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Trimetazidine as a Modifier of Chemotherapy-Induced Endothelium Homeostasis Disturbances

Conflict of interest: nothing to declare.
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Abstract

Introduction. Our research was determined to investigate the link between endothelial dysfunction, AC-mode of chemotherapy and trimetazidine-driven vasoprotection.
Purpose. To investigate the AC-mode of chemotherapy-induced endothelium dysfunction and rationality of TMZ prescription.
Materials and methods. The conducted study is characterized as a fundamental, randomized, controlled, experimental in vivo study. To carry out the experimental work, 80 inbred Wistar rats were involved, which were randomly divided into 4 equal groups. A model of endothelial dysfunction was generated using a direct combination of doxorubicin and cyclophosphamide. Trimetazidine was used as a probable stabilizer of endothelial activity. Course dose of doxorubicin – 15 mg/kg, course dose of cyclophosphamide – 150 mg/kg, course dose of trimetazidine – 42 mg/kg. The duration of the experiment was 14 days.
Results. Within the framework of this study, deviations of endothelin-1, nitric oxide, as well as adhesion factors VCAM and ICAM, and an inflammatory marker, C-reactive protein, were evaluated.
Conclusions: 1. AC-mode of chemotherapy provide pronounced endothelium discoordinating impact. 2. TMZ has sufficient vasoprotective properties.
Keywords: cardiotoxicity, doxorubicin, atherosclerosis, endothelium, myocardial metabolism

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Триметазидин как модификатор химиотерапией индуцированной дестабилизации эндотелиального гомеостаза

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Резюме

Введение. Настоящее исследование направлено на изучение взаимосвязи между эндотелиальной дисфункцией, кардиотоксичностью АС-режима химиотерапии и триметазидином опосредованной вазопротекции.
Цель. Изучить вазотоксический потенциал АС-режима химиотерапии наряду с одновременной оценкой вазопротекторного потенциала триметазидина.
Материалы и методы. Проведенное исследование характеризуется как фундаментальное, рандомизированное, контролируемое, экспериментальное, in vivo. С целью проведения экспериментальной работы было задействовано 80 инбредных крыс линии Wistar, которые были рандомно разделены на 4 равные группы. Модель эндотелиальной дисфункции была получена с использованием химиотерапевтического коктейля, состоящего из доксорубицина и циклофосфамида. В качестве вероятного стабилизатора функционирования эндотелия был выбран триметазидин. Курсовая дозировка доксорубицина составила 15 мг/кг, циклофосфамида – 150 мг/кг, а триметазидина – 42 мг/кг. Продолжительность эксперимента – 14 дней.
Результаты. В рамках настоящего исследования оценены девиации эндотелина-1, оксида азота, а также факторов адгезии VCAM и ICAM, маркер воспаления – С-реактивный белок. Полученные данные показали, что триметазидин модифицировал усиление эндотелиальной дисфункции.
Выводы: 1. АС-режим химиотерапии оказывает выраженное эндотелий-дискоординирующее воздействие. 2. Триметазидин обладает значимым вазопротекторным потенциалом.
Ключевые слова: кардиотоксичность, доксорубицин, атеросклероз, эндотелий, метаболизм миокарда

INTRODUCTION

To date, the fact of significant improvement of the prognosis for cancer patients along with the overall increase of their life expectancy is a real breakthrough in the modern medicine, while the hospitalization rate due to the cardiovascular events among the patients with both an active phase of the tumor and after achieving the stable long-term relapse stays high and keeps increasing [1]. It is noteworthy that the risk of CVD among the patients taking chemotherapy in a 5-year perspective up to 3.6-fold has increased the risk of cardiovascular-specific mortality and has increased 1.7- to 18.5-fold the incidence of CVD risk factors, such as hypertension, diabetes mellitus, and dyslipidemia compared with age-matched counterparts with no prior cancer history [2–4]. Per the latest data of new CARDIOTOX registry, a significant number of patients receiving high-risk cancer therapies has presented objective data of myocardial injury or LVD [5].

