Etofibrate enhances ^{123}I-LDL-binding in human liver

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Abstract

Etofibrate, a combination of fibric and nicotinic acid, is successfully used for the treatment of type IIb and IV hyperlipidemia. While an up-regulation of specific low density lipoproteins (LDL) binding sites in human platelets has been demonstrated, action on LDL-binding to the liver in patients and kinetic studies rare. This study aimed to investigate the influence of twice 500mg etofibrate daily given for 6 weeks on the in vivo binding of autologous LDL to the liver in 11 patients, 6 males, 5 females; aged 37-57 years, suffering from mixed hyperlipidemia. Etofibrate enhanced in vivo liver uptake of ^{123}I-LDL by 16.1% at mean, shortened plasma decay of LDL and improved lipid profile: serum total cholesterol was lowered by 14.9%, LDL-cholesterol was lowered by 22.2% and high-density lipoprotein (HDL)-cholesterol was increased by 10.9%. These findings are documenting a beneficial effect of the drug at the LDL liver receptor level in vivo.

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Introduction

Etofibrate (2-[-(p-chlorophenoxy)-2methylproprionoxy] ethyl nicotinate) is a diester of clofibric and nicotinic acid. The synergistic effects of these components have been successfully applied for the treatment of hyperlipidemia. Beside its action on lipids and lipoproteins, a variety of other beneficial effects decreasing platelet aggregation [1], plasma viscosity [2], fibrinogen [3] and increasing fibrinolytic activity [4], have been demonstrated. Furthermore, a beneficial effect on low-density lipoproteins (LDL) -particle size [5] and remnant removal [6] was reported.

The liver LDL receptors are playing a key role in clearing cholesterol from plasma and in its excretion [7]. Dietary and drug interventions with etofibrate in patients have been shown to beneficially affect the receptor mediated metabolism of LDL in vivo in men. This LDL-binding has not yet been proven in vivo in human.

In this study we examined the effect of a 6 weeks treatment period with etofibrate on iodine-123 (^{123}I)-LDL binding by the liver and its plasma decay.

Materials and methods

Eleven patients (for characteristics see Table 1) suffering from mixed hyperlipidemia were studied after a 4 weeks dietary run-in period. Imaging as well as lipid and lipoprotein analysis was done before as well as 6 weeks after initiating treatment with the administration of twice daily tablets of 500mg etofibrate under still ongoing treatment.

Table 1. *Patients characteristics.*

No	Age	Gender	Height (cm)	Weight (kg)	Fat (%)
1	37	m	184	85	19
2	41	f	164	60	23
3	39	f	157	50	22
4	52	f	169	58	20
5	36	m	177	80	20
6	57	m	179	77	16
7	48	m	176	78	22
8	43	f	163	60	25
9	51	f	162	64	24
10	36	m	185	81	17
11	55	m	181	85	21
Mean	45.0	6 m 5 f	180.3 m 163.0 f	81.0 m 58.4 f	19.2 m 22.8 f

Isolation of LDL

Polyclonal anti-apo-B antibodies were obtained by immunizing sheep with pure LDL. Gammaglobulins were precipitated from sheep plasma with ammonium sulfate (300g/L, Sanabo, Vienna, Austria) to a final concentration of 35% and further purified by immunoaf-

finity chromatography. For this purpose, 3g of pure LDL was isolated by immunoaffinity chromatography using BrCN-activated Sepharose Cl 4B (Pharmacia, Uppsala, Sweden) to which the immunopurified antibodies were coupled and used to isolate LDL from plasma. Ten mL of anti-LDL-sepharose Cl 4B gel were placed into a glass column (22x2cm) and the gel was thereafter extensively washed with 500ml of isotonic NaCl solution. Ten mL of plasma citrate (3.8% sodium citrate, 1:10) was re-circulated for 30min over the column at a flow rate of 10mL/min. The column was then washed with isotonic saline solution until it was protein free ($E_{260/280}$ nm < 0.002). LDL was desorbed from the column with two bed volumes of 0.2M glycine (HCl, pH 3.0, and dialyzed overnight against 5L of isotonic saline. The solution was then concentrated by ultrafiltration on Amicon XM 100 filters (Vienna, Austria) until a final concentration of 10mg of LDL/mL.

