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HOE 402 lowers serum cholesterol levels by reducing VLDL-lipid production, and not by induction of the LDL receptor, and reduces atherosclerosis in wild-type and LDL receptor-deficient mice

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Abstract

Previous rodent studies suggested that the potent hypolipidemic agent 4-amino-2-(4,4-dimethyl-2-oxo-1-imidazolidinyl)pyrimidine-5-*N*-(trifluoromethyl-phenyl) carboxamide monohydrochloride (HOE 402) is an inducer of the LDL receptor (LDLR). Using wild-type and heterozygous and homozygous LDLR-deficient (LDLR+/0 and LDLR0/0) mice, fed a low or high cholesterol diet, we investigated whether HOE 402 specifically induces the LDLR and whether other pathways are affected. Upon treatment with 0.05% (w/w) HOE 402, the serum cholesterol levels of wild-type, LDLR+/0 and LDLR0/0 mice, were maximally reduced by 53, 56, and 73%, respectively ($P < 0.05$), by reducing levels in very low density-lipoprotein (VLDL), intermediate density-lipoprotein (IDL), and low density-lipoprotein (LDL) cholesterol, whereas high density-lipoprotein (HDL) cholesterol levels were increased. The observations that HOE 402 exhibited no effect on *in vivo* clearance of ^{125}I-labeled LDL in wild-type mice, and clearly reduced serum cholesterol levels in LDLR0/0 mice, indicate that the LDLR is not the main target for the compound. In wild-type mice, production of VLDL-TG, and cholesterol were reduced by more than 50% by HOE 402 ($P < 0.05$), whereas VLDL apolipoprotein B (ApoB) secretion was unaffected, indicating that HOE 402 treatment changes the size, rather than the number of the secreted VLDL particles. The reduced VLDL production was accompanied by a 22% decreased hepatic cholesterol ester concentration ($P < 0.05$). Additionally, HOE 402 treatment strongly reduced the aortic content of atherosclerotic lesions by 90 and 72% in LDLR+/0 and LDLR0/0 mice, respectively ($P < 0.01$). In conclusion, HOE 402 is a potent cholesterol-lowering compound, which inhibits VLDL production, and consequently attenuates atherosclerosis development.

Keywords: HOE 402; VLDL-production; LDL-clearance; Atherosclerosis; LDL receptor; LDL receptor knock-out mice

1. Introduction

A high LDL cholesterol level is an independent risk factor for the development and progression of atherosclerosis in humans, but the risk can be substantially reduced by different cholesterol-lowering therapeutic modalities. Nowadays, treatment is mainly focused on: (i) inhibition of endogenous HMG-CoA reductase by statins, (ii) the reduction of VLDL secretion by nicotinic acid and derivatives, (iii) inhibition of intestinal uptake of bile acids and cholesterol, and (iv) activation of the nuclear peroxisome proliferator-activated receptor by fibric acid derivatives [1–3], the latter leading to lowered serum TG and cholesterol levels, and increased HDL cholesterol levels.

4-amino-2-(4, 4-dimethyl-2-oxo-1-imidazolidinyl)pyrimidine-5-*N*-(trifluoromethyl-phenyl carboxamide monohydrochloride (HOE 402)), is a lipid-lowering compound with a mechanism that is potentially different from those described above. HOE 402 strongly reduced serum

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Abbreviations: ACAT, acyl-coenzyme A:cholesterol acyltransferase; ALAT, alanine aminotransferase; Apo, apolipoprotein; HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HOE 402, 4-amino-2-(4,4-dimethyl-2-oxo-1-imidazolidinyl)pyrimidine-5-*N*-(trifluoromethyl-phenyl) carboxamide monohydrochloride; HDL, high density-lipoprotein; IDL, intermediate density-lipoprotein; LDL, low density-lipoprotein; VLDL, very low density-lipoprotein; LDLR+/0 or 0/0, LDL receptor heterozygous or homozygous knockout.