In the present research, the vasotoxic potential of the AC-mode of chemotherapy is investigated. Along with its high efficiency and wide application, the components of this mode, doxorubicin (DOX) and cyclophosphamide (CY), possess a considerable cardiotoxicity [6, 7]. The most challenging problem cardiologists and oncologists are currently encountering is the decision about the possibility or impossibility of commencing, continuing, or terminating chemotherapy in the case of CVD.

The problem of atherosclerosis development among AC mode of chemotherapy administered cancer patient with low CV risk was grab our attention. That's why we have started to research this issue. We have already published 3 clinical cases [8–10] were we comprehensively discus this actual problem. Moreover, our group also published two narrative review which related to this problem, the first one is about pathological pathways of doxorubicin related CV toxicity [11] and the second one about the modern outlook of atherosclerosis development and progression mechanisms [12]. Also, we have obtained data, which indicate that AC-mode of chemotherapy is a hyperhomocysteinemia development trigger, and moreover we have discovered promising pleotropic effects of TMZ - homocysteine stabilizing property [13].

The present study aimed to investigate the AC-mode of chemotherapy-induced endothelium integrity impairment as a one of risk factor for atherosclerosis formation. As a modifier of chemotherapy-induced CV complications, a well-known and affordable piperine-compound myocardial protector TMZ was chosen considering its antioxidant, cytoprotective, antihypoxic, and antianginal properties [14]. The pharmacological action of TMZ is associated with the mitochondrial 3-ketoacyl-CoA thiolase inhibition [15]. It should be noted that experimental animals were administered not a single chemotherapy drug but a full chemotherapy mode. The chosen drugs administration method makes the results more extrapolative and outmost comparable with real clinical practice.

MATERIALS AND METHODS

For this experimental study, 80 inbred Wistar rats (males, weighing 280–300 g) were probed. The animals were kept in a vivarium under the conventional conditions, specifically in plastic cages with wood chips, of 5 individuals in each, at a temperature of 22–24 °C, under the conditions of 12-hour daylight, with free access to food (standard diet) and water (ad libitum). The study with experimental animals was carried out under stringent

recommendations stipulated in the "Guide for the Care and Use of Laboratory Animals – NAP, 2012". All manipulations were carried out following the European Convention's provisions for the Protection of Vertebrates Used for Experimental and Other Scientific Purposes (Strasbourg, 1986; ed. Strasbourg, 2006) and legislation on the protection of animals used for scientific purposes (Directive 2010/63/EU).

At the onset of the study, the experimental animals were randomly divided into 4 equal groups by 20 rats in each:

1. Group No. 1 – rats were injected intraperitoneally with a normal saline in a single dose of 10 ml/kg, 3 times a week within 2 weeks.
2. Group No. 2 – administrated AC chemotherapy regimen, carried out by intraperitoneal administration of DOX-hydrochloride in a single dose of 2.5 mg/kg and CY-monohydrate in a single dose of 25 mg/kg, 3 times a week within 2 weeks.
3. Group No. 3 – administered AC-mode of chemotherapy in a similar manner (DOX + CY) with additional intragastric administration of crushed TMZ dihydrochloride in a single dose of 3.0 mg/kg [16]. The preparation was carried out daily, from the beginning to the end of the experimental work.
4. Group No. 4 – rats were similarly administered TMZ dihydrochloride at the previously noted dosage, as well as intraperitoneal normal saline per the same group No. 1 scheme, during 2 weeks.

The cumulative dosage of DOX was 15 mg/kg [17] and CY 150 mg/kg [18]. On a daily basis TMZ dihydrochloride was administered intragastricly through a probe in a complete dosage of 42 mg/kg. On the days coinciding with chemotherapeutic agents' injection, TMZ was administered one hour before the intraperitoneal chemotherapy injections.

In group No. 2 (DOX+CY), by the end of the 2^{d} week of the experiment, the death of 4 rats was recorded. No animal deaths were observed in other groups. The material was collected after the 2 day of final injection.

