacrylamide gel (0.5×10 cm) prepared according to a discontinuous gradient of 5.5–10% in SDS pH 7 phosphate buffer (anhydrous $NaHPO_4 \cdot 12H_2O$, 154.65 g; SDS, 6 g in 3 l of demineralized water). An aliquot of lipoprotein proteins, 50 μg, was reduced and denatured with a mixture containing 10 μl of dithiothreitol (400 μM), 10 μl of 20% SDS, 10 μl of isopropanol and a drop of glycerol in a boiling waterbath for 5 min. The mixture, stained with 0.05% bromophenol, was then deposited on each gel. At the same time, various standard proteins with known molecular weights in the range of those contained in the samples were also deposited. Proteins were then subjected to an electric field of 1.8 mA per gel for 20 h. The gels were stained by immersion in a mixture of Coomassie blue solution (0.2%), methanol (50%) and acetic acid (7.5%). After destaining (acetic acid/methanol/distilled water) (75:80:875, v/v/v), the relative composition of each apolipoprotein in the samples was measured by band scanning (laser densitometer LKB ultrascan 2202) and signal integration (Delsi Enica 10).

2.6.1. LDL labelling

The LDL fraction was labelled with [^{125}I]tyramine cellobiose as previously described (Glass et al., 1983). Ten micrograms of iodogen in methylene chloride was added to a 30-μl microreaction vessel. The solvent was evaporated by hand warming to leave a thin coating of iodogen on the walls. The vessel was washed vigorously with water several times to remove any loosely attached iodogen particles. Tyramine cellobiose, 0.1 μmol in 10 μl of 0.4 M sodium phosphate buffer, pH 7.4 was added to the iodogen-coated vessel. Carrier-free Na–^{125}I (5–10 μCi at concentration of 100 μCi/ml) was added to bring the volume up to 200 μl. Iodination was allowed to proceed for 30 min. The solution was transferred to another vessel containing 5 μl of 0.1 M NaI and 10 μl of 0.1 N $NaHSO_3$ to stop further iodination. To activate the radioiodinated tyramine cellobiose, 0.1 μmol of cyanuric chloride in 20 μl of acetone was added to the solution of [^{125}I]tyramine cellobiose prepared as above. The reaction was allowed to proceed for less than 1 min, then 5 mg/ml of LDL protein was added for binding. This reaction was carried out for 3 h or more at room temperature. The labelled protein was then cleared of unbound LDL by dialysis with 0.2 M sodium phosphate, pH 6.8, several times before using for injection.

2.6.2. In vivo kinetics

The experimental curves for plasma radioactivity were adjusted to a pluriexponential equation with the program described earlier. The fractional catabolic rate of LDL was calculated from the equation coefficients (Matthews, 1957; Langer et al., 1972). The production rate of LDL cholesterol (expressed in μg/ml/h) was determined by multiplying the fractional catabolic rate (in pool/h) by the plasma LDL cholesterol concentration (in μg/ml).

2.7. Chemical and radioisotopic analysis

The concentration of total apolipoproteins in the lipoprotein fractions and in the labelled LDL preparation was determined by the Lowry method, using bovine serum albumin as a standard (Lowry et al., 1951). Cholesterol, phospholipids and triglycerides were assayed in whole serum and individual fractions, with enzymatic reagent kits (Boehringer Mannheim, FRG).

In order to determine whole body cholesterol turnover, cholesterol was isolated from the plasma as previously described (Ouguerram et al., 1996). The specific activity of total cholesterol was then measured by radioactivity determination in a scintillation counter (MR 300, Kontron, Milan, Italy) using scintillation liquid (lipoluma). Correction for quenching was performed with an external standard. The radioactivity of blood samples collected after labelled LDL injection was determined in a gamma counter (Cristal, Packard).

2.8. Expression of results and statistical analysis

Results were expressed as the means ± S.E.M. and statistical significance of differences between control and treated RICO rats was determined with Student's *t*-test. Paired tests were used between values obtained from different steps of treatment in the same animal.

