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Pleiotropic effects of ezetimibe/simvastatin vs. high dose simvastatin

Antonio Eduardo P. Pesaro [a,*], Carlos V. Serrano Jr. [a], Juliano L. Fernandes [a], Alexandre B. Cavalcanti [b], Alexandre H. Campos [c], Herlon S. Martins [d], Raul C. Maranhão [a], James A. de Lemos [e], Heraldo P. Souza [d], José C. Nicolau [a]

[a] Heart Institute, University of Sao Paulo, Av. Enéas de C. Aguiar, 44, Building II, 2nd Floor, Room 12, Sao Paulo, SP 05403-901, Brazil
[b] HCor (Cardiac Hospital-Hospital do Coracao), Research Institute, Hospital do Coração (HCor). R. Abílio Soares, 250, 12 Floor, São Paulo, SP 04005-000, Brazil
[c] Albert Einstein Hospital, Av. Albert Einstein, 627/701, Morumbi, Paulo-SP, Brazil
[d] Clinics Hospital, University of Sao Paulo; Av. Dr. Enéas de Carvalho Aguiar, 255, Cerqueira César, São Paulo, SP 05403-000, Brazil
[e] The University of Texas, Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390, United States

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ABSTRACT

Background: In the setting of stable coronary artery disease (CAD), it is not known if the pleiotropic effects of cholesterol reduction differ between combined ezetimibe/simvastatin and high-dose simvastatin alone.
Objective: We sought to compare the anti-inflammatory and antiplatelet effects of ezetimibe 10 mg/simvastatin 20 mg (E10/S20) with simvastatin 80 mg (S80).
Methods and results: CAD patients (n = 83, 63 ± 9 years, 57% men) receiving S20, were randomly allocated to receive E10/S20 or S80, for 6 weeks. Lipids, inflammatory markers (C-reactive protein, interleukin-6, monocyte chemoattractant protein-1, soluble CD40 ligand and oxidized LDL), and platelet aggregation (platelet function analyzer [PFA]-100) changes were determined. Baseline lipids, inflammatory markers and PFA-100 were similar between groups. After treatment, E10/S20 and S80 patients presented, respectively: (1) similar reduction in LDL-C (29 ± 13% vs. 28 ± 30%, p = 0.46), apo-B (18 ± 17% vs. 22 ± 15%, p = 0.22) and oxidized LDL (15 ± 33% vs. 18 ± 47%, p = 0.30); (2) no changes in inflammatory markers; and, (3) a higher increase of the PFA-100 with E10/S20 than with S80 (27 ± 43% vs. 8 ± 33%, p = 0.02).
Conclusions: These data suggest that among stable CAD patients treated with S20, (1) both E10/S20 and S80 were equally effective in further reducing LDL-C; (2) neither treatment had any further significant anti-inflammatory effects; and (3) E10/S20 was more effective than S80 in inhibiting platelet aggregation. Thus, despite similar lipid lowering and doses 4× less of simvastatin, E10/S20 induced a greater platelet inhibitory effect than S80.

1. Introduction

Among patients with coronary artery disease (CAD), a robust evidence base supports the beneficial effects of statin therapy on mortality and other adverse cardiovascular outcomes [1]. Previously, two large trials [2,3], have demonstrated that compared to standard dose statin therapy or placebo, high statin doses reduced low-density cholesterol lipoprotein (LDL-C) to extremely low levels and decreased coronary events, even in patients without elevated levels of LDL-C. Subsequently, recent guidelines have suggested an LDL-C treatment goal of <70 mg/dL in patients with CAD [4]. Achieving such low LDL-C levels frequently demands an intensive LDL-C reduction, often above 50%. Ezetimibe, an intestinal cholesterol absorption inhibitor, can be used as an additional therapy if statin monotherapy fails to reduce LDL-C below the treatment goal [5].

Furthermore, anti-inflammatory and antithrombotic pleiotropic effects of statins might explain, at least in part, the large benefits demonstrated in randomized trials [6,7]. For example, in hypercholesterolemic patients treated with statins, a decrease in inflammation-associated markers such as the C-reactive protein (CRP) has been described [8], although it is debated whether this effect is clearly independent of LDL-C [9].