Biological material sample preparation

After the completion of the above described administration that lasted 14 days and 12 hours prior to the blood collection, the feeding of animals was stopped. Access to the drinking bottle was ad libitum. Before taking the biomaterial, the rats were put under deep anesthesia by means of telazol 20 mg/kg intramuscularly (IM) and xylanit 6 mg/kg IM. Thereafter, a thoracotomy was performed, followed by blood sampling from the right atrium with a disposable sterile 10 ml syringe (on average, 5–6 ml of blood was received from each rat). After that, using tweezers and scissors, the heart was isolated, followed by a cutting out the left ventricle (LV) and washing out the latter in 4 °C phosphate buffered saline (PBS).

To obtain plasma, 1 ml of blood (from each animal) was divided into two 500 µL tubes covered K3EDTA (1.6 mg per 1 ml of blood) with aprotinin (500 KIU per 1 ml of blood), followed by centrifugation at 1600 g, at a temperature of 0 °C for the overall duration of 15 minutes. From each whole blood sample (500 µL), an average of 200 µL of plasma was obtained.

To obtain serum, 4 ml of blood (from each animal) was placed in 4 ml vacuum tubes with a coagulation activator (SiO2) and gel, for 30 minutes and then it was centrifuged at 2000 g at a temperature of +4 °C for 10 minutes. From each whole blood sample (4 ml), an average of 2–2.3 ml of serum was obtained.

The obtained samples were aliquoted in the following way: plasma (two 1.0 ml cryotubes, per 200 μL plasma for each), serum (four 1.0 ml cryotubes, per 500 μL serum for each) and stored in a freezer at a temperature of –80 °C. The samples were not re-frozen. SpectraMax 250 microplate reader (Molecular Devices) was used for experimental purposes.

Endothelial dysfunction assessment

Determination of the concentration of endothelin-1 in blood plasma was carried out by the enzyme immunoassay (duplicate measurements, 100 μl plasma sample for each well), using the Endothelin-1 ELISA Kit test system (ab133030, Abcam, UK). The measuring range was 0.78–100 pg/ml. The lower limit of detection was 0.41 pg/ml.

Blood serum NO concentration was determined via the colorimetric method and the Nitric Oxide (total) test system detection kit (Enzo Life Sciences, ADI-917-020, USA) using nitrate reductase (duplicate measurements, 50 μl serum sample for each well). The measuring range was 3.125–100.0 μmol/L. The sensitivity was 0.625 μmol/L.

Quantitative determination of sICAM-1 in the blood plasma was carried out by enzyme immunoassay (duplicate measurements, 50 μl plasma sample for each well), using the Rat ICAM-1/CD54 Quantikine ELISA Kit (RIC100, R&D Systems, UK). The measuring range was 31.2–2000, pg/ml. The lower limit of detection made up 4.1 pg/ml.

Quantitative determination of sVCAM-1 in the blood serum was performed by a commercial enzyme immunoassay (duplicate measurements, 100 μl serum sample for each well) using the Rat sVCAM1 (Sandwich ELISA) ELISA Kit test system (LS-F38707, Lifespan Biosciences Inc., USA). The measuring range was 0.781–50 ng/ml. The sensitivity was 0.469 ng/ml.

Statistical analysis: all determined results are presented as mean values (M) ± standard deviation of the mean (SD). The analysis of the data obtained was carried out using ANOVA test followed by Tukey's post-hoc test using SPSS 22 (Statistical package for the Social Sciences Inc., USA 14.0). P-value <0.05 was considered at a significant level.

■ RESULTS

During pairwise comparisons between groups No. 1 (control) and No. 4 (TMZ), there were no statistically significant differences in the assessed markers level (post-hoc test Tukey, $p>0.05$). The obtained results indicate the normal state of endothelial function, both in control and the TMZ treated group (Table, Figure). On the background of the AC-mode of chemotherapy administration, statistically significant intergroup differences were revealed in the blood of rats in terms of such indicators reflecting the development of ED and systemic inflammation as ET-1, NO, sICAM-1, sVCAM-1 and CRP (one-way ANOVA, $p<0.0001$).