The number of exponentials in the curve-fitting was tested using AIC (Akaike Information Criterium) (Akaike,

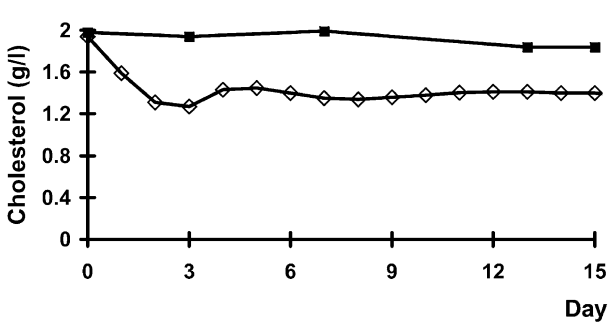

Fig. 1. Effect of F2833 on plasma cholesterol concentration (g/l) in RICO rats before (■) and after (◇) treatment with F2833.

Table 1
Effect of F2833 on lipid concentrations (mg/l) in lipoprotein fractions in RICO rats

	VLDL + IDL	LDL	HDL	Plasma
Cholesterol				
Control	190 ± 19	928 ± 50[a]	660 ± 7[b]	1990 ± 80[b]
Treated	228 ± 75	313 ± 40	490 ± 20	1319 ± 190
Triglycerides				
Control	1806 ± 314	145 ± 14	231 ± 56	3000 ± 900
Treated	1094 ± 77	125 ± 8	200 ± 29	2200 ± 700
Phospholipids				
Control	336 ± 71	769 ± 150[b]	1525 ± 332	3300 ± 600
Treated	200 ± 20	254 ± 25	899 ± 77	1700 ± 100

[a] $P < 0.001$.
[b] $P < 0.05$.

1974; Yamaoka et al., 1978) and the *F*-test (Boxenbaum et al., 1974).

3. Results

3.1. Effect of F2833 on plasma and lipoprotein composition

Fig. 1 shows the time course of cholesterolemia during the 15-day treatment. During the first 3 days, the plasma cholesterol dropped to 60% of its initial value (1.20 ± 0.10 vs. 1.99 ± 0.08 g/l, $P < 0.001$). From the 4th to 10th day after the beginning of the treatment, plasma cholesterol fluctuated and then stabilised until the end of the experiment (15 days) to 66% (1.32 ± 0.19 vs. 1.99 ± 0.08 g/l, $P < 0.001$) of the initial values. At this time (Table 1) no significant change was observed for triglyceridemia (3.0 ± 0.9 vs. 2.2 ± 0.7 g/l before and after treatment), phospholipidemia (3.3 ± 0.6 vs. 1.7 ± 0.1 g/l) and total apolipoprotein concentration (1.64 ± 0.13 vs. 1.55 ± 0.12 g/l).

The cholesterol profile of the lipoproteins (Fig. 2 and Table 1) showed a marked drop in the cholesterol concentration, in the density of LDL (fractions 8 to 14) and HDL (fractions 15 to 22). The cholesterol concentration decrease was 70% in the LDL (0.30 ± 0.04 vs. 0.92 ± 0.05 g/l, $P < 0.001$) and 25% in the HDL (0.49 ± 0.02 vs. 0.66 ± 0.007 g/l, $P < 0.01$). No significant change was observed for the other classes of lipoproteins.

3.2. Effect of F2833 on whole body cholesterol turnover

Whole body cholesterol turnover was studied during a 2-month kinetic experiment following the 1-month treatment. During this 3-month period, cholesterolemia was stable without the F2833 treatment (1.69 ± 0.07 at beginning vs. 1.69 ± 0.05 mg/ml at the end) and significantly decreased in the treated group (1.34 ± 0.08 vs. 1.66 ± 0.05 mg/ml at the beginning of treatment, $P < 0.01$).

After an intravenous injection of tritiated cholesterol, the time course of labelling (Fig. 3) showed that the specific activity of plasma total cholesterol decreased more rapidly for the treated group than for the untreated one. The curves for the decrease in plasma cholesterol specific activity were fitted to a biexponential equation (Fig. 3). Fitting to a triexponential equation provided no significant improvement and therefore was not taken into consideration in agreement with other reports (Cohn et al., 1984; Oh et al., 1976; Robins and Russo, 1979). Use of the occupancy principle showed (Table 2) that the cholesterol input flux into the system was significantly higher in the treated rats (60 ± 5 mg/day) than in the untreated ones (36 ± 4 mg/day, $P < 0.01$). The treatment significantly increased the fractional catabolic rate of whole body cholesterol (0.25 ± 0.02 vs. 0.17 ± 0.005 day^{-1}, $P < 0.01$).