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LDL-cholesterol levels in hamsters and Watanabe heritable hyperlipidemic rabbits [4,5]. The effect of HOE 402 could not be attributed to interference with intestinal cholesterol absorption or hepatic sterol synthesis [5]. LDL-clearance rate, however, was doubled in HOE 402-treated hamsters and rabbits. Moreover, LDL-binding sites and LDLR mRNA in cultured HepG2 cells incubated with HOE 402 were 3- to 4-fold increased compared to control, suggesting that HOE 402 induced the upregulation of LDLR activity [4]. However, the *in vivo* specificity of HOE 402 as a LDLR inducer remained unclear. To investigate whether HOE 402 acts *via* the induction of the LDLR, the present study was performed in wild-type, heterozygous and homozygous LDLR deficient mice. On a high fat/cholesterol diet LDLR-deficient mice exhibit elevated levels of LDL and VLDL, caused by an impaired LDLR-mediated clearance [6,7]. We argued that if HOE 402 acts directly on the LDLR, it should be ineffective in LDLR0/0 mice.

We found that HOE 402 is a potent cholesterol-lowering and anti-atherosclerotic compound that acts independently of the LDLR.

2. Materials and methods

2.1. Animals, diets and study design

Three- to five-month-old female wild-type, LDLR+/0 and LDLR0/0 mice, crossed back on a C57Bl6/J background (IFA-Credo and Jackson Labs) were used. Female mice were used because they are more responsive to the dietary intake of fat and cholesterol with respect to changes in plasma lipids and consequently to development of atherosclerosis. All animals were housed in wire-topped cages with sawdust as bedding, and all diets and water were given *ad lib*. Food consumption was assessed weekly at group level.

Three semi-synthetic pelleted diets [8] (Hope Farms), named J, W, and N were used in this study (Table 1). Diet J contained no cholesterol and no cholate, diet W contained 0.25% cholesterol and no cholate, whereas diet N contained 1% cholesterol and 0.5% cholate. Added to these diets was 0% (control diets) or 0.05% HOE 402 (Hoechst AG), which equals 0 or 50 mg/kg body weight HOE 402 per day, respectively. At the start of a 2-week run-in period, diets were changed from chow to one of the semi-synthetic diets (Table 1). Thereafter, animals were randomized in age-matched groups ($N = 6$–9), and intervention periods of 4 weeks (LDL clearance and VLDL production) or 21 weeks (atherosclerosis) started, using diets enriched with 0.05% HOE 402 (treated) or without this compound (controls).

Table 1
Composition of the semi-synthetic diets

Diet components	Type of diet (g/100g diet)		
	J	W	N
Cocoa butter	–	15.0	15.0
Cholate	–	–	0.5
Cholesterol	–	0.25	1.0
Sucrose	50.5	40.5	40.5
Corn starch	12.2	10.0	10.0
Corn oil	5.0	1.0	1.0
Cellulose	5.0	6.0	4.7

Furthermore, the diets contained 20.0% casein, 1.0% choline chloride, 0.2% methionine, and 5.1% vitamin and mineral mixture. All percentages are w/w. The energy content of the diet J was 16246 and 18226 J/g for the W and N diets.

Blood samples were drawn from the tail vein after 4 hr of fasting. At the study endpoint, fasted (4 hr) mice were anaesthetized with 2.5 mL/kg Dormicum and 2.5 mL/kg Hypnorm (Janssen Pharmaceutica), blood was collected by orbital puncture, and mice were sacrificed *via* cervical dislocation.

2.2. Serum and liver lipids

Serum cholesterol and TG levels were determined individually using commercially available enzymatic kits, and ultracentrifugation lipoprotein profiles were made of pooled sera as previously described [9]. For determination of liver lipids, livers were mechanically homogenized in PBS (pH 7.4), protein contents were measured [10], cholesterol acetate was added as an internal standard followed by lipid extraction [11], and separation by thin-layer chromatography and quantification of the bands [12].

2.3. LDL clearance and VLDL production

Human LDL, isolated by ultracentrifugation, was radioiodinated using [^{125}I]monochloride [13], to a specific activity of 100–300 cpm/ng of protein, and extensively dialysed against PBS/10 μM EDTA at 4°. Wild-type mice on diet J were injected in the tail vein with 200 μL NaCl (0.9%) containing the freshly isolated [^{125}I]-labeled human LDL (10 μg protein). Heparinized blood samples were drawn from the tail vein, 5 min (100% reference value), 40 min, and 2, 5, 10 and 24 hr after LDL injection. Radioactivity was determined in these samples; after 24 hr, 90% of the radioactivity was still associated with the ApoB fraction as determined by the method described in the VLDL-production study.