In group No. 2, the concentration of ET-1 is higher by 32.4 and 28.13%, NO is higher by 26.82 and 33.45%, sVCAM-1 is higher by 59.6 and 60.71%, sICAM-1 is more increased by 108.8 and 111.13%, CRP is more elevated by 69.34 and 69.62% than in groups No. 1 and No. 4.When comparing groups No. 3 and No. 2, it was noted that the use of TMZ is associated with a decrease in the concentration of ET-1 by 10.43%, NO by 23.17%, sVCAM-1 by 29.02%, sICAM-1 by 35, 45%, CRP by 97.62% by 53.5% (post-hoc Tukey test, $p<0.05$).

Endothelial dysfunction markers

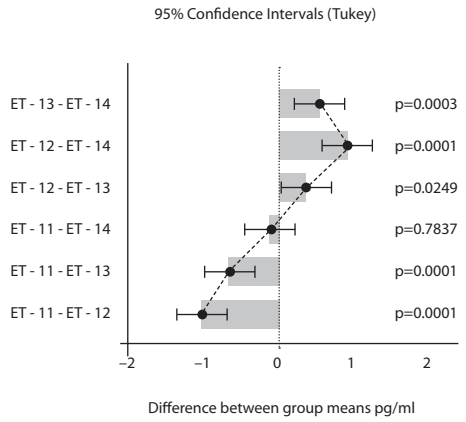
Marker	Group 1, NaCl	Group 2, AC-mode	Group 3, AC-mode + TMZ	Group 4, TMZ	Tukey's post-hoc Test
Endothelin-1/ET-1 F=28.92 P<0.001 (pg/ml)	2.69±0.44	3.73±0.51	3.36±0.42	2.81±0.15	P_{1-2}=0.0001 P_{1-3}=0.0001 P_{1-4}=0.7837 P_{2-3}=0.0249 P_{2-4}=0.0001 P_{3-4}=0.0003
Nitric-oxide/NO F=12.07 P<0.001 (mol/L)	23.31±5.10	30.53±6.70	24.19±4.12	21.78±3.22	P_{1-2}=0.0001 P_{1-3}=0.9431 P_{1-4}=0.7633 P_{2-3}=0.0007 P_{2-4}=0.0001 P_{3-4}=0.4203
sVCAM F=78.37 P<0.0001 (ng/ml)	0.79±0.12	1.46±0.17	1.09±0.19	0.78±0.16	P_{1-2}=0.0001 P_{1-3}=0.0001 P_{1-4}=0.9973 P_{2-3}=0.0001 P_{2-4}=0.0001 P_{3-4}=0.0001
sICAM F=211.90 P<0.0001 (ng/ml)	21.36±5.39	72.36±9.51	50.57±8.34	20.67±6.84	P_{1-2}=0.0001 P_{1-3}=0.0001 P_{1-4}=0.9919 P_{2-3}=0.0001 P_{2-4}=0.0001 P_{3-4}=0.0001
C-reactive protein/ CRP F=286.20 P<0.0001 (ng/ml)	374.51±64.34	772.03±79.80	265.59±37.13	373.35±44.93	P_{1-2}=0.0001 P_{1-3}=0.0001 P_{1-4}=0.9999 P_{2-3}=0.0001 P_{2-4}=0.0001 P_{3-4}=0.0001

