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EFFECTS OF COMBINED EZETIMIBE AND SIMVASTATIN THERAPY AS COMPARED TO SIMVASTATIN ALONE IN PATIENTS WITH TYPE 2 DIABETES:

A PROSPECTIVE, RANDOMIZED, DOUBLE-BLIND, CLINICAL TRIAL

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(*see Study Organization in the online Appendix available at http://care.diabetesjournals.org)

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Short running title: Ezetimibe and simvastatin in type 2 diabetes

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Objective: To assess the effects of inhibited gastrointestinal cholesterol absorption in statin-treated dyslipidemic patients.

Research Design and Methods: In a multi-center, prospective, randomized, double-blind, placebo-controlled trial we primarily compared by ANCOVA the effect of 2-month ezetimibe (10 mg/day) or placebo therapy on low-density-lipoprotein (LDL) cholesterol serum levels in 108 type 2 diabetes patients with albuminuria <200 μg/min and total cholesterol concentrations >135 mg/dL despite simvastatin treatment (40 mg/day)

Results: Unlike placebo, ezetimibe decreased LDL cholesterol from 99±31 to 66±22 mg/dL, total cholesterol from 162±36 to 124±30 mg/dL and apolipoprotein B from 83±22 to 64±18 mg/dL ($p<0.0001$ for all changes vs placebo). Seventy-two and 17% of patients on ezetimibe or placebo achieved LDL levels <70 mg/dL, respectively ($p<0.0001$). Treatment was well tolerated.

Conclusions: Adding ezetimibe to simvastatin therapy helps improving pro-atherogenic lipoprotein profile in type 2 diabetes patients who fail to reach recommended lipid targets with statin therapy alone.

Inhibited gastrointestinal cholesterol absorption by add-on ezetimibe therapy (1) reduced cholesterol levels in patients with persistent dyslipidemia despite statin therapy (1-6). Advantages of dual versus single drug lipid lowering therapy, however, could not be definitely established since the effects of ezetimibe combined with a given dosage of a statin were compared with those of monotherapy with another, competitor, statin (4,5) or, even, with the same statin but given at higher dosages (2). To address this issue, the Ezetimibe and Simvastatin in dyslipidemia of Diabetes (ESD) study (ClinicalTrials.gov Identifier: NCT00157482) compared the lipid-lowering effects of ezetimibe or placebo added-on the same background statin therapy in type 2 diabetes patients with persistent hypercholesterolemia despite HMGCoA reductase inhibition.

METHODS

ESD was an academic, multi-center, prospective, randomized, double-blind, placebo-controlled trial independently designed, conducted and monitored by the investigators of the Clinical Research Center for Rare Diseases *"Aldo & Cele Daccò"* and three Diabetology Units in Italy. The protocol was approved by the Ethical Committees of all institutions. Patients provided written informed consent according to the Declaration of Helsinki. Data were handled and reported without sponsor involvement. Authors had full access to data, critically revised and finally approved the manuscript.

Eighteen- to 70-year-old type 2 diabetes subjects with total cholesterol concentrations >135 mg/dL despite lipid-lowering therapy, serum creatinine <1.5 mg/dL and urinary albumin excretion (UAE) <200 μg/min were eligible for study participation. Those with recent cardiovascular events, primary hyperlipidemia, hepatic or muscle disease, or who were pregnant or lactating, treatedwith steroids, immunosuppressive agents, fibrates, niacin or cholestyramine or unable to provide an informed consent were excluded (ClinicalTrials.gov Identifier: NCT00157482).

Following four-week wash-out from previous lipid-lowering therapy (if any), and two-

month run-in with simvastatin 40 mg/day, eligible patients were randomly allocated to two-month add-on treatment with ezetimibe 10 mg/day or placebo. Demography, clinical and laboratory data were recorded at inclusion, randomization and study end.

Laboratory parameters were centrally measured by automatic analyzer Beckman Synchron CX9. Glycosylated hemoglobin was measured by high-performance liquid chromatography (normal laboratory range 3.53–5.21%; Beckman System Gold Chromatograph). UAE was measured in three consecutive overnight urine collections by rate nephelometry (Array 360 System; Beckman, Milano, Italy) in sterile urine.