Moreover, although inhibition of platelets by statin therapy is a well established effect [10], it has not yet been clarified whether platelet inhibition by statin therapy depends on the reduction of LDL-

* Corresponding author at: Heart Institute (InCor HCFMUSP), Av. Enéas de C. Aguiar, 44-Building II, 2nd Floor, Room 12, Sao Paulo, SP 05403-901, Brazil. Tel.: +55 11 21513011, +55 11 82441035.
E-mail addresses: eduardopesaro@hotmail.com (A.E.P. Pesaro), carlos.serrano@incor.usp.br (C.V. Serrano), juliano.fernandes@incor.usp.br (J.L. Fernandes), alexandrebiasi@hotmail.com (A.B. Cavalcanti), holthausen@einstein.br (A.H. Campos), herlonsm@gmail.com (H.S. Martins), amarans@usp.br (R.C. Maranhão), james.delemos@utsouthwestern.edu (J.A. de Lemos), corjnicolau@incor.usp.br (J.C. Nicolau).

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C or on the inhibition of intracellular signal pathways accompanied by disaggregating effects [11].

Two alternative pharmacologic strategies are equally effective in reducing LDL-C: high-dose statin alone and combined treatment with ezetimibe plus moderate-dose statin [12]. It is not known whether these two strategies have different cholesterol-independent pleiotropic effects on inflammation and platelets. We therefore compared the anti-inflammatory and antiplatelet effects of two intensive pharmacologic strategies designed to reduce cholesterol to a similar extent: ezetimibe 10 mg/simvastatin 20 mg (E10/S20) versus simvastatin 80 mg (S80).

2. Methods

2.1. Study design

From July 2006 to January 2009, we randomized 83 patients with stable CAD in a single tertiary specialized cardiology hospital. These patients had LDL-C >70 mg/dL despite ongoing treatment with 20 mg/day of simvastatin for more than four weeks. In addition, the patients met the following inclusion criteria: angiographically documented CAD, stable angina, and age between 18 and 80 years. Exclusion criteria were a history of myocardial infarction or revascularization within the last 3 months, moderate/severe left ventricular systolic dysfunction, warfarin treatment, malignancy, inflammatory diseases, severe renal insufficiency (creatinine >1.5 mg/dL), active liver disease or known liver cirrhosis and unclarified transaminase increase (>3 fold of normal).

Patients were randomly assigned in a double blind fashion and 1:1 ratio, to receive either E10/S20 or S80 for six weeks. The experimental regimen was initiated without a washout period of simvastatin. Fasting venous blood samples, drawn immediately after randomization and at the conclusions of the six week study period, were used for evaluation of the following biomarkers: CRP, interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1, oxidized LDL-C (oxLDL) and soluble CD40 ligand (sCD40L). Platelet aggregation and lipid levels (LDL-C, high density lipoprotein cholesterol [HDL-C], triglycerides [TG] and apolipoproteins [apo] A and B) were also compared between the two strategies.

An informed consent was obtained from each patient and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in an *a priori* approval by the institution's human research committee.

2.2. Platelet aggregation by PFA-100 assay

Samples were collected in 3.8% sodium citrate (buffered, pH 5.5, Vacutainer, Becton Dickinson, UK) for platelet function tests. Platelet function assays were processed within 2 h of blood collection. The PFA-100 point-of-care assay (Dade-Behring, USA) utilizes a cartridge that contains a capillary, a sample reservoir, a collagen/epinephrine-coated membrane, and an aperture that exposes platelets to high shear conditions. When platelets in whole blood come in contact with epinephrine and collagen, they become activated and aggregate at the aperture, and thus gradually reduce and finally arrest blood flow. The PFA-100 records the closure time (CT), which means the time in seconds (s) from the start of the test until the platelet plug occludes the aperture. We defined the upper limit of 150 s (collagen/epinephrine cartridge, citrate 3.8%) for a normal PFA-100 CT, according to previous investigation and label recommendation [13].

2.3. CRP, IL-6, MCP-1, sCD40L and oxLDL

Serum was separated by centrifugation from the blood samples. For high-sensitivity CRP measurement, whole venous blood was collected in tubes without anticoagulant and centrifuged at room temperature. Serum CRP was assessed with a high-sensitivity, latex microparticle-enhanced immunoturbidimetric assay (BN II analyzer, Dade Behring, USA). The minimum detectable concentration of CRP was 0.2 mg/L. For the other markers, serum samples were stored at −70 °C and were determined simultaneously by ELISA in order to avoid variation of assay conditions. Commercial ELISA assays detecting MCP-1 (R&D Systems, UK), IL-6 (Siemens, USA), sCD40L (R&D Systems, USA) and oxLDL (Mercodia, USA) were applied. Detection limits and intra-assay variability of ELISA assays were respectively, as follows: MCP-1, 5 pg/mL and 4.7%; IL-6, 2 pg/mL and 6%, sCD-40 L 15.6 pg/mL (intra-assay variability not available); oxLDL 0.3 U/l and 6.1%.