After 4 hr of fasting, wild-type mice on diet J were anaesthetized as described above. *In vivo* hepatic VLDL ApoB synthesis and VLDL-TG production were determined after intravenous [^{35}S]methionine and Triton WR-1339 injections [9]. 50 μL blood samples were drawn from the tail vein at 0.5, 10, 20, 30 and 60 min after injections. Blood, collected 60 min after injections, was used for quantification of newly synthesized ApoB in nascent

VLDL. Therefore, nascent VLDL was isolated after ultracentrifugation, ApoB was precipitated with isopropanol, as described previously [9], and radioactivity was determined in ApoB48 and ApoB100 after 4–20% SDS-PAGE gradient gel electrophoresis under reducing conditions [14].

2.4. Assessment of atherosclerosis

LDLR+/0 and 0/0 mice were sacrificed after a 5-month intervention period on diet N and W, respectively, with and without 0.05% (w/w) HOE 402. Aortas from the aortic origin to the iliac bifurcation were put in 3.7% formaldehyde at 4° until use. After adventitial fat removal, the aortas were cut open longitudinally *via* the outer curvature and pinned en face [15] on a silicone basement using insect pins. Then fat-containing lesions were specifically stained with Oil Red O (Aldrich Chemical Co), and lesion areas were measured quantitatively by using a drawing tube connected to a light microscope (MOP-videoplan, Kontron). Lesion areas and total aortic areas were selected by hand, using a digitialized tablet, and a computer (Videoplan evaluation software for image analysis) calculated the size of the selected areas. The same operator blindly scored all thoracic aortas, from origin to the diaphragm.

2.5. Statistical analysis

Data are expressed as mean ± SD. For comparisons of two groups, data were evaluated by the nonparametric Mann–Whitney test for unpaired data. Differences between control and HOE 402-treated groups were considered as statistically significant when $P < 0.05$.

3. Results

3.1. Effect of HOE 402 on serum lipid levels in wild-type, LDLR+/0 and LDLR0/0 mice

The cholesterol- and TG-lowering potencies of HOE 402 were evaluated in wild-type, LDLR+/0, and in LDLR0/0 mice. For this purpose, these mice were put on the following diets, with or without HOE 402 added: The wild-type and LDLR+/0 mice received for 4 weeks a lipogenic sucrose-containing diet J and a high fat/cholesterol-cholate containing diet N. In addition, LDLR+/0 and LDLR0/0 mice received for 21 weeks, high fat/cholesterol containing diets N and W, respectively. LDLR0/0 mice did not receive diet N, but received diet W, which contains no sodium cholate and less cholesterol than diet N. This was done because serum cholesterol levels of LDLR0/0 mice on diet N would become too high, and these mice would develop a severe hypercholesterolemia with additional pathologies such as cutaneous xanthomatosis [16].

In all mouse strains used, the applied dietary HOE 402 dose 0.05% (w/w) had no effects on body weight, weight gain, food intake and on serum enzyme activities of the liver damage parameter ALAT (data not shown).

The serum cholesterol and triglyceride levels of the wild-type, LDLR+/0 and LDLR0/0 mice are given in Table 2. Compared to controls, HOE 402 reduced serum cholesterol levels in wild-type and LDLR+/0 mice fed diet J, but this effect was more pronounced on diet N. HOE 402 potently reduced serum cholesterol levels in LDLR+/0 and LDLR0/0 mice after 21 weeks of treatment. These cholesterol-lowering effects of HOE 402 were more pronounced than after 4 weeks in LDLR+/0 mice, and after 11 weeks in LDLR0/0 mice. Furthermore, HOE

Table 2
Effect of HOE 402 on serum cholesterol and triglyceride levels in wild-type, LDLR+/0 and LDLR0/0 mice on diets J, W and N

