Effect of Proprotein Convertase Subtilisin/Kexin Type 9 Inhibitors on Plasma Ceramide Levels

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Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors are novel drugs that provide striking lowering of low-density lipoprotein cholesterol (LDL-C) when added to maximum tolerated therapy in patients with hypercholesterolemia. Ceramides, novel cardiac risk markers, have been associated with increased cardiovascular mortality, independent of traditional cardiovascular risk factors. The Ceramide Risk Score (CRS) predicts the likelihood of adverse cardiovascular events within 1 to 3 years in patients with coronary artery disease. The effect of PCSK9 inhibition on plasma ceramides is not well known. The study examines the effect of PCSK9 inhibitors on plasma ceramides and CRS in patients with clinical indication for this therapy. Retrospective chart review of consecutive patients with hypercholesterolemia on PCSK9 inhibitors was conducted (n = 24; Mayo Clinic 2015 to 2018). Plasma ceramides were measured before the initiation of PCSK9 inhibitors and 2 to 12 months after treatment. CRS was calculated before and after therapy based on individual plasma concentrations of 4 ceramides. Treatment with PCSK9 inhibitors was associated with significant reduction in mean CRS and individual ceramides levels ($p < 0.0001$). CRS significantly improved with PCSK9 therapy. PCSK9 inhibitors significantly decreased LDL-C levels by 63% ($p < 0.0001$). The absolute reduction in CRS did not correlate with the absolute reduction in LDL-C ($r = 0.31$; confidence interval −0.10 to 0.64), indicating that CRS may evaluate a different pathway for risk reduction beyond LDL-C lowering. In conclusion, treatment with PCSK9 inhibitors is associated with significant reduction in CRS and distinct ceramide levels. **(Am J Cardiol 2020;128:163−167)**

Proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors have gained wide interest as new therapeutic agents for lowering cholesterol in patients with atherosclerotic cardiovascular disease. Ceramides are complex lipids that are associated with inflammation and vulnerable arterial plaque.[1] The Ceramide Risk Score (CRS), which was proposed by Laaksonen et al, is a novel prognostic cardiovascular marker that incorporates the values of the cardiovascular death associated ceramide species.[2] The CRS predicts the likelihood of plaque-rupture events within 1 year in patients with coronary artery disease.[2−4] Plasma ceramides can be decreased by statins[5,6] and exercise.[7] The effect of PCSK9 inhibitors on plasma ceramides is not well studied. This study examines the effect of PCSK9 inhibitors on plasma ceramides in patients with hypercholesterolemia and atherosclerotic cardiovascular disease who are on maximally tolerated statin therapy but require additional low-density lipoprotein cholesterol (LDL-C) lowering.

Methods

We conducted a retrospective chart review of consecutive patients with hypercholesterolemia who were clinically evaluated and treated with PCSK9 inhibitors at the Mayo Clinic between January 2015 and December 2018. Patients who had plasma ceramides measured before the initiation of PCSK9 inhibitor therapy (pretreatment) and at 2 to 12 months after treatment (post-treatment) were included in this study. Patients who did not have plasma ceramide measurement at baseline and/or after treatment were excluded. This study was approved by the Mayo Clinic Institutional Review Board (IRB# 17-007924).

Mayo Clinic Cardiovascular Laboratory Medicine performed all testing. Total cholesterol, high-density lipoprotein cholesterol, triglycerides, and apolipoprotein (apo) B were measured using a Cobas c501 analyzer (Roche Diagnostics, Indianapolis, Indiana). Plasma ceramides were measured by high-pressure liquid chromatography coupled tandem mass spectrometry (LC-MS/MS). EDTA plasma samples were diluted in ethyl acetate and isopropanol (20:80 v:v) with 0.1% formic acid before addition of deuterium-labeled internal standards (Avanti Polar Lipids, Alabaster, Alabama). Ceramides were separated using an X-bridge C18 3.5, 3.1 × 50 mm column (Waters, Milford, Massachusetts) and detected using an API 5000 MS/MS (AB Sciex, Framingham, Massachusetts). Laboratory analytical imprecision ranged between 5.1% and 11.8%.[8]

Untargeted lipidomic analysis identified 3 plasma ceramide (Cer) species, Cer(16:0), Cer(18:0), and Cer(24:1),

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as highly associated with cardiovascular mortality, independent of conventional cardiovascular risk factors such as LDL-C.[5] Additional predictive value was found when these ceramides were normalized to Cer(24:0), a highly abundant plasma ceramide that is not influenced by disease.[2] The CRS incorporates values of these 3 ceramide species and their normalized ratios into a 12-point scale.[2] To calculate CRS, 1 or 2 points were added to the score for each result above the median or the 3rd quartile, respectively. In our study, the CRS was calculated before and after the PCSK9 inhibitor therapy. We also analyzed the change in LDL-C, lipoprotein(a), lipoprotein-associated phospholipase A2 activity (Lp-PLA_2), apo B, apo A-1, and apo B/apo A-1 ratio before and 2 to 12 months after treatment with PCSK9 inhibitor.