2.4. End points

The primary endpoint was CRP and the secondary end points were PFA-100, IL-6, MCP-1, sCD40L, oxLDL and lipid profile measures at 6 weeks after randomization.

2.5. Statistical analysis

The sample size was determined as 72 patients. This would enable the study to have a power of 80%, with a 2-tailed type I error of 0.05, to detect a difference of 2 mg/L between the geometric means of CRP of the study groups, assuming a standard deviation of 3 mg/L [9,14] Continuous data were presented as means ± SD, or median (25th and 75th percentiles) when the distribution was non-normal. For qualitative variables, we presented counts and relative frequencies. Paired *t*-test was used for before–after intra-group comparisons when the distribution was normal, otherwise we used the Wilcoxon signed-rank test. For comparison between treatment groups we used multiple regression with adjustment only for the baseline values of the outcome variable (ANCOVA) or Wilcoxon rank-sum test when the variable was not normally distributed. Correlations between continuous variables were analyzed with Pearson's *r* statistics. Differences between the results with a p value of less than 0.05 were considered to be statistically significant. We did not adjust P values for multiple comparisons. All analyses were performed using STATA/SE 9.2 (Stata Corp LP, USA).

3. Results

Premature discontinuation of the study drugs occurred in five patients (2 in the E10/S20 group and 3 in the S80 group). Therefore, 78 patients (62% men, mean age 63 ± 9 years) remained for statistical analysis (38 in the S80 group and 41 in the E10/S20 group). Baseline characteristics, as well as lipid and inflammatory profiles were similar between the two treatment groups (Tables 1, 2 and 3). Notably, the initial cholesterol and inflammatory levels were relatively low in the whole population, due to the fact that all patients were previously on 20 mg of simvastatin for at least 4 weeks.

3.1. Change in serum lipid levels and biochemical variables

Total cholesterol, LDL-C and apo-B plasma concentrations were significantly reduced by treatments in both groups, while HDL-C and apo-A plasma concentrations remained unchanged (Table 2). The TG levels were reduced significantly by E10/S20, but not by S80. However, there were no significant differences between the changes in all lipid parameters among the two treatment groups, indicating a comparable effect of the study medications on lipid levels (Table 2). Liver and muscle parameters were unaffected.

3.2. Change in inflammatory markers and oxLDL

No significant differences were observed at baseline comparing the two groups. No significant reduction was noted within or between groups for CRP or any of the other inflammatory markers (IL-6, sCD-40L and MCP-1; Table 3 and Fig. 1). Oxidized LDL-C was equally

Table 1
Baseline characteristics of the patients, according to treatment group.

Characteristic	Simvastatin 80 mg (S80) (n = 38)	Ezetimibe 10 mg/simvastatin 20 mg (E10/S20) (n = 40)
Men (%)	21 (55)	27 (68)
Median age, in years	61.7 ± 10	64.5 ± 9
Body mass index, in kg/m^2	27.8 ± 2.7	28.6 ± 3.7
Coronary risk factor		
Diabetes mellitus (%)	20 (52)	16 (40)
Hypertension (%)	26 (68)	36 (90)
Habitual smoker (%)	8/35 (23)	5/39 (13)
Previous myocardial infarction (%)	29 (76)	24 (60)
Previous percutaneous coronary intervention (%)	16 (42)	16 (40)
Previous coronary artery bypass grafting (%)	13 (34)	16 (40)
Previous stroke (%)	3 (8)	3 (8)
Concomitant medication (%)		
ACE inhibitor or AT-1 receptor blocker (%)	32 (84)	35 (88)
ASA, 100 mg/day (%)	35 (92)	37 (93)
Clopidogrel 75 mg/day (%)	1 (2.6)	1 (2.5)

Data are expressed as mean ± SD or *n* (%). ASA, acetyl salicylic acid; ACE, angiotensin converting enzyme inhibitor; AT, angiotensin.

Table 2
Treatment effects on plasma lipid levels.