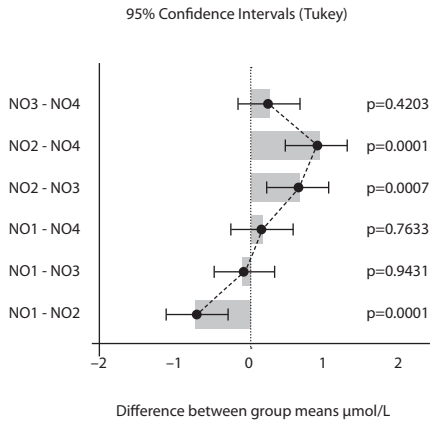
■ DISCUSSION

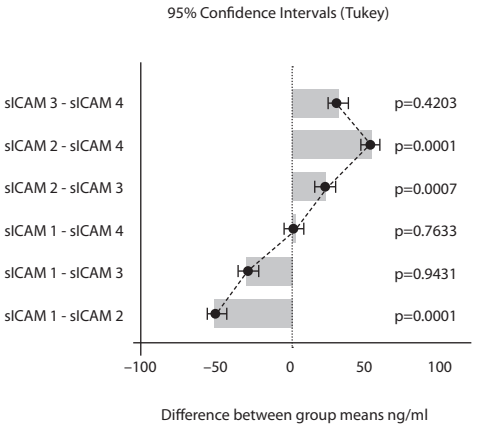
Considering the tremendous role of endothelium alteration and functional state of endotheliocytes in atherosclerosis, we decided to evaluate possible AC-mode of chemotherapy-induced endothelium dysfunction and research the possibility of TMZ-driven vasoprotection. It is known that the endothelium plays an important homeostatic role, supporting adequate vascular lumen and low thrombogenic potential [19]. However, under the flow of cardio- and vasotoxic metabolites of DOX (DOXol) [20] and CY (acrolein and 4-hydroxycyclophosphamide) [21], endothelial cells apparently transform from the mode of "protection" to the way of "emergency alert".

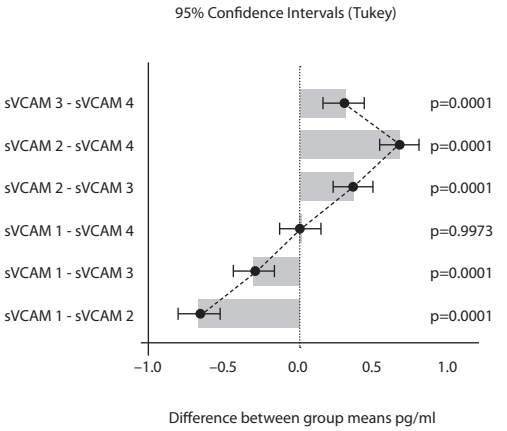
To assess the molecular targets of DOX and CY-induced ED, we investigated the main parameters of ED [24, 25], which are widely used in the clinic and available in many diagnostic laboratories of medical facilities. Also, there is a piece of evidence that TMZ modulates the human aortic vascular smooth muscle cell (HASMC) proliferation [26], and our group finds it an interesting additional pivotal point of TMZ-induced morpho-functional state of the endothelium normalization.

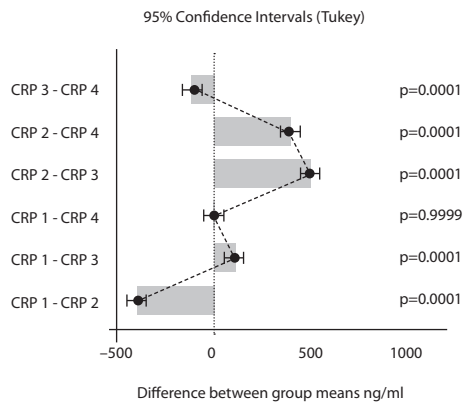
On the part of endotheliocytes, endothelin (proendothelin) is produced. Under the impact of the endothelin-converting enzyme, it is transformed into three isomers, the











Intergroup differences in the concentration of ET-1, NO, sVCAM, sICAM and CRP after 2 weeks from the start of AC-mode chemotherapy, U/mg (difference in mean, ±95% CI, one-way ANOVA, post-hoc Tukey test)

most active of which is endothelin-1 (ET-1) [27]. When endothelin-1 interacts with the receptors located on smooth myocytes, vasoconstriction, activation of mitogenesis, cellular proliferation, and intimal fibrosis occur with the vascular wall stiffness aggravation [28]. The effects of endothelin are also identified with a rearrangement of the secretory profile of nitric oxide [29].

Analyzing the abovementioned, it is worth noting that the fact of ET-1-associated vasoconstriction manifested by an increase in the concentration of this marker in the group No. 2 is very alarming since any vasoconstriction can cause an exacerbation of CHD and HF, as well as serve as the instigator of the initiation of the first attack of angina. Moreover, the TMZ-induced correlation of ET-1 concentration should be interpreted as an endotheliocyte-protective effect of this drug.

