THE DYNAMICS AND INTERACTION OF ENDOTHELIUM STATE AND IMMUNE STATUS VARIATIONS IN PROGRAM HEMODIALYSIS PATIENTS AGAINST THE BACKGROUND OF ATORVASTATIN THERAPY

UDC 615.03:615.2:616.61–078
Received 26.11.2012

© **A.L. Barsuk,** PhD, Associate Professor, the Department of General and Clinical Pharmacology;
A.M. Vozova, Postgraduate, the Department of General and Clinical Pharmacology;
E.V. Malinok, Postgraduate, the Department of General and Clinical Pharmacology;
L.V. Lovtsova, D.Med.Sc., Associate Professor, the Department of General and Clinical Pharmacology;
I.E. Okrut, PhD, Tutor, the Department of Clinical Laboratory Diagnostics, the Faculty of Doctors' Advanced the Training;
V.B. Kuzin, D.Med.Sc., Professor, Head of the Department of General and Clinical Pharmacology

Nizhny Novgorod State Medical Academy, Minin and Pozharsky Square, 10/1, Nizhny Novgorod, Russian Federation, 603005

The aim of the investigation was to study the interaction of endothelium state and immune status variations in program hemodialysis (PHD) patients against the background of atorvastatin therapy.

Materials and Methods. 54 patients included in the study were divided into two groups in a random manner. The main group patients (n=28) received atorvastatin, 20 mg a day, for 30 days against the background of PHD, the main patients (n=26) had PHD alone. Endothelin level (1–21) and Willebrand factor activity were determined by enzyme immunoassay, the content of active nitric oxide metabolites was estimated spectrophotometrically. The immune status indices (CD3, CD4, CD8, CD16, CD19, CD56) were studied by monoparametric analysis using monoclonal antibody technique. The content of interleukins (IL-6, IL-10, IL-1β) and tumor necrosis factor-α (TNF-α) were investigated using enzyme immunoassay.

Results. PHD patients within the observation period (30 days) were found to have the following changes: high concentration of nitric oxide and endothelin (1-21), reduced Willebrand factor activity; the decrease of relative number of T helpers and the increased number of cytotoxic T lymphocytes, as well as the increased IL-10 level and reduced TNF-α level in relation to the initial values. Atorvastatin administration for 30 days does not have any significant effect on endothelium condition, but reduces total lymphocyte count and immunoregulatory index, as well as proinflammatory cytokine IL-6 level in PHD patients.

The main patients in the observation period were found to have the interaction of the changed values of endothelium state (nitric oxide level) with the levels of IL-6 and IL-10, TNF-α, as well as Willebrand factor activity — with relative number of T helpers and immunoregulatory index.

The patients with atorvastatin therapy were recorded to have nothing but the interaction of the Willebrand factor activity changes with IL-6 content.

Conclusion. Due to the necessity of long-term administration of statin preparations in program hemodialysis patients, it is recommended to control the changes of endothelium state and immune status values in the course of the treatment.

Key words: program hemodialysis (PHD); endothelium state in PHD; immune status in PHD; atorvastatin.

To date, the efficacy and safety of applying statins in patients being on program hemodialysis (PHD) are not well understood, although the relevance of reducing cardiovascular mortality in these patients is enormous — more than half of them die from cardiovascular pathology [1].

One of the modern classes of drugs used in various cardio-vascular pathologies is statins. Numerous studies of statin therapy show high efficacy of this group of drugs for the prevention and treatment of cardiovascular disease.

The main mechanism of action of statin drugs has traditionally been associated with inhibition of the key enzyme in cholesterol synthesis of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase) [2]. However, this mechanism does not exhaust all the positive properties of this group of drugs. Pleiotropic effects of statins that are not related to their influence on the synthesis of cholesterol (endothelium protection, antioxidant and anti-inflammatory activity, the effect on hemostasis, etc.), in some cases, come to the fore as the assessment and prediction of the therapeutic effect of statins [3].

The mechanisms of anti-inflammatory action and the protection of endothelium with statins mediated through inhibition of HMG-CoA reductase, leading to the reduction

For contacts: Barsuk Alexandr Lvovich, phone: 8(831)436-54-01, +7 910-131-16-05; e-mail: farmnnov@mail.ru

of mevalonic acid — isoprenoid precursor (geranyl-geranyl pyrophosphate and farnesyl pyrophosphate), which determine the activation of Rho-protein initiating transcription of NF-κB. The latter takes part in the induction of specific proinflammatory genes and in the initiation of apoptosis mechanism. Rho-protein also inhibits the synthesis of nitric oxide and also it is required for adhesion of monocytes on the surface of endothelial cells [4]. Effect of statins on endothelial function in patients PHD is not practically investigated [5, 6].

