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Influence of Dinitrosyl Iron Complexes on the Activity of Enzymes, Indicators of Cardiovascular Diseases

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Abstract: Increased enzyme activities of matrix metalloproteinase, myeloperoxidase, renin, histone deacetylase, and poly (ADP-ribose) polymerase are indicators of the development of cardiovascular diseases. Therefore, the search for new inhibitors of these enzymes is relevant. The effect of iron dinitrosyl complexes and nitric oxide donors on these enzymes' activity was studied. It has been shown by fluorescence spectroscopy that DNICs (at concentration $2x10^{-4}$M) inhibit the activity of these enzymes. The constants of half-maximal inhibition of enzymes under the action of DNICs were determined (10^{-4}-10^{-5}M). The results showed that DNICs are effective inhibitors of matrix metalloproteinase, myeloperoxidase, renin, and polyadenine-ribose polymerase but do not affect histone deacetylase activity. These DNICs have been shown to have therapeutic potential for treating cardiovascular diseases.

Keywords: dinitrosyl iron complexes; nitric oxide; enzymes; cardiovascular diseases.

1. Introduction

Cardiovascular diseases (CVD) have been the leading cause of death worldwide for over 20 years. However, they have never claimed so many lives as they do today. Since 2000, the number of deaths from cardiovascular diseases has increased by more than 2 million, and in 2019 reached almost 9 million. Heart diseases account for 16% of worldwide deaths today [1]. Therefore, the search for new cardioprotectors, and the study of their mechanism of action, is highly relevant.

Nitric oxide (NO) is known to be involved in various physiological and pathophysiological processes in mammalian organisms [2-4]. Therefore, the obtained data on the diverse biological activities of this mediator radical and its reactions with biological substrates in cells are used in the development of effective drugs - NO donors (NO-therapy) or inhibitors of NO synthase activity (anti-NO-therapy) [5-8].

In recent years, there has been an exponential growth of interest in studying nitrosyl complexes of transition metals, particularly iron complexes [9-12]. Previously, synthetic

approaches were developed to create structural analogs of active sites of nitrosyl non-heme [2Fe-2S] and [1Fe-2S] proteins, which are hybrid molecules containing two pharmacologically significant fragments simultaneously: sulfur-containing ligands and NO groups– the dinitrosyl iron complexes (DNICs) [13]. The molecular crystalline structures of DNICs were established by X-ray diffraction analysis, and the properties of these new NO donors were studied by NGR and IR spectroscopy [14,15]. However, there are no data in the literature on the molecular targets of DNICs action.

Many enzymes are now known to be biomarkers and mediators for the development of cardiovascular disease. Several studies have shown that myeloperoxidase (MPO), a heme-containing protein with peroxidase activity, is an exceptional indicator of the development of cardiovascular diseases. An elevated level of myeloperoxidase in the body indicates the risk of developing atherosclerosis and indicates the progression of this disease [16–18]. Poly (ADP-ribose) polymerase (PARP) is a biomarker for the development of coronary heart disease [19–21]. Increased levels of matrix metalloproteinases (MMPs) correlate with many pathological processes and diseases, including arthrosis, heart attack, fibrosis, arthritis, glaucoma, cirrhosis, multiple sclerosis, aortic aneurysm, and many others [22-26]. Renin is a biomarker for the development of hypertension [27–30]. Histone deacetylase (HDAC) is involved in multiple processes associated with cardiovascular and metabolic diseases, including cardiac hypertrophy and remodeling, fibrosis, calcium processing, inflammation, and energy metabolism [31,32]. Therefore, the search for effective inhibitors or activators of these enzymes is an urgent task. The results of this work can become the basis for the creation of a new generation of drugs for NO-therapy of cardiovascular diseases.

The purpose of the study was to study the effect of DNICs on the activity of enzymes that are biomarkers/mediators for the development of cardiovascular diseases: matrix metalloproteinase, myeloperoxidase, renin, histone deacetylase, and poly(ADP-ribose) polymerase.