	Simvastatin 80 mg (S80) (n = 38)	Ezetimibe 10/simvastatin 20 mg (E10/S20) (n = 40)	p
Total cholesterol, mg/dL			
Pre-treatment	170.5 (155–212)	175 (157–195)	0.74
Post-treatment	143 (117–160)	143 (127–157)	
p: post vs. pre	<0.01	<0.01	
Median change (%)	−20 (9–27)	−20 (13–27)	0.76
LDL-C, mg/dL			
Pre-treatment	101 (85–130)	99 (89–117)	0.83
Post-treatment	76 (61–90)	72 (62–80)	
p: post vs. pre	<0.01	<0.01	
Mean change (%)	−28 ± 30	−29 ± 13	0.46
HDL-C, mg/dL			
Pre-treatment	45 (38–50)	42 (37–48)	0.87
Post-treatment	42 (38–48)	43 (38–49)	
p: post vs. pre	0.16	0.38	
Mean change (%)	−1 ± 14	2 ± 11	0.45
TG, mg/dL			
Pre-treatment	117 (85–150)	139 (108–168)	0.07
Post-treatment	104 (91–127)	112 (77–149)	
p: post vs. pre	0.07	0.01	
Mean change (%)	−4 ± 32	−14 ± 31	0.67
Apo A, mg/dL			
Pre-treatment	1.6 (1.4–1.7)	1.6 (1.4–1.7)	0.26
Post-treatment	1.58 (1.4–1.7)	1.5 (1.4–1.7)	
p: post vs. pre	0.97	0.17	
Mean change (%)	0.8 ± 13	−2 ± 12	0.34
Apo B, mg/dL			
Pre-treatment	0.9 (0.7–1.0)	0.9 (0.8–1.0)	0.58
Post-treatment	0.7 (0.6–0.8)	0.7 (0.7–0.9)	
p: post vs. pre	<0.01	<0.01	
Mean change (%)	−22 ± 15	−18 ± 17	0.22

Data are expressed as mean ± SD or median (interquartile range). HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglyceride; Apo, apolipoprotein.

Table 3
Change in inflammatory markers and platelet aggregation.

	Simvastatin 80 mg (S80) (n = 38)	Ezetimibe 10/simvastatin 20 mg (E10/S20) (n = 40)	p
hs-CRP, mg/L			
Pre-treatment	2.3 (0.7–5.5)	1.7 (0.72–3.65)	0.27
Post-treatment	1.8 (0.5–4.1)	1.27 (0.8–3.7)	
p: post vs. pre	0.1	0.5	
Median change (%)	−16 (−42 to 7)	−11 (−37 to 26)	0.30
IL-6, pg/mL			
Pre-treatment	2.9 (2.0–4.3)	2.1 (2.0–3.5)	0.18
Post-treatment	2.3 (2.0–4.2)	2.0 (2.0–4.3)	
p: post vs. pre	0.55	0.23	
Median change (%)	0 (−22 to 24)	0 (−14 to 0)	0.8
sCD-40 L, pg/mL			
Pre-treatment	10.8 (8.9–15.5)	11.0 (9.5–15.1)	0.63
Post-treatment	10.8 (8.6–14.8)	11.2 (9.4–16.4)	
p: post vs. pre	0.53	0.56	
Mean change (%)	6 ± 43	6 ± 34	0.48
MCP-1, pg/mL			
Pre-treatment	230 (190–170)	200 (175–295)	0,52
Post-treatment	240 (185–275)	235 (190–285)	
p: post vs. pre	0.47	0.11	
Mean change (%)	11 ± 47	10 ± 21	0.85
oxLDL, ui			
Pre-treatment	75 (54–99)	67 (54–100)	0.93
Post-treatment	55 (37–76)	58 (38–83)	
p: post vs. pre	<0.01	<0.01	
Mean change (%)	−18 ± 47	−15 ± 33	0.65
PFA-100 CT, seconds			
Pre-treatment	131 (94–204)	145 (103–191)	0.83
Post-treatment	145 (89–198)	171 (122–244)	
p: post vs. pre	0.9	<0.01	
Mean change (%)	8 ± 34	27 ± 43	0.02

Data are expressed as mean ± SD or median (interquartile range). CRP, C-reactive protein; IL, interleukin; MCP-1, monocyte chemotactic protein-1; oxLDL, oxidized LDL; sCD40L, soluble CD40 ligand; PFA-100 CT, *Platelet Function Analyzer* closure time.