Diet	Wild-type		LDLR+/0		LDLR0/0	
	Control	HOE 402	Control	HOE 402	Control	HOE 402
Serum cholesterol mmol/L						
J (4 weeks)	2.5 ± 0.3	1.8 ± 0.4 (72)*	6.8 ± 0.9	5.3 ± 0.9 (78)*		
W (11 weeks)					50.4 ± 10.2	33.6 ± 13.3 (67)
W (21 weeks)					49.2 ± 13.0	13.1 ± 2.4 (27)**
N (4 weeks)	7.3 ± 0.7	3.4 ± 0.3 (47)**	18.4 ± 4.1	12.4 ± 3.1 (67)*		
N (21 weeks)			29.4 ± 4.3	12.9 ± 3.1 (44)**		
Serum triglycerides mmol/L						
J (4 weeks)	0.12 ± 0.07	0.10 ± 0.04 (83)	0.17 ± 0.05	0.15 ± 0.04 (88)		
W (11 weeks)					5.78 ± 2.46	2.84 ± 1.76 (49)
W (21 weeks)					1.91 ± 1.27	0.73 ± 0.27 (38)
N (4 weeks)	0.08 ± 0.04	0.03 ± 0.03 (38)	0.02 ± 0.03	0.19 ± 0.08 (950)		
N (21 weeks)			0.12 ± 0.09	0.26 ± 0.12 (217)		

Serum cholesterol and triglyceride concentrations were measured in wild-type and LDLR+/0 mice on diets J and N after 4 and 21 weeks (LDLR+/0 mice) of feeding and in LDLR0/0 mice on diet W after 11 and 21 weeks of feeding with or without 0.05% (w/w) HOE 402 ($N = 6$–8 per group). At baseline, serum cholesterol and triglyceride levels of control and HOE 402-treated mice were the same (data not shown). Values between parenthesis represent the percentage of the value obtained in mice on the control diet. HOE 402 had no effect on serum TG levels.

* Statistically significant differences between control and treated mice are indicated by $P < 0.05$.

** Statistically significant differences between control and treated mice are indicated by $P < 0.005$.

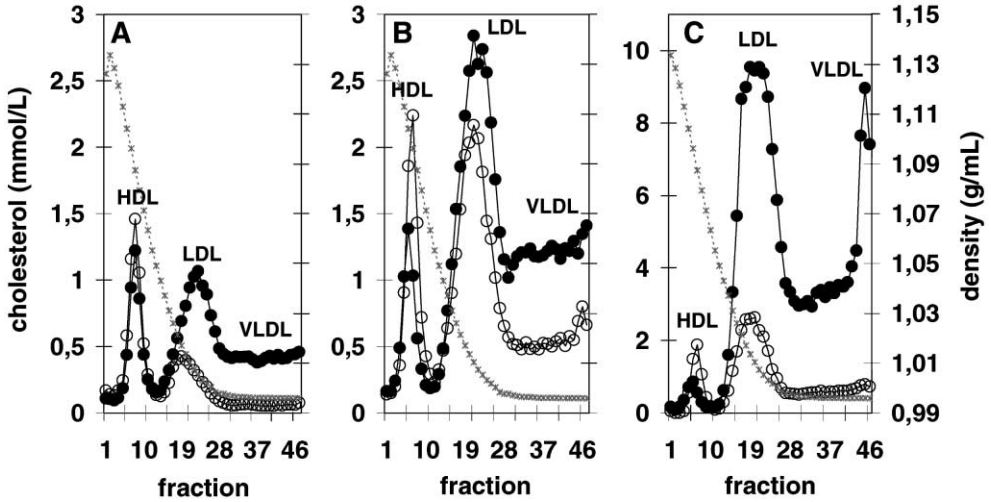

Fig. 1. Effect of HOE 402 on lipoprotein profiles in wild-type (A), LDLR+/0 (B), and LDLR0/0 (C) mice. Ultracentrifugation profiles of pooled serum of wild-type ($N = 6$ per group) and LDLR+/0 mice ($N = 8$ per group), fed diet N, were made after 4 weeks of treatment. The profiles of LDLR0/0 mice, fed diet W ($N = 8$), were made after 21 weeks of treatment. Control (●) and HOE 402 treatment (○); × indicates the density (g/mL) gradient. The lipoproteins are classified by their density, being VLDL: $d < 1.006$, IDL: 1.006–1.019, LDL: 1.019–1.063, and HDL: $d > 1.063$ g/mL.

402 had no statistically significant effect on serum TG levels in all mice strains on the different diets.

In wild-type (Fig. 1A), LDLR+/0 (Fig. 1B), and in LDLR0/0 mice (Fig. 1C), HOE 402 reduced serum cholesterol levels of VLDL/IDL/LDL by 86, 57 and 63%, respectively, whereas HDL-cholesterol levels were increased by 21, 54 and 47%, respectively. The findings that HOE 402 treatment resulted in a potent reduction of VLDL cholesterol whereas the serum TG level had not been changed, indicate that the VLDL particles had been depleted of cholesterol whereas TG were abundantly present in VLDL, resulting in a less β-VLDL-like, less atherogenic particle. The consequence of this effect of HOE 402 for the metabolism of VLDL is a more rapid clearance of the particle and a reduced delivery of cholesterol to the periphery.