2. Materials and Methods

2.1. Materials.

For cell culture, we used Dulbecco's Modified Eagle Medium (DMEM) with low content of glucose (1 g/L), L-glutamine, and 25 mM HEPES (Biowest, France), or DMEM with high content of glucose (4.5 g/l) (PanEko (Russia). Fetal bovine serum (FBS; ultra-low endotoxin content), as well as 0.25% trypsin solution, 0.02% EDTA solution in HBSS, and gentamicin (10 mg/mL) were purchased from Biowest (France). Various plastic labware (culture flasks, Petri dishes, tubes, disposable pipettes) for cell culture were purchased from Corning-Costar (USA). Recombinant human PARP-1 was kindly provided by Prof. Olga I. Lavrik (Institute of Chemical Biology and Fundamental Medicine of the Siberian Branch of the RAS, Novosibirsk).

2.2. Cell lines.

The human lung embryonic fibroblasts (cell line HLEF-104) and African green monkey renal epithelial cells (line Vero) were purchased from the Russian Collection of Vertebrate Cell Cultures (BioloT, St. Petersburg, Russia). Rat cardiomyoblast culture (cell line H9c2) was

purchased from the ATCC cell culture collection (American Type Culture Collection; LGC Standards, UK).

2.3. Cell culture.

The HLEF-104 cells and Vero cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS, vol/vol), glutamine (0.15%), HEPES (10 mM, pH 7.2) and gentamicin (50 mg/ml). The rat H9c2 cardiomyoblasts were cultured in DMEM medium containing 10% fetal calf serum, L-glutamine (2 mM), and antibiotics (50 U/ml penicillin and 50 μg/ml streptomycin).

All cell types were cultured in plastic flasks (Corning Incorporated, Corning, NY, USA) in an incubator at 37°C, 5% CO_2, and 90% humidity. Cells were grown to a density of 90%, and then they were detached from the surface using 0.25% trypsin. The cell suspension was centrifuged at 3000 g for 5 min.

The supernatant was removed, and the cell sediment was used to obtain cell lysates.

In all experiments, we utilized cultures of HLEF-104, H9c2, and Vero cells that underwent not more than 20 and 25 passages, respectively.

2.4. Preparation of cell lysates.

Human lung embryonic fibroblasts (cell line HLEF-104), rat cardiomyoblasts (cell line H9c2), and African green monkey renal epithelial cells (cell line Vero) were cultured in DMEM medium with 10% FBS at 37°C and 5% CO_2. Cells were grown to a density of 90 % in culture flasks. When a confluence of 90 % was achieved, cells were treated with 0.25% trypsin-EDTA, precipitated by centrifugation at 3000 g for 5 min. Thereafter, the cell pellet was resuspended in phosphate buffer (PBS, 0.1 M, pH 7.4), and cell lysates were prepared. Lysates were prepared by squeezing cells five times through a syringe needle (needle diameter 25 G). An aliquot was taken from the obtained lysates, and the protein concentration was measured and was 1.5 mg/ml.

The cell lysates of HLEF-104 were used to determine the activity of MMP. The cell lysates of H9c2 were used to determine the activity of MMO. The cell lysates of cell line Vero were used to determine the activity of HDAC.

2.5. Synthesis of the dinitrosyl iron complexes (DNICs).

Water-soluble cationic dinitrosyl iron complexes DNICs (DNIC#1-$[Fe(SC(NH_2)_2)_2(NO)_2]Cl$; DNIC#2-$[Fe(SC(NH_2)_2)_2(NO)_2]_2SO_4$; DNIC#3-$[Fe(SC(NH_2)_2)_2(NO)_2]_2Fe_2(S_2O_3)_2NO_4$; DNIC#4-$[Fe(SC(NH_2)(NHC_2H_5))_2(NO_2)]Cl[Fe(SC(NH_2)(NHC_2H_5))Cl(NO_2)]$; DNIC# 5-$[Fe(SC(NH_2)_2)_2(NO)_2]ClO_4$; DNIC #6-$[Fe(SC(NH_2)_2)_2(NO)_2]ClO_4Cl$), DNIC#7-$[Fe(SC(NHCH_3)_2)_2(NO)_2]BF_4$ with functional sulfur-containing ligands, thiourea were synthesized as described in protocol [33, 34]. The structures of DNICs has been confirmed by X-ray analysis, Mössbauer, IR and EPR spectroscopy [33, 35-37]. When dissolved in water solvents, these DNICs release NO as a result of complex dissociation [33].

