wjpmr, 2022,8(2), 16 – 20.

SJIF Impact Factor: 5.922

WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

DEVELOP INTEGRATED DIAGNOSTIC BIOMARKERS OF ATHEROSCLEROSIS BASED ON EXPERIMENTAL STUDIES

Azizova D. M.*, Sabirova R. A., Islamova N.U., Rahmanova Z.T., Ahmatova K. and Alimuhamedova M.P.

Tashkent Medical Academy, Uzbekistan.

***Corresponding Author: Azizova D. M.**
Tashkent Medical Academy, Uzbekistan

Article Received on 05/12/2021 **Article Revised on 26/12/2021** **Article Accepted on 16/01/2022**

ABSTRACT

The study examined the effect of Biomaysa on the content of apoprotein in and total cholesterol, lipoprotein cholesterol and Lp FLA $_2$ in the dynamics of experimental hypercholesterolemia. Apoprotein B. of combined administration Biomaysa and ultroksa installed significant difference in the reduction of total cholesterol levels in ApoB 2.2 and 1.5, indicating good cholesterol reducing the effectiveness of the combination of these drugs by activating LDL receptor capture.

KEYWORDS: experimental hypercholesterolemia, blood, atherosclerosis, lipoproteins, cholesterol, apoprotein B, Lp FLA $_2$.

Hypercholesterolemia (CHS) plays an important role in the pathogenesis of atherosclerosis and coronary heart disease (CHD),[1] the incidence and mortality from which remain high in Russia and in the CIS countries.[2] Prescription of drugs that reduce cholesterol is a priority in the treatment of coronary artery disease and GHS.[3] Apo protein B (ApoB) is a major apolipoprotein from the group of atherogenic lipoproteins, including very lowdensity lipoproteins (VLDL), intermediate density lipoproteins (LDL) and low-density lipoproteins (LDL).[4] The concentration of ApoB significantly reflects the amount of these particles in the plasma. This is especially important in the case of a high concentration of fine, dense LDL particles in the blood. In several prospective studies, the level of ApoB was shown to be a prognostic indicator of a risk equivalent to X LDL level.[5] Studies of statins has shown that the level of ApoB was not defined as the main goal of treatment exposure, however, during the retrospective analysis it was revealed that the level of ApoB is not only a risk marker, but also the purpose of exposure during treatment, even better than cholesterol level LDL. The enzyme Lp-PLA2 is a subtype of the superfamily of phospholipases A2, which hydrolyze phospholipids of oxidized LDL.[6] Lp-PLA2 levels are associated with atherosclerosis, including coronary artery disease and MI.[7]

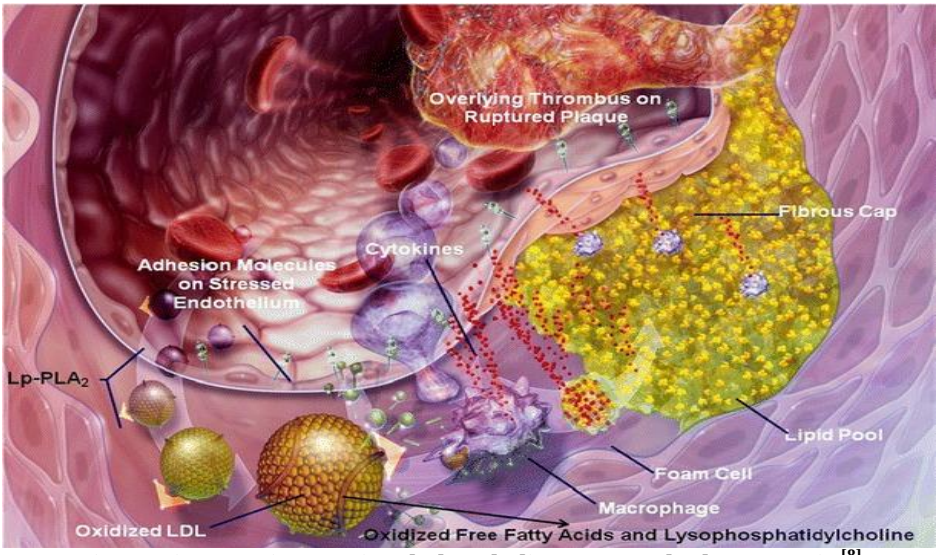

Fig. 1: Lipoprotein-associated phospholipase A2 and atherogenesis.[8]