The ED development is also verified by an increase in the NO concentration. In the presence of an inflammatory process, an iNOS-synthase-mediated rise in NO concentration is observed, resulting in nitrosative stress induction [30]. Notably, the cardiac and vasotoxic effects of NO are manifested through the secondary messenger, c-GMP as follows: 1) by CV homeostasis destabilization and the HF development with either preserved or reduced LVEF, implemented by NO [31]; 2) by a decrease in glucose transport into cardiomyocytes, causing the impairment of intramyocardial metabolism [32]. Moreover, it was found that the generation of ROS (as noted above) and the NO dysbalance destabilize the functional constant of blood vessels, acting as a trigger for the vasomotor imbalance caused by the rearrangement of the secretory profile of endotheliocytes [33]. The latter often causes a decrease in coronary perfusion [34], which is also associated with a high level of reactogenic activity of NO with superoxide anion [35]. The myocardial perfusion and metabolism suppression should be considered as a direct indication for TMZ administration; it makes the use of previously noted cardioprotective agent pathogenetically aforethought. In this context, TMZ-mediated NO metabolism normalization seems encouraging.

Toxic effects of DOX and CY on the CV integrity lead to increased C-reactive protein concentration (CRP). The production of this acute-phase protein is induced by macrophage and T-lymphocyte-mediated IL-6 secretion [36]. Consequently, along with an increase in CRP concentration, there is also an increase in IL-6, a cytokine involved in the myocardial remodeling with a high risk of contributing to HF [37]. Thus, an increase in CRP indicates a systemic inflammatory process, a risk factor for CV and oncological disorders [38]. In the given case, the C-reactive protein concentration increase was anticipated, but the data obtained in group No. 3, were rather impressive. Indeed, TMZ-induced limitation of inflammatory processes has a beneficial effect; hence this significance is essential for preventing chemotherapy-induced CVD and the formation of secondary tumors. Thus, further research on the anti-inflammatory properties of TMZ is highly appropriate.

One of the earliest stages of atherosclerotic inflammation is the adhesion of monocytes to the activated endothelial cells due to the excessive expression of adhesion molecules on their surface [39]. We have analyzed changes in sICAM-1, a type 1 intercellular adhesion molecule, and sVCAM-1, a type 1 vascular endothelial adhesion molecule [40]. Both molecules are not expressed under physiological conditions; therefore, an increase in the latter's concentration of soluble forms indicates ED development. TMZ associated lowering of the mentioned biomarkers concentration could assume that TMZ slows down the course of proatherogenic reactions, showing an atheroma-stabilizing effect, which may be associated with a specific normalization of the redox system.

■ CONCLUSION

The AC-mode of chemotherapy is an inducer for endothelium dysfunction development, and TMZ could play a required role in the limitation of this process. However, despite the obtained statistically proven and significant results, future studies remain unavoidably necessary to strengthen evidence of TMZ administration to AC-mode of chemotherapy cancer patient.

