РОЛЬ МОРФОГЕННЫХ БЕЛКОВ WNT-СИГНАЛЬНОГО ПУТИ ПРИ ИШЕМИЧЕСКОЙ БОЛЕЗНИ СЕРДЦА

Снимщикова И.А., Плотникова М.О.

ФГБОУ ВО «Орловский государственный университет имени И.С. Тургенева», г. Орел, Россия

Резюме. Исследования последних лет доказывают сложность патофизиологических процессов, участвующих в развитии острых форм ишемической болезни сердца и патологического ремоделирования миокарда. В последние годы внимание исследователей направлено на изучение WNT-сигнального пути, регулирующего процессы эмбриогенеза и участвующего в развитии патологических состояний. При этом роль морфогенных белков WNT-сигнального пути в генезе кардиоваскулярной патологии практически не выяснена. Целью исследования явилось комплексное изучение основных белков WNT-сигнального пути (β-катенина, склеростина, GSK-3α, GSK-3β, WIF-1 и DVL-1) сыворотки крови 353 больных острыми формами ишемической болезни сердца, находившихся на лечении в региональном сосудистом центре Орловской области с 2019 по 2021 гг., и 50 здоровых лиц. Комплексный анализ включал оценку клинико-лабораторных и инструментальных показателей в рамках действующих клинических рекомендаций, а также иммунологическое обследование по определению морфогенных белков WNT-сигналинга методом иммуноферментного анализа. Результаты исследований показали широкую вариабельность значений морфогенных белков WNT-сигнального пути в сыворотке крови больных. При этом уровень β-катенина, WIF-1 и DVL-1 значительно превышал аналогичные показатели, полученные у здоровых лиц, а концентрации склеростина и GSK-3β не имели с ними достоверных отличий. Наряду с этим уровень GSK-3α в сыворотке крови пациентов был в 2 раза ниже, чем у здоровых лиц. Максимально высокие концентрации склеростина были выявлены у пациентов с имеющимся кальцинозом створок аортального клапана и стенок аорты. Неблагоприятное течение острого коронарного синдрома наблюдалось у пациентов на фоне как крайне высоких, так и максимально низких показателей WIF-1 сыворотки крови. Установлены значимые корреляционные зависимости между уровнем морфогенных белков WNT-сигнального пути и показателями липидного обмена, а также ремоделирования миокарда. Полученные данные об изменении продукции агонистов и антагонистов WNT-сигнального пути позволяют расширить представления о молекулярных аспектах иммунопатогенеза миокардиального ремоделирования при ишемической болезни сердца, повышают предиктивный потенциал диа-

Адрес для переписки:

Снимщикова Ирина Анатольевна
ФГБОУ ВО «Орловский государственный университет имени И.С. Тургенева»
302026, Россия, г. Орел, ул. Комсомольская, 95.
Тел.: 8 (910) 748-29-29.
E-mail: snimshikova@mail.ru

Address for correspondence:

Irina A. Snimshchikova
Federal State Budgetary Educational Institution of Higher Education "Orel State University named after I.S. Turgenev"
95 Komsomolskaya St
Orel
302026 Russian Federation
Phone: +7 (910) 748-29-29.
E-mail: snimshikova@mail.ru

Образец цитирования:

И.А. Снимщикова, М.О. Плотникова «Роль морфогенных белков WNT-сигнального пути при ишемической болезни сердца» // Медицинская иммунология, 2023. Т. 25, № 4. С. 985-990.
doi: 10.15789/1563-0625-ROM-2835

For citation:

I.A. Snimshchikova, M.O. Plotnikova "Role of morphogenic proteins of the WNT signaling pathway in coronary artery disease", Medical Immunology (Russia)/Meditsinskaya Immunologiya, 2023, Vol. 25, no. 4, pp. 985-990.
doi: 10.15789/1563-0625-ROM-2835

DOI: 10.15789/1563-0625-ROM-2835

гностики сердечно-сосудистых заболеваний и определяют вектор дальнейшего развития кардиоиммунологии.

Ключевые слова: WNT-сигнальный путь, β-катенин, склеростин, GSK3α, GSK3β, WIF-1, DVL-1, инфаркт миокарда, ишемическая болезнь сердца

ROLE OF MORPHOGENIC PROTEINS OF THE WNT SIGNALING PATHWAY IN CORONARY ARTERY DISEASE

Snimshchikova I.A., Plotnikova M.O.