A growing body of evidence suggests that Lp-PLA2 plays a critical role in the development of atherosclerosis. The main role of Lp-PLA2 in atherogenesis is the hydrolysis of oxidized LDL in the medium of the artery wall. The result is the pro-inflammatory, atherogenic byproducts of lysophosphatidylcholine and oxidized fatty acids. The former play by acting as a chemoattractant for monocytes, disrupting endothelial function, causing cell death, disrupting plasma membranes, and inducing apoptosis in smooth muscle cells and macrophages. The latter begin to influence the growth of AT. It is generally accepted that Lp-PLA2 can concentrate in unstable atherosclerotic plaques, especially plaques prone to rupture.[9]

The purpose of this study is develop integrated diagnostic biomarkers of atherosclerosis based on experimental studies

MATERIAL AND METHODS OF RESEARCH

The experiments were carried out on 30 male rabbits of the Shinshelle line weighing 2500–3000 g, divided (depending on the purpose of the study and method of treatment) into 5 groups (6 rabbits each): 1st (control) - intact rabbits; 2nd — animals with simulated experimental hypercholesterolemia; 3rd — correction of experimental hypercholesterolemia and mystatin; 4th correction of experimental hypercholesterolemia and bibiomix; 5th — Correction of experimental hypercholesterolemia mystatin and Biomaysa. A model of experimental hypercholesterolemia was reproduced by daily intragastric injection of cholesterol at 0.2g per kg body weight for 2 months.[10] Treatment of experimental animals was carried out after 2 months of cholesterol injection. The Ultrox was used as a statin (Nobel Farm), which was injected at 0.6 mg/kg for 30 days, also as a statin was used Biomaysa - by 142 mg/kg for 30 days. Biomaysa is a wheat sprouts and it was represented by OOO ORION-SKORPION. The studied drugs were injected intragastrically using an atraumatic probe daily in the morning and evening hours, an amount of injection was calculated on the basis of the body weight of the rabbit. The object of the study was blood serum. All studies were conducted in compliance with the principles set in the ―Convention on the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes‖ (Strasbourg, 1986).

To establish a comparative evaluation of plant-derived drugs Biomaysa and Statin Ultrox in the blood serum on an automated biochemical analyzer (RXDaytona /Randox, United Kingdom) of total cholesterol (total cholesterol), high-density lipoprotein cholesterol (LDL), very low density (VLDL) the atherogenic coefficient (CA) was calculated. ApoB was evaluated by the method of turbidimetry on a biochemical automatic analyzer. The level of lipoprotein-associated phospholipase A2 was determined by the enzyme immunoassay with RayBio® kits (RayBio® Rabbit Lp-PLA2 / PLA2G7 / PAF-AH ELISA Kit for Serum, Plasma, and Cell Culture Supernatants). Digital material was processed statistically on a personal computer using a software package for statistical analysis.

RESULTS AND DISCUSSION

To get more accurate characterization of the early stages of lipedema, besides determination the level of blood lipids, the levels of ApoB were determined as well. In the group 1, the ApoB blood concentration was 81±0.8 mg/l, which corresponded to the normal values given in the literature.[11] Therefore, all the results of studies conducted by 2-5 groups were compared with the data of group 1. In the group 2, the development of experimental atherosclerosis is indicated by a tendency for blood cholesterol increase in the blood (295 ± 1.45, P 0.05), a decrease in the HDL cholesterol atomic fraction (17.8±0.8, P<0.05), a significant increase in atherogenicity (15.6±0.43, P<0.05). In the group 2, hypercholesterolemia (GHS) was detected in the blood with a significant excess of cholesterol-LDL compared with group 1 6.6 times (P<0.05). ApoB levels in the second group averaged 179±1.59 mg/dl., I.e. 2.2 times exceeding the normal level (Fig.1). An increase in the ApoB fraction indicates a violation of ApoB-100 receptor endocytosis and uptake of VLDL cells,[12] VLDL, which is not absorbed by the cells, forms hypertriglyceridemia.