Labeling of LDL

LDL was then labeled with ^{123}I using the iodine monochloride technique (ICl) [8]. Free radioiodine was removed by dialysis against 0.9% sodium chloride containing 0.1mg/mL of EDTA at a pH of 7.2. An ICl stock solution (34mM/6M HCl) was purified before labeling by three extractions with $CHCl_3$ and diluted 1:100 with 2M NaCl. To a microvial kept at 4°C, approximately 1mg of LDL, 1M glycine buffer, pH 10, about 1.85MBq or 50μCi $Na^{123}I$, and freshly diluted ICl solution were added to give a molar ratio of ICl/apoprotein of 10/1. The reaction mixture (0.5-1mL) was slowly stirred for 10 min at 4°C and filtered into a dialysis bag that was kept in dialysis buffer (0.15M $CaCl_2$, 0.01M PO^{2-}_4, pH 7.5, 0.2 mM EDTA) at 4°C until injection. Extensive investigations concerning the in vitro stability of iodine labeled LDL have been carried out previously [9], showing that the radiolabel is stable for at least 48h. Oxidative modification was excluded by TBARS-determination and electrophoretic mobility.

Liver and blood activity were monitored for 240min and 24h (after the injection of ^{123}I-LDL), respectively. The liver/blood pool ratio was calculated at 10, 20, 30, 40, 50, 60, 90, 120 and 240min after ^{123}I-LDL injection. Maximum LDL uptake by the liver was calculated from the activity trapped by the organ vs. whole body activity after correcting for physical decay.

Binding of the radiolabel

One, 2, 4, 6, 8, 10, 12, 16, 20 and 24h after re-injection of ^{123}I-LDL, blood samples were drawn to determine the recovery and the lipoprotein fraction the lipoprotein was bound to. The respective fractions were separated by ultracentrifugation and the radioactivity in the various fractions was measured in a gamma-well counter (Cobra II, Canberra Packard, III, USA). In addition, the disappearance of ^{123}I-LDL was determined from blood samples drawn during the initial 12h after injection.

In vivo uptake

During reinjection of autologous radiolabeled LDL, patients were placed in the supine position under a LFOV gammacamera. Serial images at a rate of 1 frame / minute (matrix 64x64) were obtained to monitor the kinetics. In order to assess the LDL-uptake by the liver quantitatively, total liver radioactivity as compared to whole body activity was determined by whole body scanning. After inserting regions of interest over the liver and the whole body and a background region over the left shoulder radioactive counts over the liver ROI were expressed as a percentage of whole body radioactivity after background subtraction. In vivo liver uptake was assessed at 90min after LDL injection.

Lipid and lipoprotein parameters

Lipids (cholesterol and triglycerides) were determined using enzymatic tests. Lipoproteins were measured after separation by means of ultracentrifugation. Apolipoproteins A and B were determined by means of nephelometry using a BNA (Behring nephelometer analyzer, Behring, Marburg, Germany).

Statistical analysis

All the results are presented as mean ± SD. Statistical significance was calculated using Student´s t-test for paired data and ANOVA.

Results

The recovery and lipoprotein binding of the tracer was more than 90% at the different intervals (Fig. 1) up to 6h after re-injection. Initially, more than 90% of the label was with LDL decreasing to about 80% after 6h. With increasing time more ^{123}I becomes bound to HDL. Etofibrate treatment was beneficial (Table 2). Total cholesterol decreased by mean: 15.3%, LDL-cholesterol by 22.1% and HDL-cholesterol increased by 23.0%. Initial liver uptake was 27.9% and well below normal (≥ 40%). Etofibrate treatment increased liver uptake by 16,1% at mean. Plasma decay was significantly faster after etofibrate (Fig. 2) reaching the level of significance at 4h after re-injection of autologous radiolabeled LDL, as well as thereafter. Individual initial uptake curves over the liver before and during etofibrate therapy from 8 patients (No1-8) are shown in Figure 3. A typical image of liver demonstrating LDL-trapping is shown in Figure 4.

Discussion

In patients receiving 500mg etofibrate for 3 months, beside hypolipidemic actions, antithrombotic and antiatherosclerotic properties were reported [3]. Etofibrate was found effective in rendering LDL less susceptible to oxidation [10]. There are only 2 studies available examining etofibrate on lipoprotein binding.