Federal State Budgetary Educational Institution of Higher Education "Orel State University named after I.S. Turgenev", Orel, Russian Federation

Abstract. In recent years, researchers' attention has been directed to the WNT signaling pathway study, which regulates embryogenesis processes and is involved in pathological condition development. The role of morphogenic proteins of WNT signaling pathway in the cardiovascular pathology genesis is practically not clear. The research aim was a comprehensive study of the main proteins of WNT signaling pathway (β-catenin, sclerostin, GSK-3α, GSK-3β, WIF-1 and DVL-1) in the blood serum of 353 patients with coronary artery disease acute forms who were treated at the Orel regional vascular center from 2019 to 2021, and 50 healthy individuals. A comprehensive analysis included an assessment of clinical, laboratory and instrumental parameters in the framework of current clinical guidelines, as well as an immunological examination to determine the morphogenic proteins of WNT signaling by enzyme immunoassay. The results showed a wide variability in the values of morphogenic proteins of WNT signaling pathway in the patient's blood serum. The levels of β-catenin, WIF-1 and DVL-1 significantly exceeded those obtained in healthy individuals, while the concentrations of sclerostin and GSK-3β did not differ significantly from them. The level of GSK-3α of patients was twice lower than in healthy individuals. The highest sclerostin concentrations were found in patients with existing calcification of the aortic valve leaflets and aortic walls. Acute coronary syndrome unfavorable course was observed in patients with both extremely high and extremely low WIF-1 levels. Significant correlations were established between the level of morphogenic proteins of WNT signaling pathway and lipid metabolism, as well as myocardial remodeling. The obtained data on changes in the protein production of WNT signaling pathway allow us to expand our understanding of the molecular aspects of the immunopathogenesis of myocardial remodeling in coronary artery disease, increase the predictive potential for cardiovascular disease diagnosis and determine the vector for further development of cardioimmunology determination.

Keywords: WNT signaling pathway, β-catenin, sclerostin, GSK3α, GSK3β, WIF-1, DVL-1, myocardial infarction, coronary heart disease

Introduction

Cardiovascular diseases remain one of the most acute problems of modern medicine, due to their high prevalence and high mortality among young and middle-aged people. Recent studies prove the complexity of pathophysiological processes involved in the development of acute forms of coronary artery disease (CAD) and pathological myocardial remodeling, among the chief causes of which are immune dysfunction, activation of signaling pathways, oxidative stress, and mitochondrial disorders [2, 3, 4, 10].

At the same time, the molecular mechanisms associated with inflammation and reflecting various aspects of the pathological process remain the subject of discussion and need to be clarified. Over recent years, the attention of researchers has been focused on the study of the WNT signaling pathway, which regulates the processes of embryogenesis and is involved in the development of various pathological conditions. At the same time, the role of morphogenic proteins of

the WNT signaling pathway in the pathogenesis of cardiovascular pathology is practically not clear.

In this regard, **the aim of the study** was a comprehensive study of the main proteins of the WNT signaling pathway (β-catenin, sclerostin, GSK-3α, GSK-3β, WIF-1 and DVL-1) in the blood serum of patients with acute forms of coronary artery disease and healthy individuals (HI).

Materials and methods

The study included 353 young and middle-aged patients (from 18 to 59 years old) with acute forms of CAD who were treated in the cardiology departments of the regional vascular center of the Orel Regional Clinical Hospital in the period from 2019 to 2021. All patients with coronary artery disease were divided into 2 groups: group I consisted of patients with myocardial infarction (MI) (165 people), group II included patients with unstable angina (UA) (188 people). The average age of patients with CAD and myocardial infarction was 50.8±7.4 years and with unstable angina 53.2±5.7 years. There were no statistical differences in age ($p > 0.05$). In both groups, the number of men was predominant and amounted to 79.4% of all subjects. A comprehensive analysis included an assessment of the anamnesis and objective status of patients, a general clinical laboratory examination, echocardiography, within the current clinical guidelines for the management of an exacerbation of CAD, as well as an immunological examination to determine morphogenic WNT signaling proteins. Blood sampling for research from a peripheral vein was carried out in the first 24 hours after hospitalization. The concentration of morphogenic WNT proteins (β-catenin, sclerostin, WIF-1, GSK-3α and GSK-3β, DVL-1) in blood serum was determined by enzyme-linked immunosorbent assay (ELISA) on a STAT FAX 2100 photometer using reagent kits Sunlong Biotech Co (China) in the Laboratory of Clinical Immunology of the Medical Institute of the "Orel State University named after I.S. Turgenev".

To determine the values of the immunological parameters of the WNT signaling pathway, taken as the physiological norm, we conducted a survey of 50 healthy individuals who did not have CAD, were comparable in age and gender with patients in the research groups.