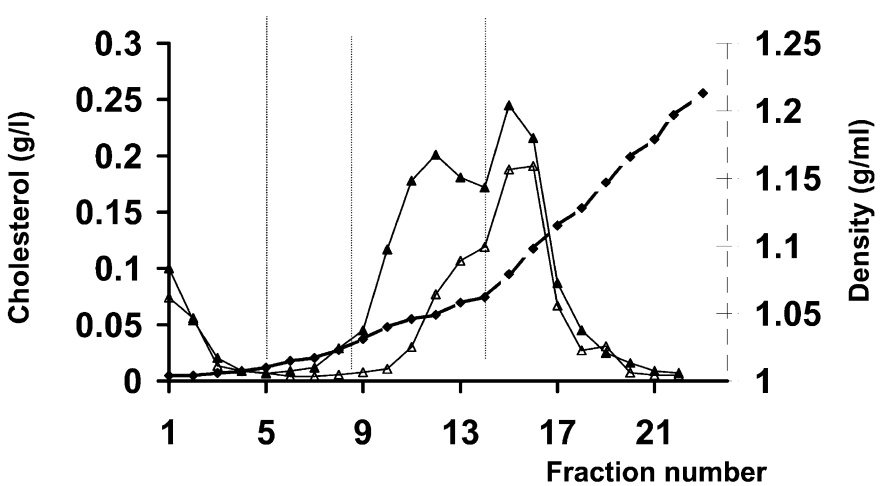

Fig. 2. Plasma cholesterol (g/l) distribution in RICO rats before (▲) and after (△) treatment. Dashed curve represents density measured (g/ml) in fractions.

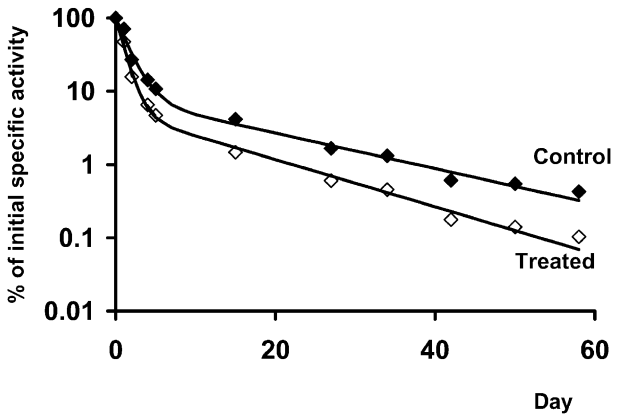

Fig. 3. Plasma cholesterol disappearance (in % of initial specific activity) in RICO rats before (♦) and after (◇) treatment following intravenous injection of [^{3}H]cholesterol. Symbols represent experimental data and lines are curves fitted using a biexponential equation.

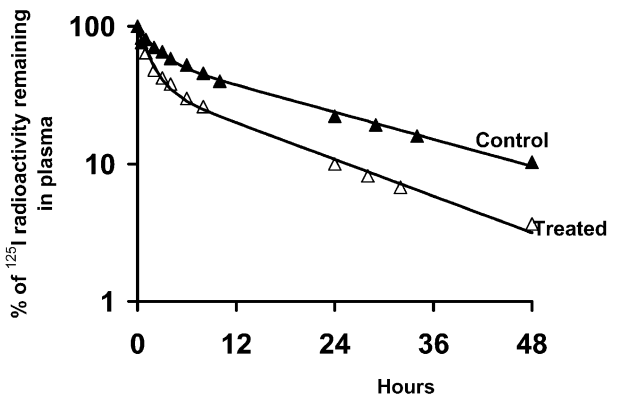

Fig. 4. Plasma decay of [^{125}I]labelled LDL in control (▲) and treated (△) RICO rats. Points represent data values. Lines represent curves fitted using a biexponential equation.