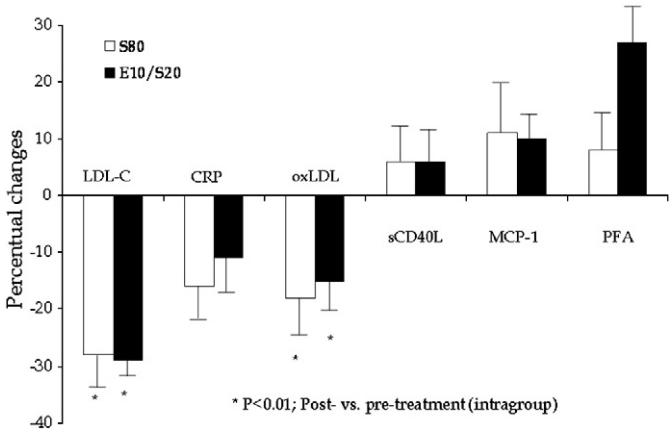

Fig. 1. Percentual changes of LDL-C and inflammatory markers between randomized treatment groups. The figure shows similar effects of both treatments on LDL-C and inflammatory markers. E10/S20, ezetimibe 10 mg/simvastatin 20 mg; S80, simvastatin 80 mg; LDL-C, cholesterol LDL; CRP, C-reactive protein; MCP-1, monocyte chemoattractant protein-1; oxLDL, oxidized LDL; sCD40L, soluble CD40 ligand. Error bars indicate standard error. Asterisks indicate that the p value was <0.01 for the difference post vs. pre-treatment intra-group. IL-6 was not changed in both groups (data in Table 3).

reduced by E10/S20 and S80 (15 ± 33% vs. 18 ± 47%, p = 0.30; respectively). We found a modest correlation between oxLDL and LDL-C reductions (r = 0.27, p = 0.02).

3.3. Change in platelet aggregation

The baseline PFA-100 median closure time levels were similar and in the normal range in both groups (145 s vs. 131 s for E10/S20 and S80 respectively, p = 0.83, Table 3), despite the fact that 95% of the patients were previously receiving treatment with acetyl salicylic acid (ASA). The PFA-100 CT increased significantly with E10/S20 but not with S80 mg (Fig. 2). The comparison between groups demonstrated a higher increase of the PFA-100 CT with E10/S20 than with S80 (27 ± 43% vs. 8 ± 34%, p = 0.02). Additionally, the changes in PFA-100 CT were not correlated to LDL-C reduction in both groups (r = 0.01; p = 0.93), but modestly correlated to TG reduction (r = 0.25; p = 0.04).

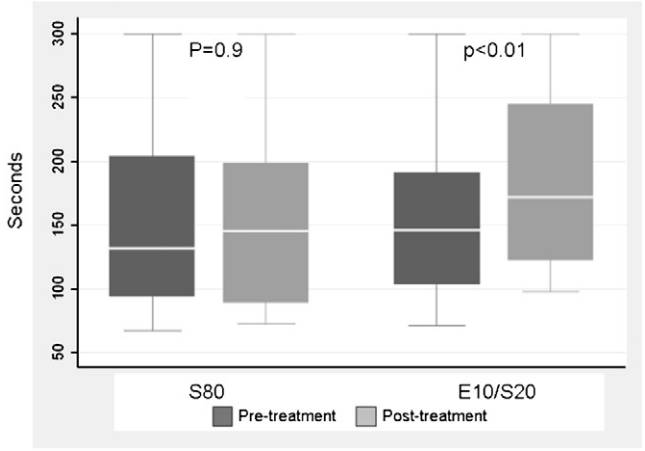

Fig. 2. Platelet Function Analyzer-100 closure times (seconds) at baseline (pre-treatment) and 6 weeks (post-treatment) in both groups. The figure shows a significant increase of the PFA-100 closure time with E10/S20 but not with S80. Data is presented as medians, 10th, 25th, 75th and 90th percentiles. E10/S20, ezetimibe 10 mg/simvastatin 20 mg; S80, simvastatin 80 mg.

4. Discussion

Our study has three main findings. First, among stable CAD patients previously treated with S20 and with an average LDL-C of 100 mg/dL, E10/S20 and S80 were equally effective in further reducing LDL-C. Second, neither treatment had any further significant anti-inflammatory effect. Finally, E10/S20 was more effective than S80 in inhibiting platelet function.

The study population included patients with CAD in a common clinical situation, in that they were already taking moderate intensity statin therapy (S20), but still above the optimal LDL-C therapeutic goal according to recent guidelines [4]. The study was designed specifically to evaluate pleiotropic effects, and as expected from prior studies, E10/S20 and S80 had similar effects on lipids [12]. Therefore, we hypothesized that any differences observed between groups in other parameters would not be related to LDL-C decrease, but to specific pharmacologic features.

4.1. Anti-inflammatory effects of E10/S20 and S80

In previous studies, differences on CRP reduction were described between statins and the combination of ezetimibe/statin [12]. However, these studies did not compare CRP changes between equivalent lipid lowering doses of these agents and therefore the LDL-C was often differentially reduced between groups. Consequently, pleiotropic effects could not be appropriately evaluated, since any difference in anti-inflammatory effect between therapies could be related mainly to distinct LDL-C reductions. In fact, a recent clinical trial that compared ezetimibe/simvastatin and simvastatin strategies to achieve aggressive cholesterol goals (target LDL-C <70 mg/dL) demonstrated equivalent CRP reduction between groups [15].