LDL-cholesterol was the primary outcome. Based on average levels observed in statin-treated type 2 diabetes patients with normo or microalbuminuria on statin therapy referred to our Research Center, we predicted LDL serum levels at randomization of 90±12 mg/dL. Assuming a 20% reduction on ezetimibe and no change on placebo, 51 patients per group provided the study a 80% power to detect as statistically significant ($p<0.05$) the expected differences in LDL changes between groups at study end. To account for a 5% drop-out rate, 54 patients per group had to be randomized.

Patients were centrally randomized to ezetimibe or placebo on a 1:1 ratio within blocks of 4 according to a computer-generated randomization list. Patients and investigators were blinded to treatment. Analyses were by intention-to-treat. Characteristics of patients were compared by chi-squared test, Fisher's exact test, unpaired t-test or Wilcoxon rank-sum test as appropriate. Within-group treatment effects were assessed by repeated-measures analyses of variance (ANOVA) followed by paired t-tests or by McNemar chi-squared test; between-group effects by analysis of covariance (ANCOVA), adjusting for the measurement at randomization or by chi-squared test. The multiple pair-wise comparison issue was addressed using Bonferroni adjustment. Analyses were performed using SAS version 9.1 (SAS Institute Inc, Cary, NC). Data were expressed as mean ± standard deviation or median (interquartile range) or number (%) unless otherwise specified. All P values were two-sided. Statistical significance was set at the 0.05 level.

RESULTS

Of 114 screened subjects, 108 fulfilled the selection criteria and were randomized. One patient randomized to ezetimibe eventually withdrew his consent, thus 53 patients on ezetimibe and 54 on placebo completed the study. Main characteristics at randomization (Table) including age (65.7 vs 65.2 years), proportion of males (63.0% vs 55.6%) and of current smokers (44.6% vs 55.6%) were similar in the ezetimibe and placebo group, respectively, whereas the proportion of subjects with a family history of coronary heart disease was higher (51.9% vs 29.6%) in the ezetimibe group.

LDL and total cholesterol, and apolipoprotein B significantly decreased by 30.9%, 21.6%, and 19.6%, respectively, compared to randomization in patients given ezetimibe, but did not change appreciably in those on placebo (Table). These trends were significantly different between groups ($p<0.0001$ for all comparisons). At study end 4.2-folds more subjects on ezetimibe than on placebo achieved an LDL level of 70 mg/dL or less (Table). Serum HDL cholesterol significantly decreased on ezetimibe, but not on placebo. Serum triglycerides decreased on ezetimibe and were significantly different from placebo ($P<0.05$). After randomization UAE did not appreciably change in both groups (Table).

Treatment was well tolerated. No significant increases in serum creatinphosphokinase and transaminase levels were observed in both

arms (Table). Four patients on ezetimibe and 1 on placebo had transient sinus bradycardia that recovered spontaneously without treatment withdrawal.

DISCUSSION

In type 2 diabetes patients with normo- or micro-albuminuria and persistent dyslipidemia despite background therapy with a fixed dose of simvastatin, two-month add-on treatment with ezetimibe significantly ameliorated the lipid profile and, compared to placebo, increased by 4-folds the proportion of patients achieving the LDL target currently recommended for people with diabetes (7,8). This effect exceeded the lipid lowering effect of combined therapy reported in previous series, most likely because here we compared ezetimibe with placebo in subjects given the same dosage of simvastatin whereas previous studies compared combined therapy with simvastatin given at higher dosages (2) or with another more powerful statin (4,5). However, our study was underpowered to assess whether cholesterol reduction may affect albuminuria *per se,* (9-11) independent of HmGCoA inhibition (12). As previously reported (13), combined treatment was remarkably well tolerated. However, since there were four cases of transient sinus bradycardia in the ezetimibe arm, this therapeutic option should be considered with caution in subjects with brady-arrhythmias. In conclusion, adding ezetimibe to simvastatin therapy helps improving pro-atherogenic lipoprotein profile in type 2 diabetes patients avoiding the drawbacks of maximizing statin doses.