3.3. Effect of F2833 on LDL turnover

Electrophoresis analysis of the lipoprotein fraction (density 1.020–1.050) used for labelling shows that virtually no apolipoprotein A-I (contamination index of LDL fraction by HDL particles) was present (less than 5% of the total apolipoproteins). The apolipoproteins recovered in this fraction were almost exclusively apolipoproteins B100 (22 ± 2 et 40 ± 4%) and E (67 ± 3 et 51 ± 3%), in control and treated rats, respectively.

At the time of killing (48 h after the injection), more than 80% of plasma radioactivity was recovered in the LDL fraction (density 1.020–1.050) in control and treated rats. The rest was recovered in the 1.050–1.21 fraction.

Fig. 4 shows the time course of plasma radioactivity 48 h following labelled LDL injection. The curves were fitted to a biexponential equation (Fig. 4). Fitting to a triexponential equation provided no significant improvement and therefore was not taken into consideration, in agreement with similar analyses for this strain of rats (Ouguerram et al., 1996).

Table 2 shows the values of LDL turnover parameters for treated and untreated rats after injection of labelled LDL. The fractional catabolic rate calculated after the injection of autologous LDL was greater ($P < 0.001$) in the treated group (10.6 ± 0.1%/h) than in the untreated one (5.2 ± 0.1%/h). Treatment with F2833 slightly lowered the production rate of LDL cholesterol: 15.6 ± 0.3 in treated rats vs. 20.9 ± 0.2 μg/ml/h in control rats ($P < 0.01$). The injection of heterologous LDL yielded FCR values that were not significantly different for treated or untreated recipient rats (13.9 ± 0.9 and 9.9 ± 0.5%/h, respectively) and not significantly different from those found after an autologous LDL injection in treated rats (10.6%/h noted above).

Table 2
Effect of F2833 on the parameters of whole body cholesterol and plasma LDL turnover in RICO rats

	Control rats	Treated rats
Cholesterol turnover		
Production rate (mg/day)	36 ± 4[a]	60 ± 5
Fractional catabolic rate (day^{-1})	0.17 ± 0.005[a]	0.25 ± 0.02
LDL turnover		
Production rate (μg/ml/h)	20.9 ± 0.2[a]	15.6 ± 0.3
Fractional catabolic rate (%/h)	5.2 ± 0.1[b]	10.6 ± 0.1

[a] $P < 0.01$.
[b] $P < 0.001$.

4. Discussion

4.1. Cholesterol concentrations

F2833 is an effective hypocholesterolemic drug that decreases cholesterolemia by a third after 2 days of treatment. This effect lasts for 3 months, as demonstrated during whole body cholesterol kinetic experiments. This hypocholesterolemic effect acts essentially on the LDL fraction. Hypercholesterolemia in the RICO rat strain concerns mainly this class of lipoproteins (Ouguerram et al., 1996). Thus, F2833 appears to be a convenient hypocholesterolemic agent for durable normalisation of a hypercholesterolemic situation with elevated LDL.

4.2. Whole body cholesterol kinetics

Whole body kinetics for untreated RICO rats yielded values similar to the ones previously obtained by the same approach in this strain of rats (Ouguerram et al., 1996). More particularly, a major increase was still observed in the cholesterol production flux of the RICO rat vs. that of the normocholesterolemic strain: cholesterol production rate, 18.5 mg/day in the normocholesterolemic strain (Ouguerram et al., 1996) vs. 36 mg/day in Rico rats from the present study and 29 mg/day in the previous study