In patients with type II hyperlipoproteinemia upregulation of specific Indium-111 (^{111}In)-LDL as well as ^{111}In-HDL binding sites of human platelets, paralleled by reduced platelet activation were monitored after a 6 weeks treatment period with twice 500mg etofibrate daily [11].

Table 2. *Lipids and lipoproteins of the subjects studied.*

No	CH (in mg/L)		LDL(in mg/L)		HDL (in mg/L)		Liver binding (in %)	
	b	a	b	a	b	a	b	a
1	286	241	184	161	36	40	21.4	24.7
2	271	252	176	155	41	43	34.6	34.5
3	264	239	202	110	37	42	27.7	31.8
4	293	237	191	142	42	46	31.1	35.0
5	257	222	166	149	36	41	29.5	34.4
6	268	216	178	133	33	35	31.6	36.9
7	274	229	166	126	39	43	26.2	32.1
8	251	221	174	139	36	42	22.7	29.8
9	298	242	200	157	31	38	25.3	30.4
10	317	265	223	168	42	44	33.2	36.3
11	285	243	188	154	38	43	24.5	28.6
Mean	278.5	237.0	186.2	144.9	37.4	41.5	27.9	32.4

b: before; a: after; values in mg/dl (except liver binding in % of total injected LDL)
CH: cholesterol; LDL: low density lipoproteins; HDL: high density lipoproteins

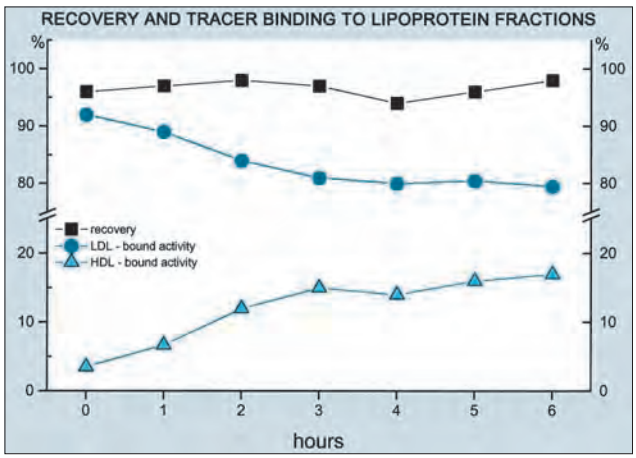

Figure 1. Recovery of the radiolabel is high. Plasma radioactivity decreases in LDL and moves to HDL.

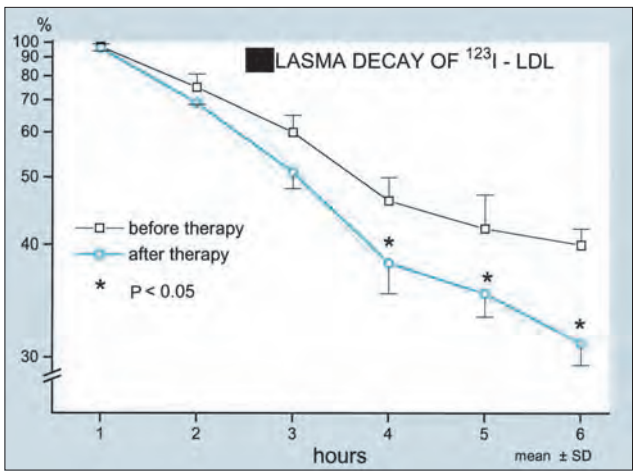

Figure 2. Plasma decay of ^{123}I-LDL is significantly enhanced after 6 weeks of etofibrate treatment.