The research was performed in accordance with the standards of clinical practice (Good Clinical Practice) and the principles of the Declaration of Helsinki, the study protocol was approved by the Ethics Committee of the Orel State University.

Inclusion Criteria: young (18-44 years) and middle-aged (45-59 years) people with a clinically established diagnosis of acute forms of CAD, the patient's consent to participate in the research.

Exclusion Criteria: age younger than 18 years and older than 59 years, cardiogenic shock, hypertension above 2 stage, chronic heart failure above I stage, verified oncological, autoimmune, neuropsychiatric diseases, the presence of a pathology affecting lipid metabolism, decompensated diabetes mellitus, exacerbation of chronic diseases, pathologies of hemostasis, pregnancy and lactation, acute infectious diseases, refusal of the patient to participate in the research.

Results and discussion

It is known that the WNT signaling pathway is traditionally divided into two types: canonical and non-canonical. Although the modalities of WNT signaling in the embryonic stages of heart development are fairly well understood and experimental evidence identifies canonical WNT/β-catenin signaling as a "key factor" in the regulation of cardiac function and dysfunction, however, in a few different studies conflicting data have been obtained on the involvement of morphogenic proteins of the canonical and non-canonical WNT signaling pathway in the pathogenesis of cardiovascular diseases, including coronary heart disease [4].

There is growing evidence that reactivation of the canonical WNT pathway negatively affects myocardial healing after ischemic exposure, causing death of cardiomyocytes and the development of the heart muscle fibrosis [10].

In addition, the clinical significance of serum concentrations of morphogenic proteins of the WNT signaling pathway remains debatable and needs to be clarified in order to determine them as possible potential predictors in the pathology of the circulatory system and to develop new approaches to targeted therapy.

Taking this into account, it was of interest to study the features of the production of the main proteins of the WNT signaling pathway (β-catenin, sclerostin, GSK3α, GSK3β, WIF-1 and DVL-1) in acute forms of CAD, as well as to establish the relationship of the studied morphogenic proteins with clinical and laboratory data and indicators of the structural and functional state of the myocardium in patients with MI and UA.

According to current data, β-catenin is an integral structural component and the main effector

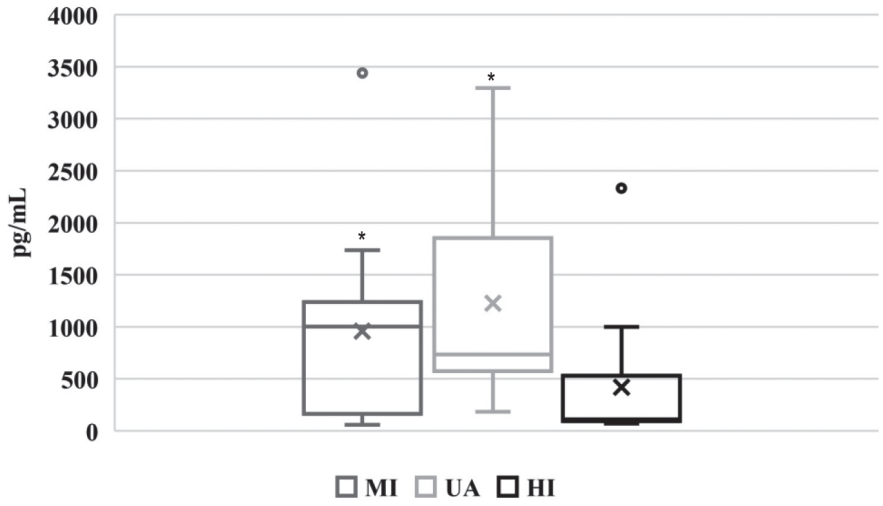

Figure 1. Level of DVL-1 in blood serum, pg/mL

Note. For distributions that differ from normal in Figures 1 Me (median) is given; 25-75 percentiles (upper and lower quartiles $Q_{0.25}$-$Q_{0.75}$); minimum and maximum sample values; outlier. *, $p < 0.05$, compared with HI.

of canonical WNT signaling involved in tissue homeostasis, myocardial remodeling, and control of the proliferative capacity of cardiomyocytes [10].

The results of our studies have shown a wide variability in the values of β-catenin in the blood serum of patients with coronary artery disease. At the same time, its level in patients with MI and unstable angina was 418 (293-579) pg/mL and 462 (384-588) pg/mL, respectively, which was several times higher than similar indicators obtained in HI 63.5 (57.25-86) pg/mL, $p < 0.001$. It is worth noting the revealed data on a significant increase in serum β-catenin in patients with coronary heart disease with concomitant hyperlipidemia ($p = 0.007$), which was also confirmed by the results of correlation analysis: a moderate direct correlation between the level of β-catenin and total cholesterol, low density lipoprotein (LDL) and a noticeable direct correlation with high-density lipoprotein, which is consistent with literature data indicating a correlation between impaired cholesterol metabolism and WNT/β-catenin signaling [7].