Measured parameters are expressed as mean ± standard deviation. Two-sided *t* test was used to assess statistical significance which is defined as p value ≤0.05. A multivariate correlation analysis was performed to assess whether the absolute change in CRS correlated with the absolute change in LDL-C, and apo B pre- and post-PCSK9 inhibitor treatment, respectively. Correlation is expressed as correlation coefficient "r" with confidence interval (CI).

Results

Twenty-four patients with hypercholesterolemia who were clinically treated with PCSK9 inhibitor therapy were included in this study. Baseline characteristics of the study cohort are listed in Table 1. Familial hypercholesterolemia (FH) was diagnosed in 18 patients (75%). Eleven of these patients had negative genetic testing for FH and 7 patients carried heterozygous pathogenic mutations. Twenty-two patients (92%) were treated with evolocumab, 140 mg every 14 days, and 2 patients were treated on alirocumab, 75 mg every 14 days. Baseline plasma ceramide levels were measured 0 to 157 days (mean of 52 ± 49 days) before initiation of PCSK9 inhibitor therapy. Post-treatment measurements were obtained 48 to 461 days (mean of 161 days) after the initiation of therapy. All patients had pretreatment and post-treatment plasma ceramides and LDL-C measured. A subset of patients also had measurements of lipoprotein(a) (n = 11), Lp-PLA_2 (n = 8), apo B (n = 12), and apo A-1 (n = 12) before and after initiation of PCSK9 inhibitor therapy.

Table 1
Baseline characteristics of study cohort

Variables	n = 24
Age (years)	63 ± 12
Men	9 (38%)
Non-Hispanic white	24 (100%)
BMI (kg/m^2)	30 ± 5
Smoker, current and former	12 (50%)
Familial hypercholesterolemia	18 (75%)
Coronary artery disease	20 (83%)
History of cardiac stents or coronary artery bypass graft surgery	16 (67%)
Hypertension	14 (58%)
Diabetes	3 (13%)
Chronic kidney disease	2 (8%)
Statin	16 (67%)
Ezetimibe	14 (58%)
Median number of antihypertensives (interquartile range)	1.5 (0-2)
Total cholesterol (mg/dl)	263 ± 76
High-density lipoprotein cholesterol (mg/dl)	55 ± 17
Low-density lipoprotein cholesterol (mg/dl)	180 ± 71
Triglycerides (mg/dl)	148 ± 79
Lipoprotein(a) (mg/dl)	49 ± 55

Values are mean ± standard deviation except for the number of antihypertensives.

Before initiation of PCSK9 inhibitor therapy, 15 patients (63%) were on stable maximal tolerated statin therapy ranging from rosuvastatin 5 mg weekly to atorvastatin 80 mg daily and 14 patients (58%) were on ezetimibe. One patient was started on new statin therapy (atorvastatin 20 mg weekly) during the study period after the measurement of baseline plasma ceramides. Patients were encouraged to stay on their statin therapy and ezetimibe after the initiation of PCSK9 inhibitor therapy. The majority of patients (13 of 15) continued with the same dose of statin. One patient had a dose reduction due to myalgia and 1 patient self-discontinued statin therapy after the initiation of PCSK9 inhibitor therapy.

Treatment with PCSK9 inhibitors was associated with 48% significant reduction in mean CRS and 30% to 37% reduction in plasma ceramides (p <0.0001; Table 2). The mean CRS of the cohort significantly improved from 5.8 ± 3.2 to 3.0 ± 2.0 following PCSK9 therapy (p <0.0001) (Figure 1). By comparison, LDL-C levels decreased by 63% (pretreatment 180 ± 71 mg/dl, post-treatment 67 ± 53 mg/dL) (p <0.0001; Figure 1). Notably, the CRS remained unchanged or increased in 5 patients (21%) whereas LDL-C was reduced in all cases (Figure 1). In order to evaluate the pattern of ceramide reduction, the study cohort was also stratified into previously established CRS risk categories (Lower, Moderate, Increased, and Higher Risk) based on their baseline CRS (Table 3).

The absolute reduction in CRS did not correlate with the absolute reduction in LDL-C (r = 0.31; CI −0.10 to 0.64) (Supplemental table 1). The mean reduction of LDL-C was 95 ± 52 mg/dl among the 5 patients with steady or increasing CRS, compared to 117 ± 60 mg/dl among the 19 subjects with reduced CRS. The absolute reduction in CRS appeared to have some correlation with the absolute reduction in apo B with a very wide CI (r = 0.73; CI 0.26 to 0.92). As expected, absolute reduction in LDL-C had a very strong correlation with the absolute reduction in apo B (r = 0.95; CI 0.83 to 0.99).