In recent years, the role of inflammation (including its immune component) in the pathogenesis of cardiovascular diseases attracted the attention of many researchers. The laboratory signs of active inflammatory reaction are found to detect an untoward prognosis in healthy middle-aged and elderly people, as well as the presence of cardiovascular disease [7].

The effect of statins on the processes of inflammation and immune reactivity in PHD patients is of particular interest. Biocompatibility of dialysis systems, along with the loss of antioxidant substances, contributes to the activation of the peripheral blood mononuclear cells and the production of a number of inflammatory mediators such as cytokines by means of dialysis [8]. The high levels of interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) in dialysis patients are proved to be associated with increased risk of vascular events, mortality due to cardiovascular cause and total mortality. The most important high-risk marker is IL-6 [9].

Activation of mononuclear cells and neurotransmitter systems of immune reactivity in patients undergoing PHD, leads to significant changes in cellular component of immune system that may affect all populations of lymphocytes. However, information on the direction of these changes and inconsistent, is largely based on the experimental data, or a small number of research observations [10].

Thus, incomplete and contradictory data on the effect of different statins on endothelial and immune status of dialysis patients are the basis for research in this area.

The aim of the investigation was to study the interaction of endothelium state and immune status variations in program hemodialysis (PHD) patients against the background of atorvastatin therapy.

Materials and Methods. The studies were performed using a clinical basis of the branch "FESFARM NN" of LLC "FESFARM" and laboratory facilities of the Nizhny Novgorod Regional Clinical Diagnostic Center (Nizhny Novgorod, Russia). The study included 54 patients.

Inclusion criteria were the following: a terminal chronic renal failure, PHD is not less than 6 months, dyslipidemia, age 35–70 years, hemoglobin level ⩾100 g/L, the urea reduction rate (URR) ⩾65%, ⩾ dialysis dose 1.2.

Exclusion criteria were diabetes mellitus and rheumatoid arthritis.

Using the method of simple randomization, patients were divided into two groups: the patients in Group 1 (the main group, n=28) received atorvastatin — Atoris (KRKA, Slovenia), 20 mg once a day, for 30 days along with PHD. The patients in comparison group 2 (n=26) were only on PHD.

The level of endothelin (1–21) and von Willebrand factor activity was determined by the method of enzyme immunoassay using photometer tablet "Efos 9305" (Russia). Nitric oxide (NO) was evaluated by the total concentration of its stable metabolites (nitrates and nitrites), measurement was conducted using a spectrophotometer APEL PD 303 (Japan).

The evaluation of the immune status included certain subpopulations of T and B lymphocytes and NK cells by markers CD3, CD4, CD8, CD16, CD19, CD56 (using multiparameter analysis with cytometry Beckman Coulter (USA) using the method of the monoclonal antibodies). Furthermore, immunoregulatory index (IRI), the ratio of lymphocyte markers CD3+CD4 to CD3+CD8 were calculated.

Contents of interleukin-6, -10, -1 beta (IL-6, IL-10, IL-1β), tumor necrosis factor-α (TNF-α) was studied using enzyme immunoassay with ELISA photometer tablet "EFOS 9305" (Russia).

Biochemical parameters were studied before treatment (I examination) and 30 days (II examination). The object of the study was blood serum.

Statistical processing of the results was carried out using the Statistical Package License STADIA 7.0/prof. The level of significance of differences between the two samples was assessed using parametric and non-parametric criteria (for samples having a distribution, which does not differ from the normal one — by means of the Student and Fisher's t test, for samples having a distribution different from the normal one — by means of the Wilcoxon and Van der Waerden). The research results are also processed by the method of correlation analysis (a method of non-parametric correlation, criteria of Kendall and Spearman).

The results are presented in the form of M±m, where M — arithmetic average, m — standard error of the arithmetic average.

Results. After 30 days of therapy with atorvastatin an increase of the NO concentration from baseline to 80.92% ($p<0.001$) was recorded in the PHD patients, which at the same time did not differ from the changes in the comparison group (an increase in relation to the results of examination I by 71.10%, $p<0.05$) (Table 1).