Given the important role of β-catenin in the processes of myocardial remodeling, its relationship with the parameters of intracardiac hemodynamics and the structural and functional state of the myocardium was evaluated. The most significant inverse correlations were found between the level of β-catenin and end-diastolic and systolic heart sizes ($p = 0.015$, $p = 0.018$, respectively).

The search for possible early markers of the development of cardiovascular complications led to the interest in the study of sclerostin, on the one hand, as the main inhibitor of the WNT signaling pathway, and on the other hand, as a potential participant in extraosseous calcification [5]. Taking this into account, we carried out the determination of the level of sclerostin in the blood serum of young and middle age patients with coronary artery disease and in HI.

According to the results of the research, the level of sclerostin in patients with CAD did not differ significantly from HI ($p > 0.05$). However, the analysis of the data obtained showed that the highest concentrations of sclerostin (above 215 pg/mL) were detected in patients with calcification of the aortic valve leaflets and aortic walls according to ECHO-CG data ($p = 0.002$; $p = 0.004$, respectively). It should also be noted that there was a direct statistically significant correlation between the level of sclerostin and the indicator of cardiovascular conjugation ($r = 0.7$; $p \leq 0.01$).

In recent years, data have appeared that the extracellular antagonist of the WNT signaling pathway WIF-1 is an important modulator of an adequate inflammatory process after myocardial injury, and the absence of this factor can lead to an increase in the inflammatory response and the development of pathological myocardial remodeling [8].

Analysis of the level of WIF-1 in the blood serum showed that in patients with acute forms of coronary artery disease, the concentration of WIF-1, on average, was 15.5 times higher than in healthy individuals ($p < 0.001$), and MI was characterized

by an even higher content of WIF-1 in blood serum. At the same time, in patients with a history of postinfarction cardiosclerosis, the ischemic process proceeded against the background of both extremely high (more than 3000 pg/mL) and extremely low (less than 1400 pg/mL) WIF-1 serum levels, which was combined with an unfavorable course of acute coronary syndrome.

Noteworthy are the results of a statistically significant high correlation between the concentration of WIF-1, the level of leukocytes and erythrocyte sedimentation rate ($r = -0.81$, $r = -0.70$, $p < 0.001$, respectively), as well as the content of β-catenin ($r = 0.743$; $p < 0.001$).

The study of DVL-1, which is involved in both canonical and non-canonical transmission of WNT signals, showed that in patients with acute forms of coronary artery disease, the level of DVL-1 significantly exceeded (8 times) the level of HI ($p = 0.009$), however, no statistically significant intergroup differences were found. In patients with transmural MI, maximum DVL-1 values were recorded at the level of 3400-3440 pg/mL (Figure 1). The analysis of the serum level of DVL-1 with clinical and laboratory data in patients with coronary artery disease made it possible to establish statistically significant direct correlations between DVL-1, total cholesterol ($p = 0.008$) and LDL ($p = 0.006$), as well as the presence of a direct relationship with levels of β-catenin ($p < 0.001$) and WIF-1 ($p < 0.001$), indicating the important role of DVL-1 as an integrator of canonical and non-canonical WNT signaling [11].

Recently it has been shown that glycogen synthase kinase-3 (GSK-3α and GSK-3β) plays an important role in the regulation of cell proliferation processes, including cardiomyocytes [1].

Considering the involvement of GSK-3α in the pathophysiology of cardiometabolic diseases [6, 9], in this study, we analyzed the obtained data on the content of GSK-3α in the blood serum of patients with acute forms of coronary artery disease, the level of which was 2 times lower than in healthy people (277 (238.25-875) pg/mL; $p = 0.021$); no statistically significant differences were found in patients of groups I and II. It should be noted that at normal levels of LDL and cholesterol, lower concentrations of GSK-3α were recorded, and patients with atherosclerosis had higher GSK-3α values, the highest level of GSK-3α was noted in patients with coronary artery disease and grade 3 obesity. The study of the content of GSK-3β in blood serum, which has an antifibrotic effect, did not reveal statistically significant differences in patients of the research groups and healthy individuals.

The absence of statistically significant differences in the concentrations of GSK-3α and β between the studied groups, along with the revealed changes in the level of other morphogenic proteins of the WNT signaling pathway, apparently, may be due to dysregulation of other signaling pathways in which GSK-3 plays a key role [9].