2.6. Determination of the effect of DNICs on the activity of matrix metalloproteinases.

The Amplite™ Universal Fluorimetric MMP Activity Assay Kit (AATBioquest, Inc., USA) was used to determine the effect of DNICs on the activity of matrix metalloproteinases by the fluorescent method in accordance with the manufacturer's protocol.

Analysis of the effect of DNICs (#1-7) was performed on HLEF-104 cell culture lysates. Briefly, cell lysates containing the MMP enzyme (50 µl, protein content 162 µg/well) were added to each well of a 96-well plate. Next, 50 µl of the tested DNICs were added to each well (the final concentration of DNICs was 6.6 x 10^{-5}M). As a control, a cell lysate (50 µl) containing the MMP enzyme was used, supplemented with 50 µl of buffer (no test compound). The control without enzyme contained buffer solution (50 µl) and test compounds (50 µl) per well. The plate was incubated at room temperature for 15 min. After 15 min, 50 µl of the working solution of MMP substrate GreenTM was added to each well, and the plate was incubated at room temperature for 60 min in the dark. Then the fluorescence intensity was measured at E_{ex}/E_{em}=490/525 nm on a spectrofluorimeter, using a Cary Eclipse spectrofluorimeter (Varian Medical Systems, USA).

The IC_{50} value (DNIC concentration causing 50% inhibition of MMP enzymatic activity) was determined by constructing concentration-effect curves showing the change in the effect of DNICs on MMP activity depending on their concentrations (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2}M).

2.7. Determination of the effect of DNICs on the activity of myeloperoxidase.

To assess the effect of DNICs on myeloperoxidase activity, a fluorescent method was used using the Amplite™ Fluorometric myeloperoxidase Assay Kit (AATBioquest, Inc., USA) according to the manufacturer's protocol.

Lysates of H9c2 cells (protein content 150 µg/well) were added to the wells of a 96-well plate. As a control, we used the studied DNIC compounds and a buffer solution (without the enzyme). A cell lysate containing MPO and buffer (no test compound) was used as a positive control. The test DNICs (compounds 1–7, final concentration 6.6 × 10^{-5} M/well) were added to the wells to the cell lysates and incubated at room temperature for 15 min. To assess the enzymatic activity of MPO, a reaction mixture containing substrates (10 µl H_2O_2, chloride ions, and 20 µl Amplit Red substrate in test kit buffer (pH 7.4)) was added to the wells. The plate was then incubated at room temperature for 60 minutes in the dark.

Fluorescence was measured on a Cary Eclipse spectrofluorimeter (Varian Medical Systems, USA) at E_{ex}/E_{em}=530/570 nm.

The IC_{50} value (DNIC concentration causing 50% inhibition of MPO enzymatic activity) was determined by constructing concentration-effect curves showing the change in the effect of DNICs on MPO activity depending on their concentrations (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2}M).

2.8. Determination of the effect of DNICs on the activity of renin.

To analyze the effect of DNICs on renin activity, a fluorescent method was used (Amplite Universal Fluorometric Renin Red Activity assay Kit, AAT Bioquest, Inc., USA). All procedures were performed according to the manufacturer's protocol. To recombinant renin (12.5 µl/well, concentration 1 µg/ml) was added 10 µl of the tested DNICs (DNIC#3, 4 and 6, final concentration 2.0 x 10^{-4}M/well). For control, 10 µl of Assay Buffer (no test compound)

was added to renin. The plate was incubated for 15 min at 37°C. After 15 min, 10 µl of the Renin Red substrate working solution was added to each well, and the plate was incubated at 37°C for 60 min in the dark. Then the fluorescence intensity was measured at E_{ex}/E_{em}=540/590 nm using a Cary Eclipse spectrofluorimeter (Varian Medical Systems, USA).

The IC_{50} value (DNIC concentration causing 50% inhibition of Renin enzymatic activity) was determined by constructing concentration-effect curves showing the change in the effect of DNICs on Renin activity depending on their concentrations (10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2}M).