***Author Contributions*:** Piero Ruggenenti and Giuseppe Remuzzi had the original idea; Dario Cattaneo and Piero Ruggenenti contributed to data analyses; Stefano Rota, Ilian Iliev, Aneliya Parvanova, Antonio Bossi, Roberto Trevisan and Antonio Belviso were in charge of patient selection, monitoring and care; Bogdan Ene-Iordache implemented the data base; Silvia Ferrari performed the laboratory analyses; Olimpia Diadei monitored the study; Dario Cattaneo wrote the initial draft and Piero Ruggenenti the final version of the manuscript. All the authors revised and approved the final version.

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Table: Patients characteristics at randomization (Pre) and at the end of the treatment period (Post) according to study treatment.

Variable *(unit)*	Ezetimibe		Placebo	
	Pre *(N=54)*	**Post** *(N=53)*	**Pre** *(N=54)*	**Post** *(N=54)*
Clinical parameters				
BMI *($Kg.m^{-2}$)*	29.0 ± 4.2	28.8 ± 4.3	28.8 ± 4.1	28.9 ± 4.1
SBP *(mmHg)*	136 ± 13	133 ± 13	131 ± 15	131 ± 15
DBP *(mmHg)*	79 ± 7	78 ± 7	78 ± 8	77 ± 7
Metabolic parameters				
Serum glucose *(mg/dL)*	160 ± 44	168 ± 43	162 ± 50	159 ± 44
HbA_{1C} *(%)*	5.8 ± 1.5	5.5 ± 1.2	5.5 ± 1.1	5.5 ± 1.2
Renal function				
Serum creatinine *(mg/dL)*	0.90 ± 0.18	0.91 ± 0.17	0.87 ± 0.20	0.88 ± 0.21
Serum urea *(mg/dL)*	42 ± 12	43 ± 12	39 ± 12	39 ± 11
UAE *(μg/min)*	5.2 (2.8 to 10.3)	4.7 (3.3 to 11.8)	4.6 (3.4 to 7.2)	4.7 (3.3 to 9.6)
Lipids				
Total cholesterol *(mg/dL)*	162 ± 36	124 ± 30*	154 ± 30	158 ± 32°
HDL-cholesterol *(mg/dL)*	48 ± 11	45 ± 12	50 ± 12	50 ± 11§
LDL-cholesterol *(mg/dL)*	99 ± 31	66 ± 22*	91 ± 28	94 ± 32°
LDL <70 mg/dL - n(%)	7 (13.0)	38 (71.7)**	14 (25.9)	9 (16.7)° **
Triglycerides *(mg/dL)*	123 ± 95	108 ± 77	106 ± 65	104 ± 62§
Apolipoprotein A *(mg/dL)*	134 ± 23	138 ± 27	139 ± 20	143 ± 23
Apolipoprotein B *(mg/dL)*	83 ± 22	64 ± 18*	81 ± 23	81 ± 22°
Safety parameters				
AST (IU/L)	23 ± 7	24 ± 9	21 ± 5	21 ± 6
ALT (IU/L)	25 ± 10	27 ± 10	23 ± 9	23 ± 8
GGT (IU/L)	33 ± 39	35 ± 37	30 ± 30	29 ± 27
CPK (IU/L)	147 ± 140	143 ± 120	113 ± 60	115 ± 70

Data are mean ± standard deviation or median (interquartile range) or number (%).
*p<0.01 and **p<0.0001 vs pre (paired t-test or McNemar chi-squared test). °p<0.0001 and § vs ezetimibe (ANCOVA or chi-squared test).
Abbreviations: BMI: body mass index, SBP: systolic blood pressure; DBP: diastolic blood pressure; UAE: urinary albumin excretion rate; HDL: high density lipoprotein, LDL: low density lipoprotein; AST: aspartate transaminase; ALT: alanine transaminase; GGT: gamma-glutamyl transferase; CPK: Creatine phosphokinase