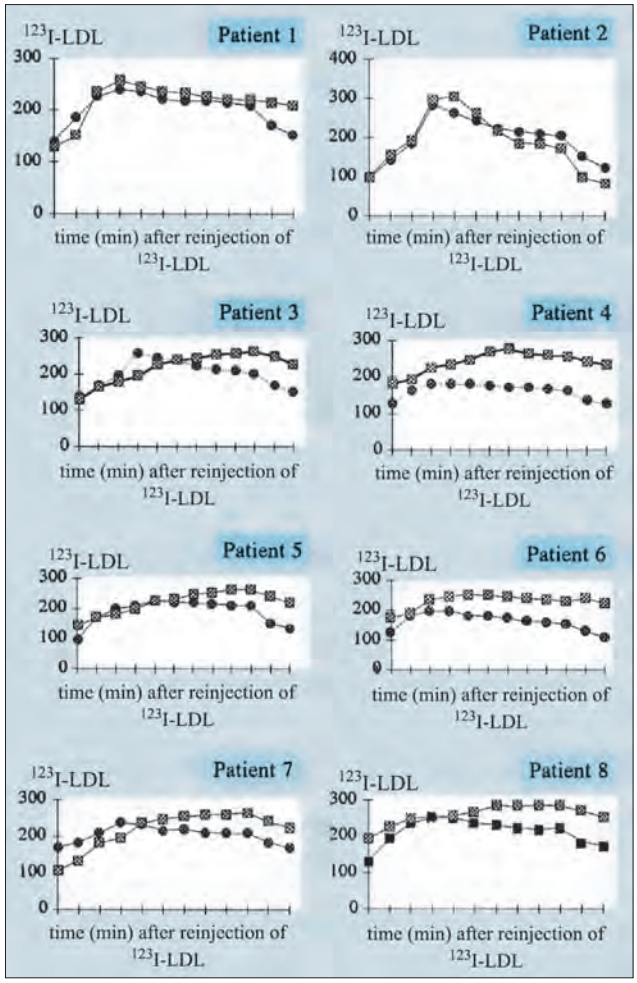

Figure 3. Calculated liver uptake during the initial 60min from patients 1-8 (time scale in 5min intervals). Black symbols are before and grey symbols are 6 weeks after (still ongoing) etofibrate treatment. Already after 60min in all patients but one (No 2), liver trapping of LDL was increased as compared to pre-values. Mean increase was 16.1% at time of maximum uptake ($P<0.01$).

In a study with single dose etofibrate (1.0g/d) in 11 hypercholesterolaemic individuals CH decreased by 14%. It has been shown that the increased clearance resulted from activation (64%) of the LDL-receptor pathway, while synthesis was not affected [12]. Imaging and quantification of LDL-uptake in the liver has been proven to be a reliable tool for receptor identification [13]. Especially the ^{111}In- and ^{123}I-label proved to be useful in human in vivo. Identification of defects at the receptor level [14] as well as follow-up monitoring after various therapeutic interventions [15], have been reported. In this study we demonstrated an enhanced uptake of LDL by the liver paralleled by an enhanced plasma clearance induced by etofibrate treatment. They confirm earlier data on fractional catabolic rate calculation [16] and platelet binding [11]. Therefore, we approached the in vivo labeling of autologous LDL in human.

Improvement in lipid parameters was similar in our patients as compared to a large multicenter study (n=1943)

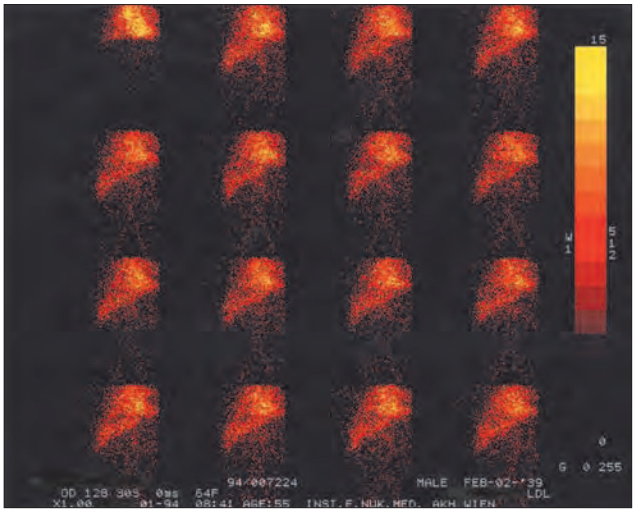

Figure 4. Kinetics of LDL uptake over the liver. Imaging started at time of injection. Each image reflects an acquisition period of 1min.

showing a decrease in total CH (19.9%), LDL (14.9%) and very low-density lipoprotein (VLDL) (14.5%) as well as an increase in HDL (18.1%) [17].

Our investigation showed, that in vivo imaging of radiolabeled LDL and monitoring of kinetics is a reliable tool for diagnosis of (qualitative and/or quantitative) receptor defects as well as for the assessment of therapeutic benefit at the receptor level in human liver.

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