2.9. Determination of the effect of DNICs on the activity of histone deacetylase.

To analyze the effect of DNICs on histone deacetylase activity, a fluorescent method for determining HDAC activity was used (Amplite Fluorometric HDAC Activity assay Kit, AAT Bioquest, Inc., USA). All procedures were performed according to the manufacturer's protocol. Testing the effect of DNICs (#3, 4, and 6) on HDAC activity was performed on lysates obtained from African green monkey renal epithelial cells (cell line Vero).

Lysates (50 µl, protein content 62.5 µg/well) were added to each well of a 96-well plate. Next, the tested DNICs were added to the lysates (DNIC#3, 4, and 6; 10 µl, final concentration $2x10^{-4}$M/well). Next, cell lysate supplemented with 10 µl of buffer (no test compound) was used as a control. The plate was incubated at room temperature for 20 min. After 20 min, a solution of GreenTM substrate (50 µl) was added to each well, and the plate was incubated at room temperature for 60 min. The fluorescence intensity was then measured on a spectrofluorimeter at E_{ex}/E_{em}=490/525 nm using a Cary Eclipse spectrofluorimeter (Varian Medical Systems, USA).

2.10. Determination of protein in cell lysates.

The protein content in the samples was determined by the Bradford method, using bovine serum albumin as a standard [38].

2.11. Determination of the effect of DNICs on the activity of poly(ADP-ribose) polymerase-1.

The principle of the method is based on the analysis of the rate of histone protein poly(ADP-ribosyl)ation using the original substrate-biotinylated NAD+. Briefly, reaction mixtures (10 µl) consisting of recombinant PARP-1 (100 nM), decamer oligonucleotide (50 µg/ml), DNICs (100 µM) or 3-aminobenzamide (2 mM 3-AB, PARP-1 inhibitor was used as a reference drug) in 50 mM Tris buffer (pH 7.8) containing KCl (100 mM), $MgCl_2$ (10 mM), dithiothreitol (1 mM) and BSA (1 mg/ml) were prepared in 200 µl PCR vials. After the samples were preincubated for 5 min at 37°C, 1 nmol NAD (a substrate-donor of ADP-ribosyl residues) was added to the reaction mixture to initiate the auto poly(ADP-ribosyl)ation reaction. After 15 min of incubation at 37°C, the reaction was stopped by adding an equal volume of stop solution to the test tubes. The amount of the poly(ADP-ribose) formed was determined by dot-blot analysis on a nitrocellulose membrane using primary antibodies to PAR (mouse anti-pADPr antibody 10H, sc-56198S; Santa Cruz Biotechnology, USA), secondary antibodies conjugated with HRP (goat anti-mouse antibody P-GAM, #90724; Imtek, Russia), and HRP chemiluminescent substrate (Pierce ECL Western Blotting Substrate; Thermo Scientific, USA). Chemiluminescence was measured on a Cary Eclipse spectrofluorimeter (Varian Medical Systems, USA) at an excitation wavelength of 530 nm.

2.12. Statistical analysis.

All experiments were duplicated three times, and data are presented as averages of three replicate experiments. Statistical analysis of the data was performed using the One-Way ANOVA program. An asterisk indicates a statistically significant difference between the positive control and all other treatments. Differences were considered significant at * - $p \leq 0.05$, ** - $p \leq 0.01$, *** - $p \leq 0.001$.

3. Results and Discussion

3.1. Effect of DNICs on the activity of matrix metalloproteinases.

The study results showed that DNICs (#1-7, at a concentration of $2x10^{-4}$ M) inhibit the activity of MMPs in the human fibroblast lysate in the range from 38 to 98% (Figure 1). The most effective MMP inhibitors are DNIC#3, 4, and 6 (MMP inhibition by 96, 98, and 76%, respectively).