Conclusions

Thus, the obtained data on changes in the production of agonists and antagonists of the WNT signaling pathway (β-catenin, sclerostin GSK-3α, GSK-3β, WIF-1 and DVL-1) allow us to expand our understanding of the molecular aspects of the immunopathogenesis of myocardial remodeling in CAD, increase the predictive potential of diagnosing cardiovascular diseases and determine the vector of further development of cardioimmunology.

There is no conflict of interest.

References

1. Ahmad F., Woodgett J.R. Emerging roles of GSK-3α in pathophysiology: Emphasis on cardio-metabolic disorders. *Biochim. Biophys. Acta Mol. Cell Res., 2020, Vol. 1867, no. 2, 118616.* doi: 10.1016/j.bbamcr.2019.118616.

2. Bravo-San Pedro J.M., Kroemer G., Galluzzi L. Autophagy and mitophagy in cardiovascular disease. *Circ. Res., 2017, Vol. 120, no. 11, pp. 1812-1824.*

3. Förstermann U., Xia N., Li H. Roles of vascular oxidative stress and nitric oxide in the pathogenesis of atherosclerosis. *Circ. Res., 2017, Vol. 120, no. 4, pp. 713-735.*

4. Foulquier S., Daskalopoulos E.P., Lluri G., Hermans K.C.M., Deb A., Blankesteijn W.M. (2018). WNT signaling in cardiac and vascular disease. *Pharmacol. Rev., Vol. 70, no. 1, pp. 68-141.*

5. Hernandez P., Whitty C., John Wardale R., Henson F.M. New insights into the location and form of sclerostin. *Biochem. Biophys. Res. Commun., 2014, Vol. 446, no. 4, pp. 1108-1113.*

6. Kulakova A.S., Snimshchikova I.A., Plotnikova M.O. Role of GSK-3 in Wnt/β-catenin signaling pathway in obesity. *Medical Immunology (Russia), 2021, Vol. 23, no. 4, pp. 775-780.* doi: 10.15789/1563-0625-ROG-2287.

7. Mermelstein C.S., Portilho D.M., Mendes F.A., Costa M.L., Abreu J.G. Wnt/beta-catenin pathway activation and myogenic differentiation are induced by cholesterol depletion. *Differentiation, 2007, Vol. 75, no. 3, pp. 184-192.*

8. Meyer I.S., Jungmann A., Dieterich C., Zhang M., Lasitschka F., Werkmeister S., Haas J., Müller O.J., Boutros M., Nahrendorf M., Katus H.A., Hardt S.E., Leuschner F. The cardiac microenvironment uses non-canonical WNT signaling to activate monocytes after myocardial infarction. *EMBO Mol. Med., 2017, Vol. 9, no. 9, pp. 1279-1293.*

9. Roca C., Campillo N.E. Glycogen synthase kinase 3 (GSK-3) inhibitors: a patent update (2016-2019). *Expert Opin. Ther. Pat., 2020, Vol. 30, no. 11, pp. 863-872.*

10. Stylianidis V., Hermans K.C.M., Blankesteijn W.M. Wnt signaling in cardiac remodeling and heart failure. *Handb. Exp. Pharmacol., 2017, Vol. 243, pp. 371-393.*

11. Zhao H.D., Sun M.N., Li M.D., Li F.L., Li H. Dishevelled-1 (Dvl-1) protein: a potential participant of oxidative stress induced by selenium deficiency. *Biol. Trace Elem. Res., 2014, Vol. 157, no. 1, pp. 45-50.*

Авторы:

Снимщикова И.А. *– д.м.н., профессор, заведующая кафедрой иммунологии и специализированных клинических дисциплин, директор Медицинского института ФГБОУ ВО «Орловский государственный университет имени И.С. Тургенева», г. Орел, Россия*

Плотникова М.О. *– старший преподаватель кафедры иммунологии и специализированных клинических дисциплин Медицинского института ФГБОУ ВО «Орловский государственный университет имени И.С. Тургенева», г. Орел, Россия*

Authors:

Snimshchikova I.A., *PhD, MD (Medicine), Professor, Head, Department of Immunology and Clinical Specialties, Director of the Medical Institute, Federal State Budgetary Educational Institution of Higher Education "Orel State University named after I.S. Turgenev", Orel, Russian Federation*

Plotnikova M.O., *Senior Lecturer, Department of Immunology and Clinical Specialties of the Medical Institute, Federal State Budgetary Educational Institution of Higher Education "Orel State University named after I.S. Turgenev", Orel, Russian Federation*

Поступила 15.04.2023
Принята к печати 24.04.2023

Received 15.04.2023
Accepted 24.04.2023