3.2. Effects of HOE 402 on LDL clearance and VLDL production

The finding that LDLR deficiency did not influence the serum cholesterol-lowering potency of HOE 402, suggests that HOE 402 reduced serum cholesterol levels *via* another mechanism than by inducing the LDLR. To study the mechanism of action, we investigated whether HOE 402 modulated the LDLR, and whether HOE 402 could alter VLDL production in wild-type mice on the lipogenic diet J.

By measuring the serum clearance of injected human iodinated LDL in mice that had received diets without or with HOE 402 (Fig. 2), no effect of HOE 402 on the clearance of LDL was found. For this clearance study we used human LDL, because in contrast to mouse LDL, it contains no ApoE and can therefore be more specifically cleared by the LDLR than mouse LDL which can also be cleared by the ApoE requiring LDLR-related protein (LRP). The clearance of human [^{125}I]-labeled LDL in mice has previously been described by Nikoulin and Curtiss [17].

Subsequently, the effect of HOE 402 on different aspects of VLDL production after Triton WR-1339 injection, was studied by measuring the production rates of VLDL-TG and VLDL ApoB (Fig. 3), and the cholesterol content of nascent VLDL. Compared to controls, the serum TG production rate was reduced by more than 50% in HOE 402 treated mice. In contrast, the synthesis rate of VLDL ApoB was not changed. Furthermore, the cholesterol content of nascent VLDL was also diminished by HOE 402 (112 ± 2 vs. 65 ± 7 μg/mL serum, $P < 0.05$). These findings indicate that HOE 402 had no effect on the number of VLDL particles produced, but resulted in the release of TG- and cholesterol-poor VLDL in the circulation.

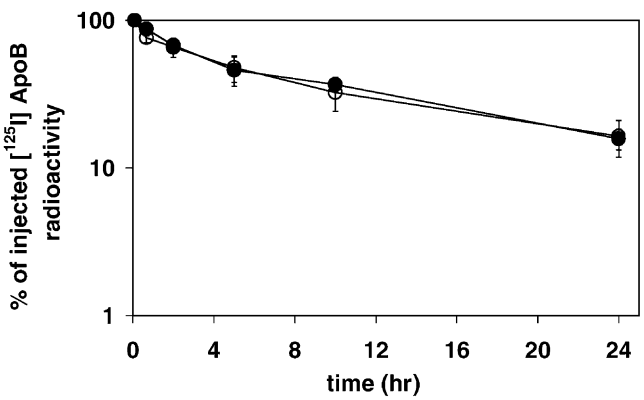

Fig. 2. Clearance of human LDL-ApoB in wild-type mice. Mice were fed the sucrose rich diet J for 4 weeks. Fasted mice were injected with 10 μg of [^{125}I]-labeled human LDL protein. Blood was collected at 40 min, 2, 5, 10, and 24 hr and [^{125}I]ApoB was measured. Control (●) ($N = 6$) and HOE 402 treated (○) ($N = 6$). Statistically significant differences between control and treated mice were not present.

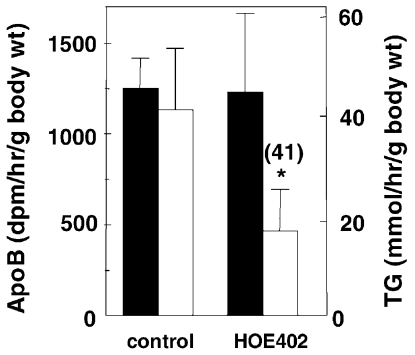

Fig. 3. VLDL-ApoB and -TG production in wild-type mice. After 4 weeks of feeding diet J, fasted control ($N = 7$) and HOE 402-treated ($N = 7$) mice were anaesthetized and intravenously injected with [^{35}S]methionine and Triton WR-1339. Thereafter, [^{35}S]ApoB (■) and TG (□) production rates were determined as described in Section 2. The value in parentheses represents the percentage of the value obtained in mice on the control diet. Statistically significant differences between control and treated mice are indicated by an asterisk (*) $P < 0.05$.