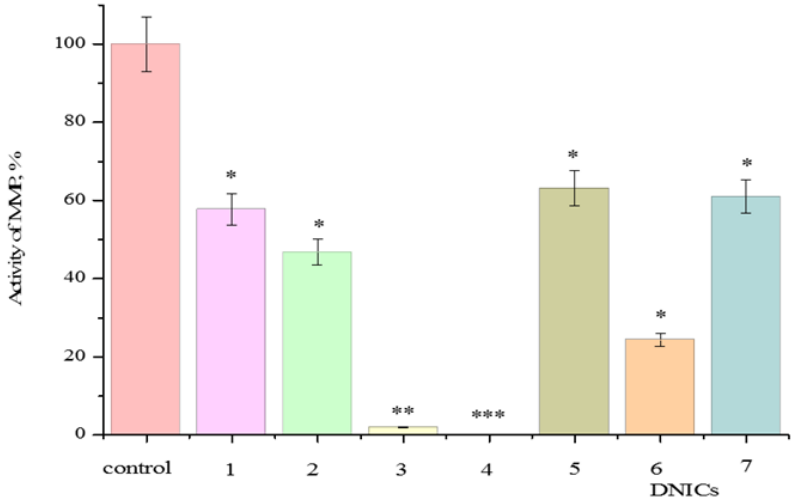

Figure 1. Effect of DNICs (#1–7) on MMP activity. M ± m, n = 3, *p < 0.05, ** - $p \leq 0.01$, *** - $p \leq 0.001$.

For the most effective DNICs, concentration dependences were plotted according to their effect on the activity of the MMP enzyme, and inhibition constants (IC_{50} values) were determined. The effectiveness of DNIC#3, 4, and 6 was assessed by constructing dose-dependent curves (Figures 2A, 2B, 2C).

It was shown that the inhibitory effect of DNICs on MMPs is clearly dose-dependent. A noticeable suppression of MMP activity occurs even at a DNICs concentration of about 10 μM. Calculation of IC_{50} values was carried out using the approximation of experimental values by the equation of the logistic curve by the method of non-linear regression method. It was shown that DNIC#3 has $IC_{50}=4 \times 10^{-5}$ M, DNIC#4 has $IC_{50}=7.5 \times 10^{-4}$M, DNIC#6 has $IC_{50} =3.6 \times 10^{-5}$M. It has been established that the most effective MMP inhibitors are DNIC#3 and #6. Thus, it has been shown that DNICs can effectively suppress the activity of MMPs at pharmacologically acceptable doses, while the effectiveness of the inhibitory action is largely determined by the structure of DNICs.

Therefore, in this study, we have shown that DNIC#3, 4, and 6 effectively inhibit MMPs and have the potential for the treatment of cardiovascular disease (e.g., atherosclerosis).