Following the therapy with the study drug improvement of endothelium (1–21) as compared to baseline was also revealed: the main group — 2.18 times ($p<0.01$), in the control group — 2.24 times ($p<0.001$). Thus after 30 days the decrease in activity relative to the starting Willebrand factor resulting at 17% ($p<0.01$) was also observed. The patients of the comparison group also showed a reduction of that indicator relative to the initial value for 18% ($p<0.01$) (See Table 1).

Along with the applying of atorvastatin in patients with PHD relative number of B lymphocytes (CD19), and the number of NK cells (CD16+CD56), both in the main and in the control group, did not change in comparison with the results of the examination I (Table 2). The relative number of T lymphocytes (CD3) in the main group decreased to 7.95% ($p<0.05$), whereas in the comparison group it did not change from the baseline ($p<0.05$ between the

Table 1

Dynamics of endothelial status (M±m)

Parameter	Stage of research	Groups of examined patients	
		main	of comparison
Nitrous oxide content, μmol/L	I examination	6.92±0.53	6.92±0.53
	II examination	12.52±1.48 p<0.001	11.84±2.36 p<0.05
Endothelin level (1-21), fmol/ml	I examination	0.90±0.13	0.90±0.13
	II examination	1.96±0.46 p<0.01	2.02±0.36 p<0.001
Activity of von Willebrand factor, %	I examination	161.60±6.07	161.60±6.07
	II examination	134.10±4.66 p<0.01	132.50±3.97 p<0.01

Note: p — level of statistical significance of difference in comparison with the results of examination I.

Table 2

Dynamics of indicators of cellular immunity (M±m)

Parameter	Stage of research	Groups of examined patients	
		main	of comparison
T lymphocytes (CD3), %	I examination	76.75±1.32	76.75±1.32
	II examination	70.65±1.87 p_1<0.05; p_c<0.05	76.76±4.46
T helpers (CD3+CD4), %	I examination	51.19±1.55	51.19±1.55
	II examination	42.05±2.98 p_1<0.05	44.66±4.98 p_1<0.05
Cytotoxic T lymphocytes (CD3+CD8), %	I examination	23.27±1.50	23.27±1.50
	II examination	24.63±2.51 p_c<0.01	28.42±5.87 p_1<0.01
B lymphocytes (CD19), %	I examination	8.57±0.70	8.57±0.70
	II examination	9.70±1.98	6.78±1.18
NK cells (CD16+CD56), %	I examination	12.81±1.15	12.81±1.15
	II examination	13.60±2.69	15.26±4.16
IRI (T helpers/cytotoxic T lymphocytes)	I examination	2.36±0.18	2.36±0.18
	II examination	1.80±0.30 p_1<0.05; p_c<0.01	1.97±0.49

Note: p_1 — level of statistical significance of difference in comparison with the results of examination I; p_c — in comparison with the changes of similar indicators in the group of comparison.

index changes main group and the comparison one). The relative number of T helper cells (CD3+CD4) also decreased in the main group for 17.86% (p<0.05), in the comparison group — for 12.76% (p<0.05) in comparison with the data of examination I. In this case, the relative number of cytotoxic T lymphocytes (CD3+CD8) along with atorvastatin treatment did not change significantly, while the comparison group showed an increase in this indicator for 22.13% (p<0.01 compared to examination I and the main group). Moreover, along with taking of atorvastatin, a reduction of the IRI relative to the data of examination I for 23.73% (p<0.05) was shown, whereas in the control group the indicated score did not change (p<0.01 between the changes in IRI in the main group and the group of comparison) (See Table 2).

The dynamics of cytokines content in serum of the patients studied category is characterized by a reduction of IL-6 along with atorvastatin treatment 7.88% relative to the baseline (p<0.05) with insignificant increase of the indicator in the control group (Table 3). The level IL-10 increases almost to the same extent in the main group (9.96 times; p<0.001) and in the comparison group (at 10.68 times; p<0.001), while the content of TNF-α in serum on the contrary is reduced in both groups (for 77.03%; p<0.001 and 68.90%; p <0.001, respectively) compared with the results of study I. The level of IL-1β changes only slightly in both the main group and the comparison group (See Table 3).