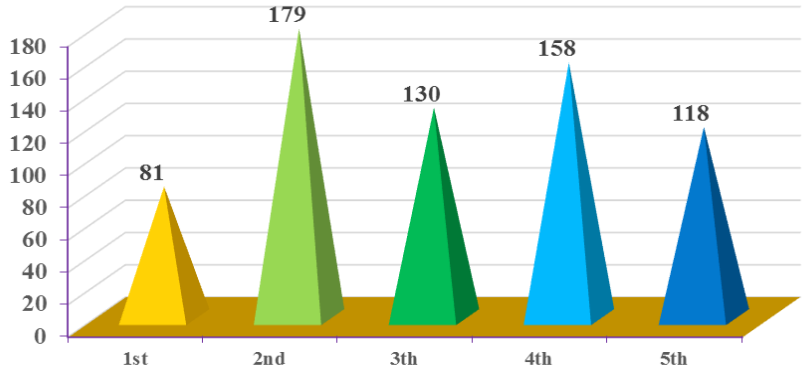

Fig.2. Influence of Biomays and Ultrax on the Apo B content (mg / dL) in experimental hypercholesterolemia (n = 6).

The research results has showed that in groups 3 and 4, the content of total cholesterol and the level of ApoB decreased by 2.1; 1.6 and 1.37; 1.13 times, respectively, compared with 2 group. The degree of reduction in total cholesterol and apo B were similar. In group 5, in comparison with the control, 30 days after the combined administration of drugs, there was a significant difference in the decrease of total cholesterol level by 2.2 ($p<0.05$) and Apo B by 1.5 times, which indicates effective reduction of cholesterol level due to the combination of drugs Ultrox with Biomaysa by activation of receptor uptake of LDL. The Ultrox and Biomaysa decreased the level of CA in this group by 4 and 2.5 times, respectively, compared with the untreated group. At the same time, the combined administration of used drugs led to a decrease in this ratio by 5.6 times compared with the untreated group. Thus, the strength of the effect of the combination of drugs Ultrox and Biomaysa exceeds together in comparison of using the drugs separately. It can be predicted that combination of these drugs increases the the capture receptor ApoB containing PL. It is known that statines normalize hyperlipidemia by: a) activating the uptake of VLDL by insulin-dependent cells and b) activating the uptake of LDL by all cells, increasing the bioavailability of PUFA, activating apoB- 100-endocytosis. Laboratory studies of Lp-PLA2 levels between groups showed no significant difference at baseline. Intergroup comparison at the end of the 2-month experiment showed that Lp-PLA2 levels significantly differed between groups ($P <0.05$), in intact and control 461.5 ± 30.5; 1928.9 ± 385.7) Several studies have shown that Lp-PLA2 is associated with plaque progression and vulnerability[13,14] ($P <0.05$). Meanwhile, in the control group, the LpPLA2 levels were significantly higher than in the intact one. In circulating blood, LpPLA2 binds to lipoproteins via apolipoprotein (Apo) B, thereby hydrolyzing oxidized phospholipids in Ox-LDL to produce lipid proinflammatory substances such as lysolecithin and oxidized free fatty acids.[15] Thus, the creation of a multicomponent biologically active substance with lipid-lowering properties, which act on the ApoB and TC, appears to be effective not only for the potential usage in the treatments of light forms of lipid metabolism disorders, but also in combination with statins in order to reduce the dose of the latter, and therefore their side effects. - these lipid pro-inflammatory substances can cause death of vascular endothelial cells and endothelial dysfunction, stimulate the production of adhesion factors and cytokines and cause atherosclerosis.[16]

Lipid metabolism disorders are associated with the development of atherosclerosis.[17] Studies explain the persistence of serum lipoproteins in the artery wall at AS.[18] The chronic proinflammatory cascade in the artery wall was initiated by the accumulation of oxidized lipoproteins.

The chronic inflammation seen in AS was caused by the recruitment of macrophages and the uptake of lipids into these cells.[19] Studies have shown that a decrease in TC and LDL-C in patients with coronary artery disease can significantly reduce mortality and recurrence rates of cardiovascular events.[20] In the present study, Biomaysa significantly reduced TG and LDL cholesterol levels in rabbits fed a high cholesterol diet.