(Ouguerram et al., 1996). Conversely, the fractional catabolic rate of cholesterol was the same in the normocholesterolemic strain, 0.15 day^{-1} (Ouguerram et al., 1996) and in Rico rats, 0.17 day^{-1} in the present study and 0.14 day^{-1} in the previous study (Ouguerram et al., 1996). Thus, the increase in cholesterol synthesis rate is the essential mechanism concerning whole body cholesterol turnover, which explains the hypercholesterolemia in RICO rats. After treatment, the hypocholesterolemic effect can only be explained by an increase in the cholesterol fractional catabolic rate (by a factor of 1.5), representing cholesterol transformation into bile acids and biliary cholesterol elimination. This fractional catabolic rate was not affected in this animal model compared to that in the normolipidemic strain (Ouguerram et al., 1996). In contrast, the production rate described as higher in the RICO rats than in the normal strain was even higher after the treatment (1.7-fold). This demonstrates that the normalisation of cholesterolemia in the RICO rats is obtained through mechanisms other than the ones disturbed by the pathology. The kinetic profile of whole body cholesterol turnover obtained with F2833 treatment (increased synthesis rate, increased fractional catabolic rate) is similar to the one obtained with a treatment acting through stimulation of bile acid synthesis, as demonstrated in humans by the study of bile acid sequestrants on whole body cholesterol turnover (Quarfordt and Greenfield, 1975).

4.3. LDL kinetics

An intracellularly trapped tracer (Pittman and Taylor, 1986) was used to minimise tracer recycling as much as possible in the studies on LDL kinetics. In order to make radioactivity measurement easier, [^{131}I] linked to tyramine cellobiose was used in the present study. This procedure had already been validated for kinetic studies concerning HDL apolipoprotein A-I (Glass et al., 1983) and LDL apolipoprotein B100 (Goldberg et al., 1991; Pittman et al., 1987). Similar labelling, using [^{14}C]sucrose, was also used and validated in the same strain of rats in our laboratory for LDL kinetics (Ouguerram et al, 1996). These two labelling procedures give similar fractional catabolic rates for LDL in RICO rats: 5.5%/h for [^{14}C]sucrose (Ouguerram et al, 1996) vs. 5.2%/h for [^{131}I]tyramine cellobiose in the present study.

Apolipoprotein composition of the lipoprotein fraction showed less than 5% of apolipoprotein A-I in the injected LDL fraction: contamination of injected LDL by HDL, particularly HDL_1 possibly found in the fraction of density < 1.063 in rats, was negligible in our study. This is also validated by the fact that the labelling remained essentially in the LDL fraction during the experiment (80% of the labelling at the end of experiment in the 1.020–1.050 density) with [^{131}I]tyramine cellobiose similar to [^{14}C]sucrose labelling (Ouguerram et al, 1996). In our study, the labelling can be considered as LDL particle labelling.

The effect of F2833 on LDL concentration was essentially due to an increase in the LDL fractional catabolic rate. A comparison of effects of LDL injection in treated and in untreated rats shows that this effect was essentially the result of enhanced activity of catabolism and not an effect on the LDL particle. Thus, F2833 could act by stimulating the LDL receptor. This could be the consequence of the enhanced catabolism of body cholesterol during the treatments as demonstrated above. It is well established that stimulation of cholesterol catabolic rate, consecutive to bile acid sequestration, results in the stimulation of LDL catabolism (Shepherd et al., 1980; Levy and Langer, 1972) together with moderately lower (Witzum et al., 1985) or unchanged (Shepherd et al., 1980) LDL production rates.

Taking into account the studies concerning LDL metabolism and those concerning whole body cholesterol turnover, the hypothesis can be proposed that F2833 normalises the lipoprotein profile of RICO rats by stimulating LDL catabolism, probably through the LDL receptor. This situation could be the result of hepatic cholesterol depletion consecutive to the increase in body cholesterol catabolic rate now observed. The enhanced production rate of cholesterol also observed in our studies is consistent with this hypothesis. The increase in cholesterol catabolic rate may be produced either through an increase in bile acid synthesis or direct biliary cholesterol elimination. Our results do not allow an answer to these questions because the methodology used only quantifies the total fractional catabolic rate of cholesterol. However, the F2833 molecule is similar to that of fibrates (Tikkanen, 1992). The latter hypolipidemic drugs are known for a PPAR (peroxisome proliferator activated receptor)-mediated effect on lipid metabolism (Staels et al., 1998). Recent studies showed a stimulating effect of PPARα on bile acid biosynthesis (Hunt et al., 2000). A stimulating effect on bile acid synthesis is then an acceptable hypothesis for F2833. However, further studies on the effects of F2833 on biliary cholesterol and bile acid metabolism are required to support this hypothesis.

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