Recently, Azar et al. [16] demonstrated that ezetimibe compared to placebo, on top of atorvastatin, strongly reduced oxLDL in patients with CAD. Evidently, the ezetimibe group had a larger reduction in LDL-C. Moreover, the change in oxLDL correlated significantly with the LDL-C decrease. On the other hand, to our knowledge, our study was the first one that demonstrated similar oxLDL reductions between comparable lipid lowering doses of the combination ezetimibe/simvastatin and simvastatin alone.

The fact that the inflammatory markers (CRP, IL-6, MCP-1 and sCD40L) were not reduced by either treatment strategy might be explained by the following reasons: (1) As described above, the initial inflammatory levels were already low in our population. This may be related to the fact that patients were receiving guideline-based treatment (aspirin, angiotensin converting enzyme inhibitor, angiotensin type 1 receptor blocker and S20) before randomization and the study did not include a washout period before randomization. (2) The period of six weeks may not be long enough to detect some of the anti-inflammatory effects of these treatments. (3) Some of the anti-inflammatory effects of statins may be concentrated in moderate doses. Blanco-Colio et al. [17], for example, demonstrated that atorvastatin 10 mg reduced the levels of the intercellular adhesion molecule-1. However, the increase of the drug to 80 mg did not show any additional reduction. Similarly, in the Aggrastat to Zocor (A to Z trial), minimal difference in MCP-1 was seen between patients receiving S80 or S20 [18].

These data do not suggest differences in pleiotropic anti-inflammatory effects between E10/S20 and S80. Moreover, the effect of these strategies on oxLDL seems to be related mainly to cholesterol reduction and not to any pleiotropic effect.

4.2. Antiplatelet effects of E10/S20 and S80

In our study, E10/S20 was more effective than S80 in inhibiting platelet aggregation, despite similar LDL-C reductions between groups. The treatment with E10/S20, differently from S80, significantly increased the PFA-100 CT, an effect that could not be entirely explained by cholesterol reduction. However, this finding should be interpreted with caution since it was one of many comparisons in our study, which may increase the chance of a spurious statistically significant association. Nevertheless, this study shows for the first time that ezetimibe/simvastatin, at therapeutic concentrations, can inhibit platelet function, a potential beneficial pleiotropic effect of this combination.

Despite the previous use of ASA (100 mg/day) in 95% of the evaluated population, the median initial PFA-100 CT was in the normal range in 56% of the patients. Other studies have also reported high residual platelet aggregation among patients receiving aspirin [19], particularly in patients using low doses [20]. On the other hand, lipids may increase platelet aggregation by several mechanisms [21]: (1) LDL-C, HDL-C and apolipoproteins bind specific receptors on the platelet membrane, promoting thromboxane synthesis, glycoprotein IIbIIIa expression and calcium influx [22]; (2) Modification of native LDL-C generates a platelet-activating particle [23]; and, (3) Triglycerides may also be related to platelet function. Kunes et al. [24] demonstrated that lowering TG by gemfibrozil increases platelet membrane microviscosity and reduces cytosolic Ca^{2+} in rats. Subsequently, Karepov et al. [25] demonstrated that hypertriglyceridemia reduces platelet response to aspirin.

Concomitantly, several investigators have evaluated the effects of statins and ezetimibe on platelet function. However, these studies were small and performed different and poorly reproducible platelet evaluation methodologies [11,19,26–29]. Moreover, it is not clear if these effects were related to cholesterol reduction, pleiotropic effects or both. In patients with hypercholesterolemia, statins decreased thrombin and thromboxane levels [26]. Tirnaksiz et al. performed the PFA-100 CT measurement in patients with CAD and demonstrated a significant reduction in aspirin resistance after treatment with atorvastatin [27]. In contrast, other investigators did not find any antiplatelet effect evaluated by flow cytometry and turbidimetric aggregometry, after treatment with E10/S10 or S80, in diabetic patients [28]. Hussein et al. [29] demonstrated that ezetimibe decreases platelet aggregation, LDL-C peroxidation and platelet cholesterol content. Interestingly, this antiplatelet effect was not present when simvastatin was associated to ezetimibe. Recently, Piorkowski et al. [11] evaluated 56 patients with CAD and demonstrated a significant reduction of P-selectin and platelet aggregation by 40 mg of atorvastatin, but not with 10 mg of ezetimibe/10 mg of atorvastatin, despite similar reduction on LDL-C. However, only 38 patients were submitted to optical aggregometry in this study and the platelet effect was not different between groups.