If in these wild-type mice, HOE 402 had a serum cholesterol-lowering effect *via* the induction of the LDLR, an increased flux of cholesterol from the circulation to the liver could lead to an increased hepatic cholesterol storage. However, we found that HOE 402 reduced the liver cholesterol ester content by 22% in these mice (6.5 ± 0.6 vs. 5.1 ± 0.7 μg/mg protein, $P < 0.05$). Additionally, no changes in the hepatic mRNA levels of HMG-CoA synthase were found (data not shown).

3.3. The effect of HOE 402 on atherosclerotic lesion size in aortas of LDLR+/0 and LDLR0/0 mice

LDLR+/0 and LDLR0/0 mice were fed the atherogenic diets N and W, for 21 weeks. In these mice HOE 402 significantly ($P < 0.005$) reduced the serum cholesterol exposures (weeks × mmol/L) by 47% in LDLR+/0 mice (controls 462 ± 53; HOE 402 246 ± 53), and by 41% in LDLR0/0 mice (controls 979 ± 143; HOE 402 574 ± 221). These reductions were accompanied by a strong decrease in the atherosclerotic lesion area in the HOE 402-treated groups (Fig. 4).

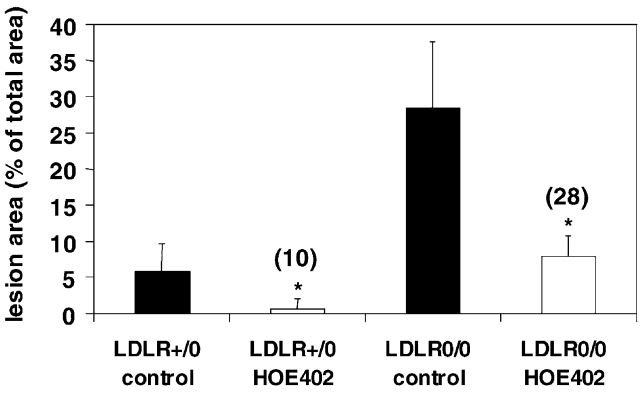

Fig. 4. Effect of HOE 402 on atherosclerotic lesion area in aortas of LDLR+/0 and LDLR0/0 mice. Aortic lesion areas were expressed as a percentage of total aortic area from its origin to the renal artery branch points. Lesions were stained with Oil Red O in aortas of LDLR+/0 mice fed diet N and LDLR+/0 mice fed diet W for 21 weeks with and without HOE 402. Filled bars (■) controls ($N = 8$ per group); open bars (□) HOE 402 ($N = 8$ per group). Values in parentheses represent the percentage of the value obtained in mice on the control diet. Statistically significant differences between control and treated mice are indicated by an asterisk (*) $P < 0.01$.

4. Discussion

The present study demonstrated the hypolipidemic activity of the compound HOE 402 in wild-type, LDLR+/0 and LDLR0/0 mice, showing strong reductions in serum levels of the ApoB-containing lipoproteins VLDL, IDL and LDL. Treatment with HOE 402 had no effect on LDL-clearance, but reduced VLDL-TG and cholesterol production without affecting ApoB production. These findings indicate that HOE 402 inhibits VLDL lipid production rather than inducing the LDLR, as suggested previously [4,5].

A similar reduction in serum cholesterol levels by HOE 402 as we found in mice was observed in hyperlipidemic Watanabe rabbits, where mainly LDL-cholesterol was lowered [4]. In cholesterol-fed hamsters cholesterol levels were similarly lowered in all lipoprotein fractions [5]. In addition, HOE 402 had no effect on serum lipids in homozygous LDLR-deficient rabbits [4]. Based on the latter data and on the fact that HOE 402 treatment upregulated LDLR mRNA levels in HepG2 cells [4], it was concluded that HOE 402 is an LDLR inducer. However, the present study shows that in mice the LDLR is not the primary target of HOE 402, since HOE 402 treatment reduced serum cholesterol levels in LDLR0/0 mice, and the compound had no effect on the clearance of human LDL in wild-type mice. In addition, we found that in wild-type mice treatment with HOE 402 resulted in the production of nascent VLDL particles that are relatively poor in TG and cholesterol. Thus, this reduced VLDL lipid secretion may explain the marked serum VLDL/IDL/LDL cholesterol-lowering properties of HOE 402 in these mice. Concomitantly, we found that HOE 402 treatment reduced hepatic cholesterol ester contents, which may form an explanation for the reduced VLDL-cholesterol secretion.

