Pharmacological Reports 70 (2018) 258–262

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Original article

Comparison of the effects of rosuvastatin monotherapy and atorvastatin-ezetimibe combined therapy on the structure of erythrocyte membranes in patients with coronary artery disease

Małgorzata Olszewska-Banaszczyk[a,*], Paulina Jackowska[a], Paulina Gorzelak-Pabiś[a], Edyta Pytel[b], Maria Koter-Michalak[b], Marlena Broncel[a]

[a] Department of Internal Diseases and Clinical Pharmacology, Medical University of Łódź, Łódź, Poland
[b] Department of Environment Pollution Biophysics, Faculty of Biology and Environmental Protection, University of Łódź, Łódź, Poland

ARTICLE INFO

Article history:
Received 29 May 2017
Received in revised form 18 September 2017
Accepted 16 November 2017
Available online 20 November 2017

Keywords:
Coronary artery disease
Rosuvastatin
Ezetimibe
Membrane fluidity
Oxidative stress

ABSTRACT

Background: Abnormalities in the physical properties of the red blood cells (RBCs) membranes may underlie the defects that are strongly linked to cardiovascular diseases (CVD).
The aim of the study was to compare the effects of two therapies of equal hipolipemic efficacy on the erythrocyte membrane fluidity, concentration of membrane cholesterol, lipids peroxidation and RBCs distribution witdh in patients with CVD.
Methods: The study included 44 patients with angiographic evidence of CVD, who despite previous 6-month hypolipemic therapy, did not achieve the concentration of LDL-C <70 mg/dl. They were randomly assigned to: rosuvastatin 20 mg/day (R20) and atorvastatin 10 mg/day combined with ezetimibe 10 mg/day (A10 + E10). The membrane fluidity, the concentration of thiobarbituric acid reactive substances –TBARS, concentration of membrane cholesterol were evaluated after 6 months therapy.
Results: An improvement in lipid parameters was observed in each of the groups studied. In R20 the treatment resulted in 33% reduction concentrations of TBARS in serum, as well as in a decrease in membrane cholesterol by 16%, fluorescence anisotropy of TMA-DPH by 17.7%, fluorescence anisotropy of DPH by 2.8%. In A10 + E10 the reduction of TBARS by 20.5% in serum, membrane cholesterol by 15.8% as well as a 14.25% increase in RBC membrane fluidity in the superficial layer (TMA-DPH) and decrease fluidity in the deep layer (DPH) were observed.
Conclusion: Rosuvastatin increases the fluidity of erythrocyte membrane and decreases the TBARS in serum to greater extent than does equal hipolipemic combined therapy atorvastatin with ezetimibe.

Introduction

Abnormalities in the physical properties of the cell membranes may underlie the defects that are strongly linked to cardiovascular diseases [1]. Our recent results suggest that the membrane fluidity of red blood cells (RBCs) was significantly lower in patients with hypercholesterolemia than in healthy due to higher level of cholesterol in membrane and lipids peroxidation [2]. Ma at al. showed that supplementation of iron alone and combined with vitamins improves haematological status and erythrocyte membrane fluidity in anaemic pregnant women [3]. Tziakas et al. observed a link between RDW – red cell distribution width (measure of RBC volume variations –anisocytosis) and total cholesterol erythrocyte membrane [4]. Previous studies have noted the significance of RDW as a predictor of mortality in patients with stable coronary artery disease and patients suffering from myocardial infarction (MI) [5]. The reduction in membrane fluidity is responsible for the deterioration of cell deformability and blood flow through the microcirculation. This mechanism may explain the relationship between RBC rheology and the lack of tissue reperfusion following PCI in patients suffering from MI [6,7]. This effect may also explain the slow flow phenomenon observed in the epicardial coronary arteries in symptomatic patients without coronary stenosis [8]. The cholesterol concentration in RBCs from patients with acute coronary syndrome (ACS) is significantly higher than in individuals

* Corresponding author.
E-mail address: greta.olszewska@wp.pl (M. Olszewska-Banaszczyk).

https://doi.org/10.1016/j.pharep.2017.11.004

with stable angina and can be considered as a potential biomarker of plaque destabilization [9,10].

The statins, most commonly used in the treatment of hyperlipidemic patients, especially in the secondary prevention have certain beneficial effects including improved endothelial function, plaque stabilization and decreased oxidative stress, inflammation, beyond their lipid lowering effect. However, the effects of statins on RBC membrane fluidity are still unclear, the results are divergent. Compared to numerous reports on statins, there are no studies on the effect of ezetimibe on RBC, RDW, used in combination with statin.

The aim of the study was to compare the effects of two different therapies of equal hipolipemic efficacy on the erythrocyte membrane fluidity, concentration of membrane cholesterol, lipids peroxidation and red blood cell distribution width in patients with coronary artery disease.

Material and methods

The study included 44 patients with a history of MI, who underwent percutaneous coronary intervention (PCI) and/or coronary artery bypass surgery within 6 months prior to randomization and did not achieve the target therapeutic concentration of LDL-C (<70 mg/dl) despite hypolipemic therapy with simvastatin (10–40 mg), lovastatin (10–40 mg), atorvastatin (10–30 mg) and rosuvastatin (5–15 mg). Exclusion criteria were following: NYHA class III or IV chronic heart failure with ejection fraction (EF) < 40%, chronic kidney disease at stage IV,V (estimated glomerular filtration rate, eGFR ≤30 ml/min/1.73 m^2) type 1 or 2 diabetes mellitus, hyper- or hypothyroidism, diseases of the liver, liver dysfunction or serum activity of hepatic enzymes >3-fold upper normal limit, myopathy, myalgia, autoimmune disorders, allergies, infectious diseases, statin and/or ezetimibe intolerance, history of acute infection within 2 weeks prior to randomization, anemia, history of cancer within 5 years prior to randomization, pregnancy or breastfeeding, alcohol abuse, tobacco smoking, treatment with atorvastatin (≥40 mg/day), rosuvastatin (≥20 mg/day) or combined therapy with a statin and ezetimibe prior to randomization.