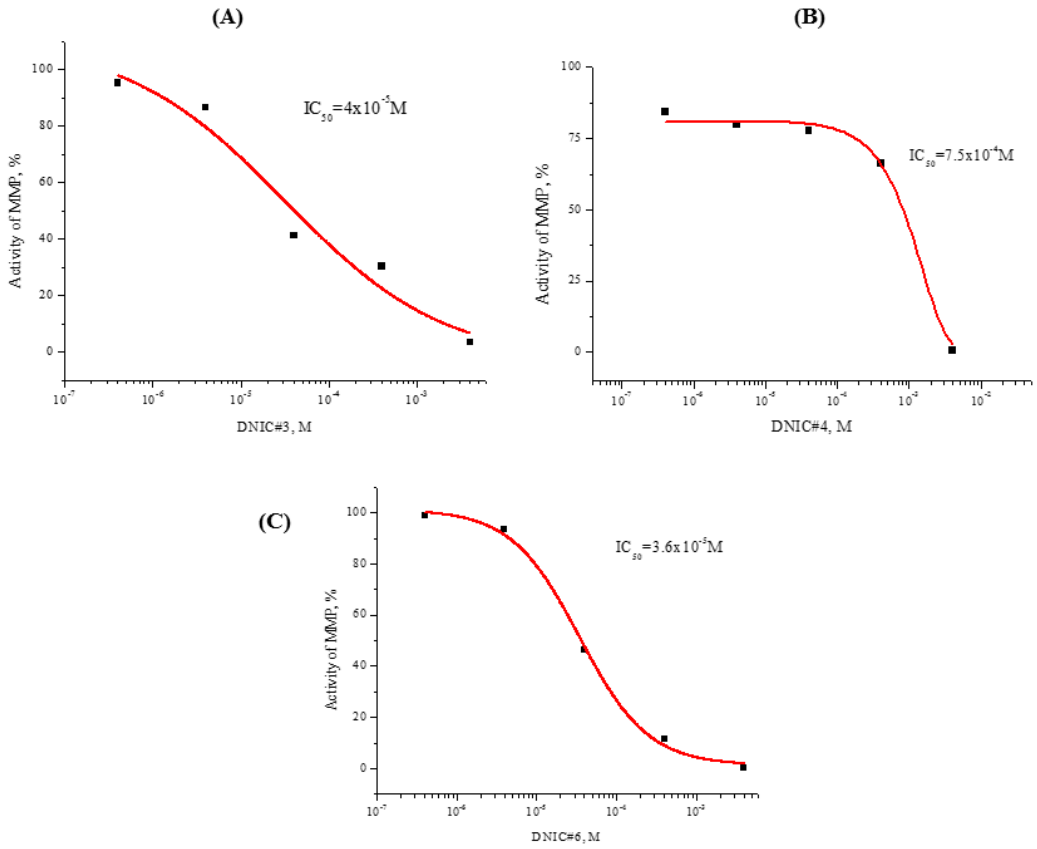

Figure 2. Effect of different concentrations of DNICs on MMP activity: A-DNIC#3, B-DNIC#4, C-DNIC#6.

3.2. Effect of DNICs on the activity of myeloperoxidase.

To identify molecular targets of DNICs action, we studied the effect of these compounds on MPO activity in the human fibroblast lysate using a fluorescent method. It was shown that six out of seven tested DNICs compounds inhibited MPO activity, and compound # 7 activated enzyme (Figure 3).

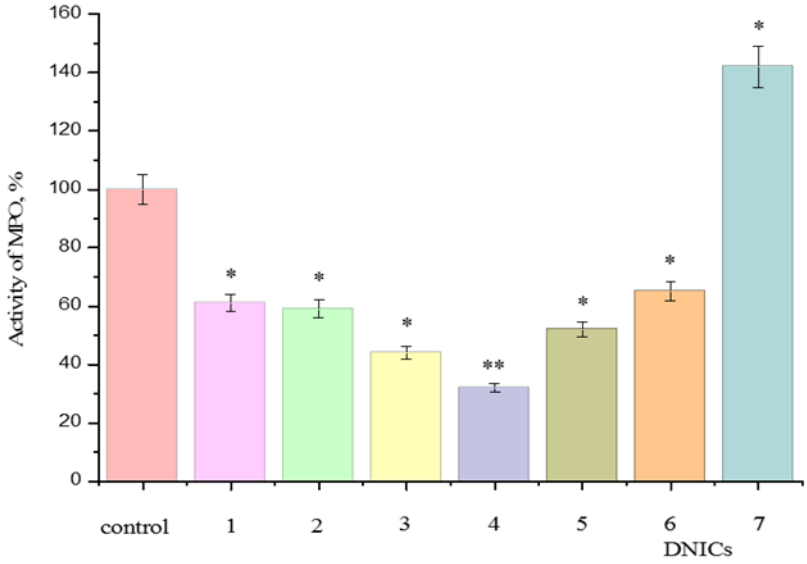

Figure 3. Effect of DNICs (#1–7) on MPO activity. M ± m, n = 3, *p < 0.05, ** - p ≤ 0.01.

Quantitative analysis of the effect of DNICs on the MPO activity showed that compounds #1, 2, 5, and 6 inhibited enzyme activity by more than 30%, and compound #7

activated MPO by 40%. Compounds #3, 4, and 5 showed the strongest inhibitory effect on MPO - 56, 68, and 48%, responsibly.

To compare the effectiveness of two DNICs (#3 and 4), most strongly inhibiting MPOs, we determined their IC_{50} values using dose-dependent curves (concentration-effect). The results showed that the IC_{50} for DNIC#3 was 4.5 $x10^{-4}$ M (Figure 4A), and for DNIC# 4 was 4.5$x10^{-7}$ M (Figure 4B).