■ REFERENCES

1. Avagimyan A, Mrochek A, Sarrafzadegan N, et al. Mitochondrial dysfunction associated with AC mode of chemotherapy intake. European Heart Journal.2020;41(2): ehaa946.3293 10.1093/ehjci/ehaa946.3293.
2. Pan J, Garza F, Lyon AR. Cardio-oncology: rationale, aims and future directions. Curr Opin Support Palliat Care.2021;15(2):134–140. doi: 10.1097/SPC.0000000000000544.
3. Al-Malky HS, Al Harthi SE, Osman AM. Major obstacles to doxorubicin therapy: Cardiotoxicity and drug resistance. J Oncol Pharm Pract.2020;26(2):434–444. doi: 10.1177/1078155219877931.
4. Gilchrist S, Barac A, Ades P, et al. Cardio-Oncology Rehabilitation to Manage: Cardiovascular Outcomes in Cancer Patients and Survivors. A Scientific Statement From the American Heart Association. Circulation.2019;139:997–1012. doi: 10.1161/CIR.0000000000000679
5. López-Sendón J, Álvarez-Ortega C, Zamora Auñon P, et al. Classification, prevalence, and outcomes of anticancer therapy-induced cardiotoxicity: the CARDIOTOX registry. Eur Heart J.2020;41(18):1720–1729. doi: 10.1093/eurheartj/ehaa006.
6. Von Hoff DD, Layard MW, Basa P, et al. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med.1979;91(5):710–717.
7. Prasanna PL, Renu K, Valsala Gopalakrishnan A. New molecular and biochemical insights of doxorubicin-induced hepatotoxicity. Life Sci.2020;250:117599. doi: 10.1016/j.lfs.2020.117599.
8. Avagimyan A, Mkrtchyan L, Abrahomovich O, et al. AC-mode of chemotherapy as a trigger of cardiac syndrome X: a case study. Curr Probl Cardiol.2021:100994. doi: 10.1016/j.cpcardiol.2021.100994.
9. Avagimyan A, Mkrtchyan L, Gevorkyan A, et al. Relationship between Chemotherapy and Atrial Fibrillation: Clinical Case. Rational Pharmacotherapy in Cardiology.2021;17(5):785–791. doi: 10.20996/1819-6446-2021-10-17.
10. Avagimyan A, Mkrtchyan L. Cardiotoxicity of Chemotherapeutic Drugs (Literature Review and Clinical Case Description). Lviv Clinical Bulletin. 2019;28(4):56–62. doi:10.25040/lkv2019.04.051.
11. Avagimyan A, Kakturskiy L, Heshmat-Ghahdarijani K, et al. Anthracycline Associated Disturbances of Cardiovascular Homeostasis. Curr Probl Cardiol. 2021:45(5):100909. doi: 10.1016/j.cpcardiol.2021.100909.
12. Avagimyan A, Ananyan E, Djndoyan Z, Navasardyan G. Modern Vision about the Mechanism of Atherosclerosis Development. The Medical Science of Armenia NAS RA.2019;59(1):3–21.
13. Avagimyan A. Hyperhomocysteinemia as a Link of Chemotherapy-Related Endothelium Impairment. Curr Probl Cardiol.2021:100932. doi: 10.1016/j.cpcardiol.2021.100932
14. Shu H, Peng Y, Hang W, Zhou N, Wang DW. Trimetazidine in Heart Failure. Front Pharmacol.2021;11:569132. doi: 10.3389/fphar.2020.569132.
15. Avagimyan A, Kononchuk N, Mkrtchyan L, et al. Chemotherapy as a possible trigger for the myocardial lipomatosis development. Arterial'naya Gipertenziya (Arterial Hypertension).2021;27(6):706–712. doi:10.18705/1607- 419X-2021-27-6-706-712
16. Albengre Ed, Tillement J, Le Louet H, Morin D. Trimetazidine: Experimental and Clinical. Update Review. Curdioiwscular Drug Rt.1998;16(4):359–390.
17. Sheibani M, Nezamoleslami S, Faghir-Ghanesefat H, et al. Cardioprotective effects of dapsone against doxorubicin-induced cardiotoxicity in rats. Cancer Chemother Pharmacol.2020;85(3):563–571. doi: 10.1007/s00280-019-04019-6.
18. Omole JG, Ayoka OA, Alabi QK, et al. Protective Effect of Kolaviron on Cyclophosphamide-Induced Cardiac Toxicity in Rats. J Evid Based Integr Med.2018; 23:2156587218757649. doi: 10.1177/2156587218757649.
19. Zhong S, Li L, Shen X, et al. An update on lipid oxidation and inflammation in cardiovascular diseases. Free Radic Biol Med.2019;144:266–278. doi: 10.1016/j.freeradbiomed.2019.03.036.
20. Prathumsap N, Shinlapawittayatorn K, Chattipakorn SC, Chattipakorn N. Effects of doxorubicin on the heart: From molecular mechanisms to intervention strategies. Eur J Pharmacol.2020; 866:172818. doi: 10.1016/j.ejphar.2019.172818.
21. Kurauchi K, Nishikawa T, Miyahara E, et al. Role of metabolites of cyclophosphamide in cardiotoxicity. BMC Res Notes.2017;10(1):406. doi: 10.1186/s13104-017-2726-2.
22. Tanaka K, Sata M. Roles of Perivascular Adipose Tissue in the Pathogenesis of Atherosclerosis. Front Physiol.2018;9:3. doi:10.3389/fphys.2018.00003.
23. FernándezAlfonso MS, Somoza B, Tsvetkov D, et al. Role of Perivascular Adipose Tissue in Health and Disease. Compr Physiol.2017;8(1):2359. doi:10.1002/cphy.c170004.
24. Vorobyeva E, Shumakher G, Khoreva M, Osipova I. Endothelial Dysfunction – A Key Factor In Atherosclerosis Pathogenesis. Russian Journal of Cardiology. 2010;(2):84–91. doi: 10.15829/1560-4071-2010-2-84-91.
25. Khripun I, Morgunov M, Vorobyov S, et al. Endothelial dysfuncton and 2 type diabetes: novel markers for earlier diagnostics. Cardiovascular Therapy and Prevention.2016;15(5):59–63. doi: 10.15829/1728-8800-2016-5-59-63.
26. Barton M, Yanagisawa M. Endothelin: 30 Years From Discovery to Therapy. Hypertension.2019;74(6):1232–1265. doi: 10.1161/HYPERTENSIONAHA.119.12105.
27. Titus A, Marappa-Ganeshan R. Physiology, Endothelin. 2020. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2020
28. Jankowich M, Choudhary G. Endothelin-1 levels and cardiovascular events. Trends Cardiovasc Med. 2020;30(1):1–8. doi: 10.1016/j.tcm.2019.01.007.
29. Bonifácio KL, Barbosa DS, Moreira EG, et al. Increased nitro-oxidative stress toxicity as a major determinant of increased blood pressure in mood disorders. J Affect Disord.2020;278:226–238. doi: 10.1016/j.jad.2020.09.040.