Qualified patients were randomly assigned to two therapeutic groups. Twenty-one patients received rosuvastatin 20 mg/day (R20); and 19 patients – combination therapy: atorvastatin 10 mg/day with ezetimibe 10 mg/day (group A10 + E10).

The study material was collected from patients at two different time points of the treatment: before and after 6 months of the therapy.

The groups were homogeneous. They did not differ in terms of lipid parameters, BMI, liver enzymes, glycemia, hsCRP, activity of liver enzymes. There were no muscular complaints, or a significant increase in creatine kinase (CK) (Table 1).

Concentrations of TC-Total cholesterol, TG-triglycerides, LDL-low density lipoprotein cholesterol, HDL-high density lipoprotein cholesterol, hsCRP- high sensitivity c reactive protein and glucose were determined enzymatically with commercially available kits from Roche Diagnostics. The results were expressed in mg/dl.

Erythrocyte preparation. Venous blood was collected to test tubes containing ACD solution (23 mM citric acid, 45.1 mM sodium citrate and 45 mM glucose) as anticoagulant. The samples were centrifuged for 10 min at 4 °C (3000 rpm) to separate plasma and leukocytes. Isolated erythrocytes were washed three times with 0.9% NaCl and suspended in buffer to obtain 50% hematocrit value. Isolation of erythrocyte membranes. Erythrocyte membranes were isolated by hypotonic hemolysis, as described by Dodge et al. [11]. Erythrocyte suspension was lysed using 20-mM phosphate buffer with EDTA (ethylenediaminetetraacetic acid disodium salt) and PMSF (phenylmethylsulfonyl fluoride) in 1:5 molar ratio, pH = 7.4.

Table 1
Characteristics of the study groups.

Parameter	Rosuvastatin group (R20) N = 21 R20 mg/d	Atorvastatin-ezetimibe group (A10 + E10) N = 19 A10 mg + E10 mg	p
Age (year)	62.1 ± 6.6	63.8 ± 7.2	ns
Body mass (kg)	80.5	84.45	ns
BMI (kg/m^2)	27.6 ± 3.2	28.2 ± 4.1	ns
Women/Men	9/13	4/18	ns
TC (mg/dl)	181.3 ± 37.3	181 ± 36.1	ns
LDL-C (mg/dl)	113 ± 28.1	109.8 ± 30.4	ns
HDL (mg/dl)	55.7 ± 16.9	52.7 ± 9.5	ns
TG (mg/dl)	132.4 ± 56.6	131.9 ± 55.9	ns
Non-HDL (mg/dl)	125.6 ± 32.7	130.8 ± 36.8	ns
hsCRP (mg/l)	1.2 ± 0.9	1.8 ± 1.7	ns
Glucose (mg/dl)	96.7 ± 11.8	98.6 ± 12.8	ns
ALT (U/l)	26.8 ± 12.9	29.2 ± 10.9	ns
AST (U/l)	25.1 ± 6.8	22.8 ± 6.1	ns
CK (U/l)	155.3 ± 112.9	159.8 ± 98.2	ns

Values are means ± SD; TC – total cholesterol; LDL-C – low density lipoprotein cholesterol; HDL- high density lipoprotein cholesterol; TG – triglycerides; non-HDL – non-high density lipoprotein cholesterol; hsCRP – high-sensitivity C-reactive protein; ALT – alanine aminotransferase; AST –aspartate aminotransferase; CK – creatine kinase.

Then, the membranes were washed with 10-mM and 5-mM phosphate buffer. All the procedures were conducted at 40 °C.

Determination of lipid peroxidation markers. Peroxidation of lipids in erythrocyte membranes was estimated on the basis of the concentration of thiobarbituric acid reactive substances (TBARS) determined according to the method of Stocks and Dormandy [12]. Erythrocyte suspensions with 50% hematocrit value were incubated at 4 °C in presence of 20% TCA (trichloroacetic acid) for 1 h, and then centrifuged at 600 × g (3000 rpm) for 5 min. After adding 0.2 ml of TBA solution to 1 ml of supernatant, the mixture was heated at 100 °C for 15 min. Absorbance was measured at λ = 532 nm wavelength.

Determination of erythrocyte membrane cholesterol. Extraction of lipids was carried out with the method proposed by Rodriguez-Vico et al. [13], using low-toxicity solvents (ethanol and chloroform mixture). Concentration of cholesterol was estimated on the basis of Liebermann-Burchard reaction, with absorbance measured at λ = 660 nm wavelength. The results were expressed as mg of cholesterol per 1 ml of packed cells (mg ml PC-1).

Fluidity of erythrocyte membranes was determined with the fluorimetric method proposed by Shinitzky and Barenholz, on the basis of fluorescence anisotropy [14].

Hemoglobin concentration. Concentration of hemoglobin was determined with Drabkin's method [15], with absorbance measured at λ = 540 nm wavelength.

Concentration of protein in the isolated erythrocyte membranes was determined according to Lowry et al. [16], with bovine serum albumin as a standard.

Statistical analysis

Normal distribution of the study variables was verified with Shapiro-Wilk W-test. Most variables lacked normal distribution. Statistical significance of intragroup and intergroup differences in the values of such variables was tested with Wilcoxon signed-rank test and Mann-Whitney *U* test, respectively. Correlations between pairs of variables were determined with Spearman's R-test.

Statistical significance of intra- and intergroup differences in the values of remaining, normally- distributed variables was verified with appropriate Student *t*-tests.