Treatment with PCSK9 inhibitors was also associated with significant reduction in lipoprotein(a) levels (p = 0.035), Lp-PLA_2 (p = 0.0003), apo B (p <0.0001), and apo B/ apo A-1 ratio (p <0.0001) (Supplemental Table 2). Apo A-1 level was significantly increased with PCSK9 inhibitors (p = 0.012). There were no deaths or cardiovascular events including cerebrovascular accidents and acute coronary syndrome during the study period.

Discussion

This is the first study to evaluate the effects of PCSK9 inhibitors on CRS. Our data shows, first, that CRS significantly improves with PCSK9 inhibitor therapy from the

Table 2
Plasma ceramides before the proprotein convertase subtilisin/kexin type 9 inhibitor therapy and 2 to 12 months after initiation of therapy in 24 patients with hypercholesterolemia

	Pretreatment mean	Post-treatment mean	Mean difference	Percentage change	p Value
Ceramide (16:0) (μM)	0.36	0.23	−0.13 ± 0.07	−37%	<0.0001
Ceramide (18:0) (μM)	0.12	0.08	−0.04 ± 0.03	−30%	<0.0001
Ceramide (24:0) (μM)	4.16	2.92	−1.24 ± 1.13	−30%	<0.0001
Ceramide (24:1) (μM)	1.42	0.98	−0.44 ± 0.26	−31%	<0.0001
Ceramide (16:0)/(24:0) (μM)	0.09	0.09	−0.01 ± 0.03	−7.6%	0.26
Ceramide (18:0)/(24:0) (μM)	0.03	0.03	0 ± 0.01	3.5%	0.74
Ceramide (24:1)/(24:0) (μM)	0.37	0.34	−0.03 ± 0.12	−7.3%	0.29
Ceramide Risk Score	5.83	3.04	−2.79 ± 2.73	−48%	<0.0001

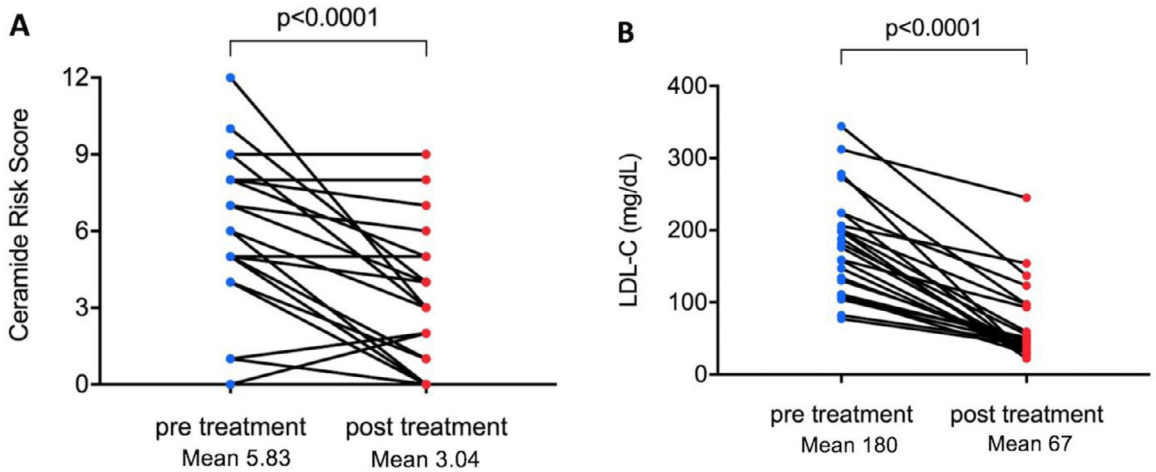

Figure 1. Change in ceramide risk score (A) and low-density lipoprotein cholesterol (LDL-C) (B) with proprotein convertase subtilisin/kexin type 9 inhibitor (n = 24).

upper limit (5.8) to the lower limit (3.04) of moderate risk category after mean treatment of 161 ± 127 days (p <0.0001). The reduction in CRS was more profound and clinically significant when looking at the mean CRS reduction within each risk category (Table 3). Patients who were at the highest risk at baseline had the largest risk reduction and there was no patient in the Higher Risk category after treatment. Previous studies in patients with coronary artery disease have shown that 5-year risk for adverse cardiovascular events increases with increase in CRS from 2.1% for CRS of 0 to 2 (Lower Risk) to 3.7% for CRS 3 to 6 (Moderate Risk), to 5.2% for CRS 7 to 9 (Increased Risk), and to 10.3% for CRS 10 to 12 (Higher Risk).[2]