The finding that a reduced rate of VLDL-TG secretion due to treatment with HOE 402 had no effect on the level of serum TG seems to be discrepant. However, TG levels in wild-type mice are very low and wild-type mice were used for the VLDL-production experiment. It is well known that lipolysis in wild-type mice is very efficient as is also witnessed in our study. Mice TG levels on a lipogenic (sucrose containing) diet were only 0.10–0.12 mmol/L. This may explain why no changes in TG levels are observed.

The precise molecular mechanism of action of HOE 402 is presently unknown, but there are some similarities with other hypolipidemic agents. Several animal studies have

shown that plant stanol esters, ACAT inhibitors, and HMG-CoA reductase inhibitors reduce serum levels of cholesterol and ApoB-containing lipoproteins by a decrease in VLDL lipid secretion, and that the latter strongly depends on the hepatic cholesterol ester content [9,17–19]. The primary mechanisms of action of these treatments are inhibition of intestinal cholesterol absorption; of endogenous cholesterol esterification, thereby also inhibiting intestinal cholesterol absorption [20]; and of endogenous cholesterol synthesis, respectively. In mice statins have been shown to decrease serum levels of ApoB-containing lipoproteins by the inhibition of the production of VLDL particles [21,22]. The ACAT inhibitors avasimibe [23] and F-1394 [24], and plant stanol esters also lowered ApoB-containing lipoproteins, the latter by inhibiting the VLDL-lipid production [9]. It is unlikely, however, that HOE 402 inhibited the intestinal cholesterol absorption, since no effect on intestinal cholesterol absorption was observed in hamsters [5], and intestinal cholesterol absorption inhibitors are effective in both hamsters and mice [9,25]. In addition, there are strong indications that inhibition of ACAT or of HMG-CoA reductase are not the primary target of HOE 402, since HOE 402 did not inhibit ACAT activity in HepG2 cells (data not shown), and HOE 402 did not inhibit endogenous cholesterol synthesis in hamsters [5] and in HepG2 cells [4]. Further, HOE 402 is an oxo-imidazolidinyl-pyrimidine which does not share homology with the structures of the inhibitors of HMG-CoA reductase or ACAT, respectively [26]. Moreover, if HOE 402 had suppressed endogenous cholesterol synthesis in these mice, an upregulation of mRNA levels of HMG-CoA synthase, an enzyme in the cholesterol synthetic pathway, could have been expected [19]. However, we found no effect of HOE 402 treatment on hepatic HMG-CoA synthase mRNA levels in wild-type, LDLR+/0 and LDLR0/0 mice (data not shown). Thus, although inhibition of intestinal cholesterol absorption, and inhibition of endogenous esterification and synthesis of cholesterol show similar hypocholesterolemic effects as HOE 402, the biochemical background of the cholesterol-lowering properties of HOE 402 is different, and needs to be further investigated.

A potential explanation for the apparent difference in mechanism between species; i.e. hamsters, rabbits and mice could be that in hamsters [5] and rabbits [4] the change in the clearance of ApoB-containing lipoproteins is not caused by a primary effect on the LDLR, but that changes in LDLR are secondary to a hitherto unknown action of HOE 402. Moreover, in mice the liver cholesterol homeostasis is mainly regulated *via* cholesterol synthesis rather than *via* LDL receptor-mediated uptake of cholesterol [18].

In this study, we found that HOE 402 potently reduced the serum levels of ApoB-containing lipoproteins in LDLR0/0, LDLR+/0 and wild-type mice, but also led to moderately increased levels of HDL in LDLR0/0 and LDLR+/0 mice. The mechanistic background for the increased HDL levels due to HOE 402 treatment is unknown and needs to await further investigation. Both changes are beneficial with respect to the development of atherosclerosis and can explain the strong reduction in aortic atherosclerosis found in mice treated with HOE 402.

In conclusion, in mice the hypocholesterolemic effect of HOE 402 was not caused by induction of LDLR activity as found in other animal models, but by reducing the VLDL-cholesterol and TG secretion. The hypocholesterolemic effect of HOE 402 resulted in a strong reduction of the atherosclerotic lesion area of the aorta in these mice.

Acknowledgments

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