The mechanism by which Biomaysa suppresses the formation developed atherosclerotic lesions, apparently due to inhibiting effect of polyconazole on LDL oxidation.[21] Polyconazole has been shown to protect lipids from peroxide decomposition and reduce the absorption of oxidized LDL by the vascular wall, depending on the concentration. We hypothesized that Biomaysa affects AS through the lipid levels of TG and LDL cholesterol. The enzymes AST and ALT are mainly concentrated in the liver and are involved in the conversion of sugars and proteins in vivo.[22] With fragmentation of the cell membrane and increased permeability caused by damage to liver cells, ALT and AST are activated and released into the bloodstream. When liver cells are damaged, cells undergo degeneration, necrosis, and cell membrane fragmentation or increased permeability.[23] At this time, the levels of ALT and AST contained in the liver cells enter the bloodstream, thereby increasing the ALT and AST activity in the blood. Currently, AST and ALT are commonly used to diagnose liver damage in the clinic.[24] In the present study, a decrease in AST levels was found when comparing between groups 3 and 4; however, this difference was not significant. Studies have shown that impaired lipid metabolism is a factor in the development of chronic liver disease and, as a consequence, liver damage can exacerbate impaired regulation of lipid metabolism.[25]

CONCLUSION

Thus, the present study suggests that Biomaysa may inhibit arterial intimal thickening. In addition, Biomaysa treatment lowers Lp-PLA2 levels in rabbits fed a high cholesterol diet, CVDs are one of the leading causes of morbidity and mortality in the world. The incidence of CVD can be greatly reduced or minimized if risk factors are identified at an early stage. One of the highly specific biomarkers is lipoprotein-associated phospholipase A2,

The increase in Lp-PLA2 levels is independent of traditional cardiovascular risk factors.

Lp-PLA2 is an enzyme produced by macrophages infiltrated in atherosclerotic plaques, therefore its determination is more specific compared to other inflammatory markers. Determination of Lp-PLA2 helps to reveal the latent risk of cardiovascular events that can be missed using standard risk factors (such as cholesterol, blood pressure, family history, tobacco smoking). The increase in Lp-PLA2 does not depend on traditional cardiovascular vascular risk factors. Lp-PLA2 is an enzyme produced by macrophages infiltrated in atherosclerotic plaques, therefore its determination is

more specific compared to other inflammatory markers. Lipid levels cannot provide enough information about the state of the artery wall, while Lp-PLA2 reflects this status independently of other cardiovascular markers. Lp-PLA2 is specific for vessels and is not produced in other inflammatory processes, in contrast to other inflammatory markers (for example, hs-CRP). Monitoring the decrease in Lp-PLA2 and LDL cholesterol in response to therapy is a better indicator of treatment efficacy compared to determining LDL cholesterol alone. Lp-PLA2 has minimal biological variability. Based on the results of determining Lp-PLA2, it is possible to determine the goal and further strategy of the patient's treatment.