Second, our data shows that PCSK9 inhibitors resulted in significant decrease in the 3 distinct cardiovascular mortality associated plasma ceramide species but not when normalized for Cer(24:0). Cer(16:0), Cer(18:0), Cer(24:1) decreased by 37% ± 20%, 30% ± 22%, and 31% ± 18%, respectively, after mean treatment of 161 ± 127 days (p <0.0001 for all measures). The data reported here are consistent with a recent study by Hilvo et al, which demonstrated that administration of a PCSK9 inhibitor (RG7652) significantly altered the lipid composition of plasma and lipoprotein particles after 29 days of therapy.[9] In addition, the authors found that ceramides with longer fatty acyl side chains were reduced to a greater extent by RG7652 when compared with their shorter fatty acyl side chain counterparts. In our study, PCSK9 inhibitors reduced all ceramide species to a similar extent. The ceramide ratios that have strong predictive value for fatal cardiovascular events were

Table 3
The study cohort (n = 24) was stratified into the 4 ceramide risk categories based on their baseline ceramide risk score in order to evaluate the pattern of ceramide risk score reduction within each category with the treatment of proprotein convertase subtilisin/kexin type 9 inhibitors

Baseline ceramide risk category	Pretreatment mean ceramide risk score	Post-treatment mean ceramide risk score	p Value
Low Risk- Score 0−2 (n = 4)	0.5	1.0	0.46
Moderate Risk- Score 3−6 (n = 10)	5.0	1.6	0.0002
Increased Risk - Score 7−9 (n = 7)	8.0	6.0	0.05
Higher Risk - Score 10−12 (n = 3)	10.7	3.7	0.02

not significantly altered by PCSK9 inhibition in either study. This is likely due to the difference in the distribution of the ceramides in the lipoprotein fractions. Cer(24:0) is much more abundant compared with the other ceramide species, leading to nonsignificant change of the ceramide ratios.[9] Our study is unique in that we are the first group to examine the effect of PCSK9 inhibitors on CRS.

In our study, the extent of LDL-C lowering in response to PCSK9 inhibition was expected and is consistent with previous studies.[10,11] Interestingly, CRS suggested no change in risk despite the reduction in LDL-C for some patients. This may indicate that CRS evaluates a different pathway for risk reduction beyond LDL-C lowering. Cholesterol-rich, apo B-containing lipoproteins are the principal actors in lipid pathogenesis of atherosclerosis. The levels of LDL-C and apo B were both significantly lowered with PCSK9 inhibitors. Although the absolute reduction in CRS appeared to have some correlation with the absolute reduction in apo B, the wide CI makes it difficult to draw any definite conclusion. Previously published study from Mayo Clinic reported that the incidence of cardiovascular events in patients with low LDL-C (<100 mg/dl) and high ceramides was 16% per person per year compared with only 3.7% in patients with low LDL-C and low ceramides.[4] Therefore, ceramides can be used to identify high-risk patients in those with LDL-C level <100 mg/dl.

In addition to significant lowering of LDL-C and ceramides, PCSK9 inhibitors are also known to affect other lipid and lipoprotein variables that are associated with cardiovascular risks.[12] In previous studies, PCSK9 inhibitors were shown to modestly improve the concentrations of triglycerides, high-density lipoprotein cholesterol, apo A-1 and lipoprotein(a) levels.[13] In our study, treatment with PCSK9 inhibitors was associated with significant reduction in lipoprotein and significant improvement in apo A-1. Lp-PLA_2 activity, which is known to predict cardiovascular risk independently of conventional risk factors,[14] was significantly reduced with PCSK9 inhibitors.

Major limitations of our study include the small sample size, retrospective study design, and homogeneous patient population in terms of ethnicity. The majority of the study cohort carried a clinical diagnosis of FH. Although we did not detect any differences in the effects of PCSK9 inhibitors on CRS between the FH patients and non-FH patients, the overall small-sample size makes subgroup analyses not very meaningful. Further studies with larger sample sizes are needed to better understand the role of different lipid species, such as ceramides, in the development of atherosclerosis and how lipid lowering therapies may alter progression of coronary disease.

Our data suggest that treatment with PCSK9 inhibitors is associated with significant reduction in the CRS and distinct ceramides, which are novel markers of cardiovascular disease. In addition to significant lowering of LDL-C and ceramides, PCSK9 inhibitors also affect other lipid and lipoprotein variables that are associated with cardiovascular risks The reduction in ceramides and CRS did not correlate with the reduction in LDL-C, indicating that the CRS may evaluate a different risk reduction pathway than LDL-C lowering and that factors other than LDL contribute to residual risk. These findings strengthen the importance of PCSK9 inhibition in targeted populations with hypercholesterolemia and may potentially contribute to our further understanding of pathophysiologic mechanisms by which PCSK9 inhibitors improve outcomes.

Disclosure

The authors have no conflicts of interest to disclose.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.amjcard.2020.04.052.

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