In our study, we performed the PFA-100, a simple and automatic point-of-care assay. Importantly, the PFA system is known to demonstrate a sensitivity of 95% and a specificity of 89% for the evaluation of platelet function status, compared with classical aggregometry [30]. We demonstrated a higher increase of the PFA-100 CT with E10/S20 than with S80 ($27 \pm 43\%$ vs. $8 \pm 34\%$, respectively, $p = 0.02$). This result could be partially explained by a significant decrease on TG levels achieved by E10/S20, but not by S80. In fact, the increase in PFA-100 was modestly, but significantly, correlated to TG reduction ($r = 0.25$; $p = 0.04$). Alternatively, platelet aggregation may be influenced by several lipid aspects, beyond LDL-C levels, as the platelet cholesterol content, platelet membrane fluidity, LDL peroxidation and HDL-C affinity to binding proteins on platelet membrane [21]. Actually, studies on the effect of ezetimibe on these parameters are lacking. Moreover, the antiplatelet effect of ezetimibe is poorly understood and larger studies are needed to confirm these antithrombotic properties.

5. Study limitations

The previous use of S20 for at least 4 weeks and the absence of a washout period before randomization may have influenced the subsequent

inflammatory changes. The PFA-100 results should be interpreted considering the fact that it was part of a multiple endpoint finding.

6. Conclusion

The present study provides evidence that among stable CAD patients treated with S20 to an average LDL-C of 100 mg/dL, (1) both E10/S20 and S80 are equally effective in further reducing LDL-C, (2) neither treatment had any further significant anti-inflammatory effects, and (3) E10/S20 is more effective than S80 in inhibiting platelet aggregation. Thus, despite similar lipid lowering and doses 4× less of simvastatin, E10/S20 induced a greater platelet inhibitory effect than S80, a finding that merits further investigation.

Disclosures

Dr. de Lemos has received consulting fees from Astra Zeneca for participation on an endpoint review committee for a drug not related to lipid lowering. Dr Nicolau received consulting fees from Merck/Schering Plough and Pfizer.

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The authors of this manuscript have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [31].