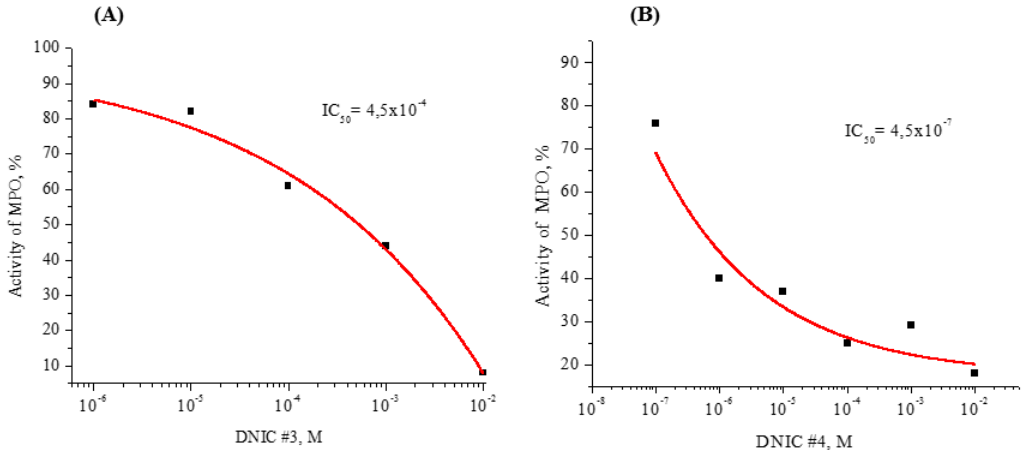

Figure 4. Effect of different concentrations of DNICs on MPO activity: A-DNIC#3, B- DNIC#4.

Since IC_{50} is an indicator concentration of the drug substance necessary required for 50% inhibition of enzyme activity *in vitro*, these data indicate that DNIC#4 has a stronger pharmacological potential for use as a cardiological agent. It is known that NO binds reversibly to many heme-containing binding proteins and can act as inhibitors inhibitor and activator of the enzymatic catalytic activity [39]. Since it was previously shown that DNICs release NO when dissolved [33], we assume that NO binds to Fe(III) in the MPO active center and forms a complex MPO–Fe(II)–NO. This iron-nitrosyl complex blocks the access of peroxide to the catalytic center of the enzyme and induces inhibitory MPO bagging.

3.3. Effect of DNICs on the activity of renin.

Renin is an angiotensinogenase - a component of the renin-angiotensin system that regulates blood pressure [40,41]. Renin is a biomarker for the development of hypertension and regulates blood pressure and arterial vasoconstriction [42].

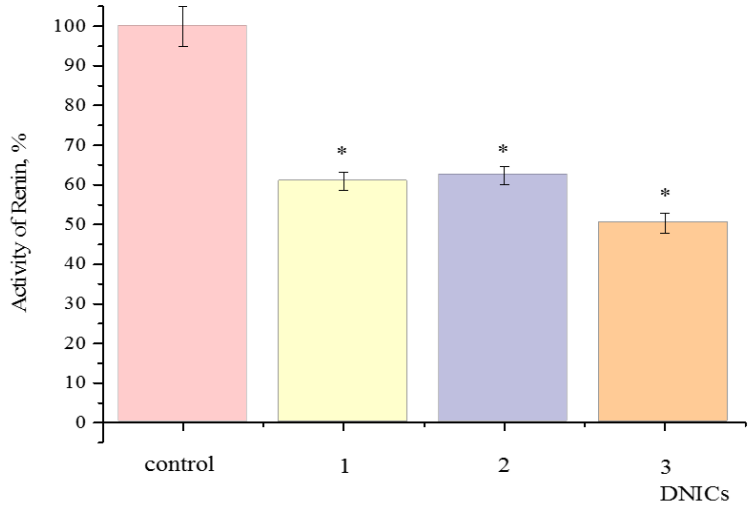

Figure 5. Effect of DNICs on renin activity: 1- DNIC#3, 2 – DNIC#4, 3-DNIC#6. Here M ± m, n = 3, *- p≤ 0.05.

Renin-inhibitors are important for creating new drugs for the treatment of hypertension and coronary heart disease [43-45]. It is known that nitric oxide regulates the tone of small and medium blood vessels, and vasodilation inhibits the production of vasoconstrictors [46–48].

Therefore, we investigated the effect of DNICs, nitric oxide donors, on human renin activity. We have shown that DNIC (#3, 4, and 6) (at a concentration of $2x10^{-4}$ M) inhibits renin activity (Figure 5). Quantitative analysis showed that DNICs inhibit renin activity by 46% (DNIC#3), 43% (DNIC#4), and by 50% (DNIC#6).