The correlation analysis showed that there is a direct correlation between the changes of IL-6, and the activity of von Willebrand factor (correlation coefficient 0.82; p<0.05) in PHD patients during the atorvastatin therapy.

In the patients of the comparison group there was registered positive correlation between changes in levels of

Table 3

Dynamics of cytokine content (pg/ml; M±m)

Parameter	Stage of research	Groups of examined patients	
		main	of comparison
IL-6	I examination	4.06±0.66	4.06±0.66
	II examination	3.74±0.21; p<0.05	4.43±0.92
IL-10	I examination	0.28±0.13	0.28±0.13
	II examination	2.79±0.85; p<0.001	2.99±0.67; p<0.001
IL-1β	I examination	1.22±0.20	1.22±0.20
	II examination	1.30±0.34	1.38±0.14
TNF-α	I examination	5.53±0.65	5.53±0.65
	II examination	1.27±0.35; p<0.001	1.72±0.42; p<0.001

Note: p — level of statistical significance of difference in comparison with the results of examination I.

nitric oxide, on the one hand, and IL-6, and TNF-α, on the other (Spearman correlation coefficients 0.81; p<0.01 and 0.51 Kendall; p <0.05, respectively). In addition, the inverse correlation between the changes in the level of nitric oxide and IL-10 (Spearman correlation coefficient –0.60, p <0.05) and between the changes of Von Willelbrand factor activity, on the one hand and relative the number of T helper cells and IRI, on the other (Spearman correlation coefficients for 0.9, p <0.05) was revealed in the patients of the comparison group.

Discussion. Comprehensive analysis of the results indicates that patients undergoing PHD without the use of atorvastatin during the observation period (30 days), the following changes: increase of the content of nitric oxide and endothelin (1–21), decreased activity of von Willebrand factor, a reduction of the relative number of T helper cells and increase the number of cytotoxic T lymphocytes and increased levels of IL-10 and lower levels of TNF-α. The drug atorvastatin when administered for 30 days had no significant effect on the state of the endothelium of patients on PHD. This is consistent with the literature [11, 12] that demonstrates that hemodialysis prevents endothelium protective effect of statins due to low molecular weight uremic toxins poorly excreted by means of dialysis (end products of accelerated glycolysis, p-cresyl and indoxyl sulfate, asymmetric dimethylarginine, and some others). At that, the total number of lymphocytes and the immune regulatory index, and the level of the proinflammatory cytokine IL-6 decrease. It should be noted that the data on the effect of statins on the levels of IL-6 are contradictory [13], and the effect of statins on cellular component of immunity in patients on dialysis is insufficiently studied [14].

In dialysis patients without atorvastatin administration within 30 days of observation there was observed the interaction of changes of such endothelial indicators as nitric oxide level (the levels of IL-6 and IL-10, TNF-α, as well as von Willebrand factor activity (the relative amount of T helpers and IRI). In the course of therapy with atorvastatin in patients surveyed category recorded only changes the relationship with von Willebrand factor activity of IL-6. This indicates that atorvastatin restores the balance between the studied parameters of endothelial and immune status of PHD patients. A similar pattern of statin effects on the correlation between the indices of endothelial and immune status was observed in patients with coronary heart disease [15].

Conclusion. In program hemodialysis patients, in the selected observation period (30 days) there were indicated: the increase of the content of nitric oxide and endothelin (1-21), the decreased activity of von Willebrand factor; a reduction in the relative number of T helper cells and an increased number of cytotoxic T lymphocytes, as well as the increased levels of IL-10 and decreased levels of TNF-α. The state of the endothelium in patients on maintenance hemodialysis drug atorvastatin when administered for 30 days has no significant effect. In this case, the background of this drug reduced the total number of lymphocytes and immunoregulatory index and the level of pro-inflammatory cytokine IL-6, which, apparently, shows a tendency to decrease the severity of the inflammation process.

In dialysis patients within 30 days of observation there is a relationship of status indicators changes endothelium as nitric oxide (the levels of IL-6 and IL-10, TNF-α), as well as the von Willebrand factor activity (the relative amount of T helper cells and the immune index). In the course of therapy with atorvastatin in patients surveyed category recorded only changes the relationship with von Willebrand factor activity of IL-6.

With regard to necessity of long-term use of statins, dynamic monitoring of indicators of endothelial and immune status is recommended within the therapy.