References

[1] Baigent C, Keech A, Kearney PM, et al. Cholesterol Treatment Trialists' (CTT) Collaborators. Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins. Lancet 2005;366:1267–78.
[2] Heart Protection Study Collaborative Group. MRC/BHF Heart Protection Study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 2002;360:7–22.
[3] Cannon CP, Braunwald E, McCabe CH, et al. Pravastatin or atorvastatin evaluation and infection therapy-thrombolysis in myocardial infarction 22 investigators. Intensive versus moderate lipid lowering with statins after acute coronary syndromes. N Engl J Med 2004;350:1495–504.
[4] Grundy SM, Cleeman JI, Merz CN, et al. Coordinating Committee of the National Cholesterol Education Program. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III Guidelines. J Am Coll Cardiol 2004;44:720–32.
[5] Kosoglou T, Statkevich P, Johnson-Levonas AO, Paolini JF, Bergman AJ, Alton KB. Ezetimibe: a review of its metabolism, pharmacokinetics and drug interactions. Clin Pharmacokinet 2005;44:467–94.
[6] Ridker PM, Cannon CP, Morrow D, et al. Pravastatin or atorvastatin evaluation and infection therapy-thrombolysis in myocardial infarction 22 (PROVE IT-TIMI 22) Investigators. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005;352:20–8.
[7] Glynn RJ, Danielson E, Fonseca FA, et al. A randomized trial of rosuvastatin in the prevention of venous thromboembolism. N Engl J Med 2009;360:1851–61.
[8] Ascer E, Bertolami MC, Venturinelli ML, et al. Atorvastatin reduces proinflammatory markers in hypercholesterolemic patients. Atherosclerosis 2004;177:161–6.
[9] Kinlay S. Low-density lipoprotein-dependent and -independent effects of cholesterol-lowering therapies on C-reactive protein: a meta-analysis. J Am Coll Cardiol 2007;49:2003–9.
[10] Puccetti L, Pasqui AL, Auteri A, Bruni F. Mechanisms for antiplatelet action of statins. Curr Drug Targets Cardiovasc Haematol Disord 2005;5:121–6.
[11] Piorkowski M, Fischer S, Stellbaum C, et al. Treatment with ezetimibe plus low-dose atorvastatin compared with higher-dose atorvastatin alone: is sufficient cholesterol-lowering enough to inhibit platelets? J Am Coll Cardiol 2007;49:1035–42.
[12] Sager PT, Melani L, Lipka L, et al. Effect of coadministration of ezetimibe and simvastatin on high-sensitivity C-reactive protein. Am J Cardiol 2003;92(12):1414–8.
[13] Sestito A, Sciahbasi A, Landolfi R, et al. A simple assay for platelet-mediated hemostasis in flowing whole blood (PFA-100): reproducibility and effects of sex and age. Cardiologia 1999;44:661–6.
[14] Ceriello A, Assaloni R, Da Ros R, et al. Effect of atorvastatin and irbesartan, alone and in combination, on postprandial endothelial dysfunction, oxidative stress, and inflammation in type 2 diabetic patients. Circulation 2005;111:2518–24.
[15] Fleg JL, Mete M, Howard BV, et al. Effect of statins alone versus statins plus ezetimibe on carotid atherosclerosis in type 2 diabetes: the SANDS (Stop Atherosclerosis in Native Diabetics Study) trial. J Am Coll Cardiol 2008;52:2198–205.
[16] Azar RR, Badaoui G, Sarkis A, et al. Effect of ezetimibe/atorvastatin combination on oxidized low density lipoprotein cholesterol in patients with coronary artery disease or coronary artery disease equivalent. Am J Cardiol 2010;106:193–7.
[17] Blanco-Colio LM, Martín-Ventura JL, de Teresa E, et al. ACTFAST investigators. Elevated ICAM-1 and MCP-1 plasma levels in subjects at high cardiovascular risk are diminished by atorvastatin treatment. Atorvastatin on Inflammatory Markers study: a substudy of Achieve Cholesterol Targets Fast with Atorvastatin Stratified Titration. Am Heart J 2007;153:881–8.
[18] de Lemos JA, Morrow DA, Blazing MA, et al. Serial measurement of monocyte chemoattractant protein-1 after acute coronary syndromes: results from the A to Z trial. J Am Coll Cardiol 2007;50:2117–24.
[19] Lordkipanidzé M, Pharand C, Schampaert E, Turgeon J, Palisaitis DA, Diodati JG. A comparison of six major platelet function tests to determine the prevalence of aspirin resistance in patients with stable coronary artery disease. Eur Heart J 2007;28:1702–8.
[20] Gurbel PA, Bliden KP, DiChiara J, et al. Evaluation of dose-related effects of aspirin on platelet function: results from the Aspirin-Induced Platelet Effect (ASPECT) study. Circulation 2007;115:3156–64.
[21] Relou IA, Hackeng CM, Akkerman JW, Malle E. Low density lipoprotein and its effect on human blood platelets. Cell Mol Life Sci 2003;60:961–71.
[22] Koller E. Lipoprotein-binding proteins in the human platelet plasma membrane. FEBS Lett 1986;200:97–102.
[23] Vlasova II. The effect of oxidatively modified low-density lipoproteins on platelet aggregability and membrane fluidity. Platelets 2000;11:406–14.
[24] Kunes J, Devynck MA, Zicha J. Chronic changes in plasma triglyceride levels do modify platelet membrane microviscosity in rats. Life Sci 2000;67:959–67.
[25] Karepov VG. Aspirin resistance, hyperlipidemia and secondary stroke prevention. Int J Stroke 2006;1(suppl 1):72–3.
[26] Fenton 2nd JW, Brezniak DV, Ofosu FA, Shen GX, Jacobson JR, Garcia JG. Statins and thrombin. Curr Drug Targets Cardiovasc Haematol Disord 2005;5:115–20.
[27] Tirnaksiz E, Pamukcu B, Oflaz H, Nisanci Y. Effect of high dose statin therapy on platelet function: statins reduce aspirin-resistant platelet aggregation in patients with coronary heart disease. J Thromb Thrombolysis 2009;27:24–8.
[28] Malmström RE, Settergren M, Böhm F, Pernow J, Hjemdahl P. No effect of lipid lowering on platelet activity in patients with coronary artery disease and type 2 diabetes or impaired glucose tolerance. Thromb Haemost 2009;101:157–64.
[29] Hussein O, Minasian L, Itzkovich Y, Shestatski K, Solomon L, Zidan J. Ezetimibe's effect on platelet aggregation and LDL tendency to peroxidation in hypercholesterolaemia as monotherapy or in addition to simvastatin. Br J Clin Pharmacol 2008;65:637–45.
[30] Mammen EF, Comp PC, Gosselin R, et al. PFA-100 system: a new method for assessment of platelet dysfunction. Semin Thromb Hemost 1998;24:195–202.
[31] Shewan LG, Coats AJ. Ethics in the authorship and publishing of scientific articles. Int J Cardiol 2010;144:1–2.