30. Zuchi C, Tritto I, Carluccio E, et al. Role of endothelial dysfunction in heart failure. Heart Fail Rev. 2020;25(1):21–30. doi: 10.1007/s10741-019-09881-3.
31. Meza CA, La Favor JD, Kim DH, Hickner RC. Endothelial Dysfunction: Is There a Hyperglycemia-Induced Imbalance of NOX and NOS? Int J Mol Sci.2019;20(15):3775. doi: 10.3390/ijms20153775.
32. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. Physiol Rev.2005;85(3):1093–129. doi: 10.1152/physrev.00006.2004.
33. Leite AR, Borges-Canha M, Cardoso R, et al. Novel Biomarkers for Evaluation of Endothelial Dysfunction. Angiology.2020;71(5):397–410. doi: 10.1177/0003319720903586.
34. Lakhani I, Wong M, Hung J, et al. Diagnostic and prognostic value of serum C-reactive protein in heart failure with preserved ejection fraction: a systematic review and meta-analysis. Heart Fail Rev.2020.doi: 10.1007/s10741-020-09927-x.
35. Hilfiker-Kleiner D, Landmesser U, Drexler H. Molecular Mechanisms in Heart Failure: Focus on Cardiac Hypertrophy, Inflammation, Angiogenesis, and Apoptosis. JAAC 2006;48(9) suppl.7:56–66. doi: 10.1016/j.jacc.2006.07.007.
36. Lino DOC, Freitas IA, Meneses GC, et al. Interleukin-6 and adhesion molecules VCAM-1 and ICAM-1 as biomarkers of post-acute myocardial infarction heart failure. Braz J Med Biol Res.2019;52(12):e8658. doi: 10.1590/1414-431X20198658.
37. Chaikijurajai T, Tang WHW. Reappraisal of Inflammatory Biomarkers in Heart Failure. Curr Heart Fail Rep.2020;17(1):9–19. doi: 10.1007/s11897-019-00450-1.
38. Lüscher TF. Cardio-oncology: low-grade inflammation as a common pathway of cancer and cardiovascular disease. European Heart Journal.2019;40(48):3871–3874 doi:10.1093/eurheartj/ehz928.
39. Asgary S, Karimi R, Joshi T, et al. Effect of pomegranate juice on vascular adhesion factors: A systematic review and meta-analysis. Phytomedicine.2021;80:153359. doi: 10.1016/j.phymed.2020.153359.
40. Avagimyan A., Kakturskiy L. Cardiotoxicity of cyclophosphamide: current state of the problem. Experimental & clinical medicine Georgia. 2022;3:84-88. https://doi.org/10.52340/jecm.2022.03.14