References

1. Rtisheva O.V., Kalev O.A., Akhmatov V.Yu. Struktura prichin letal'nykh iskhodov u bol'nykh, nakhodyashchikhsya na programmnom gemodialize. Klinicheskaya nefrologiya [The structure of causes of lethal outcomes in program hemodialysis patients]. *Klinicheskaya nefrologiya — Clinical Nephrology* 2011; 1: 43–45.

2. Susekov A.V. Giperlipidemiya — sovremennoe sostoyanie problemy i metody ee medikamentoznoy korrektsii [Hyperlipidemia — present problem state and the methods of its medical correction]. *Rus Med Z — Russian Medical Journal* 2003; 5: 267–270.

3. Fesenko E.V., Proshchaev K.I., Polyakov V.I. Pleyotropnye effekty statinov i ikh rol' v preodolenii problemy polimorbidnosti [Pleiotropic action of statins and their role in overcoming the problem of multimorbidity]. *Covremennye problemy nauki i obrazovaniya — Present problems of Science and Education* 2012; 2: 1–8.

4. Parkhomenko A.N., Lutay Ya.M. Rannee primenenie simvastatina u bol'nykh s ostrym koronarnym sindromom: vliyanie na dinamiku markerov vospaleniya i rezul'taty klinicheskikh nablyudeniy [Early simvastatin administration in patients with acute coronary syndrome: the effect on inflammation markers dynamics and the results of clinical observations]. *Ukraínskiy kardiologichniy zhurnal — Ukrainian Cardiological Journal* 2005; 3: 36–45.

5. Han S.H., Kang E.W., Yoon S.J., et al. Combined vascular effects of HMG-CoA reductase inhibitor and angiotensin receptor blocker in non-diabetic patients undergoing peritoneal dialysis. *Nephrol Dial Transplant* 2011; 26(11): 3722–3728.

6. Kishimoto N., Hayashi T., Sakuma I., et al. A hydroxymethylglutaryl coenzyme A reductase inhibitor improves endothelial function within 7 days in patients with chronic hemodialysis. *Int J Cardiol* 2010; 145(1): 21–26.

7. Karpov Yu.A., Sorokin E.V. Ateroskleroz i faktory vospaleniya: nelipidnye mekhanizmy deystviya statinov [Atherosclerosis and inflammatory factors: non-lipid mechanisms of statins]. *Rus Med Z — Russian Medical Journal* 2001; 10: 418–421.

8. Pertosa G., Grandaliano G., Simone S. et al. Inflammation and carnitine in hemodialysis patients. *J Ren Nutr* 2005; 15(1): 8–12.

9. Goicoechea M., Quiroga B., García de Vinuesa S., et al. Intraindividual interleukin-6 variations on the cardiovascular prognosis of patients with chronic renal disease. *Ren Fail* 2012; 34(8): 1002–1009.

10. Kessler M., Kano B., Pedrini L.A., et al. *Evropeyskie rekomendatsii po optimal'noy praktike gemodializa*. Chast' 1 [European recommendations for optimal hemodialysis. Part 1]. Oxford (UK): Oxford University Press; 2005; 112 p.

11. Jourde-Chiche N., Dou L., Cerini C., et al. Vascular incompetence in dialysis patients-protein-bound uremic toxins and endothelial dysfunction. *Semin Dial* 2011; 24(3): 327–337.

12. Malyszko J., Malyszko J.S., Hryszko T., et al. Simvastatin and markers of endothelial function in patients undergoing continuous ambulatory peritoneal dialysis. *Int J Tissue React* 2002; 24(3): 111–115.

13. Korybalska K., Kawka E., Breborowicz A., Witowski J. Atorvastatin does not impair endothelial cell wound healing in an in vitro model of vascular injury. *J Physiol Pharmacol* 2012; 63(4): 389–395.

14. Meier P., Meier R., Blanc E. Influence of $CD4^+/CD25^+$ regulatory T cells on atherogenesis in patients with end-stage kidney disease. *Expert Rev Cardiovasc Ther* 2008; 6(7): 987–997.

15. Atamanova T.Yu. *Vliyanie dlitel'noy terapii statinami na immunnuyu sistemu bol'nykh ishemicheskoy bolezn'yu serdtsa*. Avtoref. dis. ... kand. med. nauk [The effect of a long-term statin therapy on immune system of ischemic patients: Abstract for Dissertation for the degree of Candidate of Medical Science]. Chelyabinsk; 2006.