REFERENCES

1. Anderson K., CastelliW., LevyD. // J.Am. Med. Assoc., 1987; 257: 2176-2180.
2. Oganov R.G., Maslennikova G.Ya. //Cardiovsk. ter. n rofil, 2002; 3: 4–8.
3. Diagnosis and correction of lipid metabolism disorders for the prevention and treatment of atherosclerosis. VNOK // Cardiovsk. ter. n rofil, 2007; 6(28).
4. Langsted A, Freiberg JJ, Nordestgaard BG. Fasting and non-fasting lipid levels: lipoproteins, apolipoproteins, and car- diovascular risk prediction. Circulation, 2008; 118: 2047–2056.
5. Taylor F, Ward K, Moore TH, Burke M, Davey Smith G, Casas JP, Ebrahim S. Statins for cardiovascular disease. Cochrane Database Syst Rev., 2011; 1: CD00481642.
6. B. S. Cummings, J. Mchowat, and R. G. Schnellmann, "Role of an endoplasmic reticulum Ca2+-independent phospholipase A2 in cisplatin-induced renal cell apoptosis," Journal of Pharmacology and Experimental 8erapeutics, 2004; 308(3): 921–928.
7. C. J. Kochansky and T. G. Strein, "Determination of uremic toxins in biofluids: creatinine, creatine, uric acid and xanthines," Journal of Chromatography B: Biomedical Sciences and Applications, 2000; 747(1-2): 217–227.
8. Kenneth J. Colley, Robert L. Wolfert, Michael E. Cobble Lipoprotein associated phospholipase A_2: role in atherosclerosis and utility as a biomarker for cardiovascular riskEPMA J., Mar, 2011; 2(1): 27–38. Published online 2011 Mar 10. doi: 10.1007/s13167-011-0063-4)
9. Davidson MH, Alberts MJ, Anderson JL, et al. Consensus panel recommendation for incorporating Lp-PLA2 testing into cardiovascular disease risk assessment guidelines. Am J Cardiol, 2008; 101: 51F–57F. doi: 10.1016/j.amjcard.2008.04.019
10. Anichkov N.N., S.S. Khalatov. New data on the question of the pathology and etiology of atherosclerosis (atherosclerosis). - Rus. Doctor, 1913; 8: 184-186.
11. Choi SH, Ginsberg HN. Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. Trends Endocrinol Metab., 2011; 22: 353–363.
12. Sanders FW, Griffin JL. De novo lipogenesis in the liver in health and disease: more than just a shunting yard for glucose. Biol Rev Camb Philos Soc., 2016; 91: 452–68.
13. Min HK, Kapoor A, Fuchs M, et al. Increased hepatic synthesis and dysregulation of cholesterol metabolism is associated with the severity of nonalcoholic fatty liver disease. Cell Metab., 2012; 15: 665–74.
14. Yang Y, Jiang Y, Wang Y, An W. Suppression of ABCA1 by unsaturated fatty acids leads to lipid accumulation in HepG2 cells. Biochimie, 2010; 92: 958–63.
15. Davidson MH, Alberts MJ, Anderson JL, et al. Consensus panel recommendation for incorporating Lp-PLA2 testing into cardiovascular disease risk assessment guidelines. Am J Cardiol., 2008; 101: 51F–57F. doi: 10.1016/j.amjcard.2008.04.019
16. Corson MA, Jones PH, Davidson MH. Review of the evidence for the clinical utility of lipoproteinassociated phospholipase A2 as a cardiovascular risk marker. Am J Cardiol, 2008; 101: 41F–50F. doi: 10.1016/j.amjcard.2008.04.018.
17. Hatoum IJ, Nelson JJ, Cook NR, Hu FB, Rimm EB. Dietary, lifestyle, and clinical predictors of lipoprotein-associated phospholipase A2 activity in individuals without coronary artery disease. Am J Clin Nutr., 2010; 91: 786–793. doi: 10.3945/ajcn.2009.28870
18. Reddy KJ, Singh M, Batsell RR, et al. Effects of lifestyle counseling and combination lipid-modifying therapy on lipoprotein-associated phospholipase A2 mass concentration. J Clin Lipidol, 2009; 3: 275–280. doi: 10.1016/j.jacl.2009.06.004.
19. Gaudet D, Brisson D, Tremblay K, et al. Targeting APOC3 in the familial chylomicronemia syndrome. N Engl J Med., 2014; 371: 2200–6.
20. Miksztowicz V, Lucero D, Zago V, Cacciagiu L, Lopez G, Gonzalez Ballerga E, Sorda J, Fassio E, Schreier L, Berg G. Hepatic lipase activity is increased in non-alcoholic fatty liver disease beyond insulin resistance. Diabetes Metab Res Rev., 2012; 28: 535–541.
21. K. Rosing, M. Fobker, F. Kannenberg et al., "Everolimus therapy is associated with reduced lipoprotein-associated phospholipase A2 (Lp-Pla2) activity and oxidative stress in heart transplant recipients," Atherosclerosis, 2013; 230(1): 164–170.
22. W. R. Kim, S. L. Flamm, A. M. D. Bisceglie, and H. C. Bodenheimer, "Serum activity of alanine Evidence-Based Complementary and Alternative Medicine 7 aminotransferase (ALT) as an indicator of health and disease," Hepatology, 2008; 47(4): 1363–1370.
23. M. S. Sabatine, D. A. Morrow, and M. O'Donoghue, "Prognostic utility of lipoprotein-associated phospholipase A2 for cardiovascular outcomes in

patients with stable coronary artery disease," Arteriosclerosis, 8rombosis, and Vascular Biology, 2007; 27(11): 2463–2469.
24. N. Nakaya, K. Mizuno, Y. Ohashi, T. Teramoto, and H. Nakamura, "Low-dose pravastatin and age-related differences in risk factors for cardiovascular disease in hypercholesterolaemic Japanese," Drugs & Aging, 2011; 28(9): 681–692.
25. N. Cheng, N. Ren, H. Gao, X. Lei, J. Zheng, and W. Cao, "Antioxidant and hepatoprotective effects of Schisandra chinensis pollen extract on CCl4-induced acute liver damage in mice," Food & Chemical Toxicology, 2013; 55: 234–240.