We determined the half-maximal renin inhibition constants (IC_{50}). The results showed that the IC_{50} for DNIC#3 was 9.6×10^{-5} M (Figure 6A), for DNIC#4 was 1.25×10^{-4}M (Figure 6B), and for DNIC#6 was 1×10^{-4} M (Figure 6C).

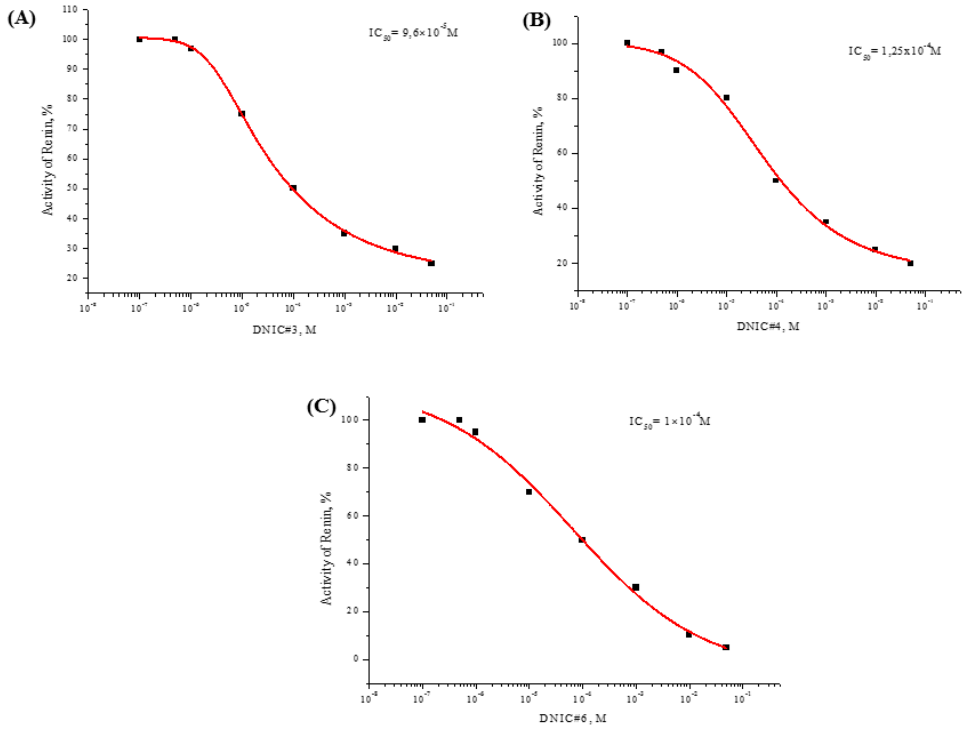

Figure 6. Effect of different concentrations of DNICs on renin activity: A-DNIC#3, B- DNIC#4, C- DNIC#6.

The data obtained indicate that DNIC#3, 4, and 6 are effective renin inhibitors and potential drugs for treating cardiovascular diseases, such as hypertension.

3.4. Effect of DNICs on the activity of histone deacetylase.

HDACs regulate gene transcription by catalyzing the removal of acetyl groups from key lysine residues in nucleosomal histones and recruiting other epigenetic regulators to the DNA promoter/enhancer region [49]. It is known that HDACs are involved in developing cardiovascular diseases, including hypertrophy and remodeling of the heart, and fibrosis [50]. Small molecule HDAC inhibitors may be powerful therapeutic agents for treating cardiovascular diseases, atherosclerosis, myocarditis, cancer, and diabetes [51 -53].

We investigated the effect of DNICs (#3, 4, and 6) on the activity of the HDAC enzyme in the monkey epithelial cell lysate. To analyze the effect of DNICs on HDAC activity, we used a fluorescent method for determining HDAC activity (Amplite Fluorometric HDAC Activity assay Kit, "Green Fluorescence" AAT Bioquest, Inc., USA) in fibroblast lysates (cell line *Vero*). It was shown that these compounds DNIC#3, 4, and 6 (at concentration $2x10^{-4}$ M)

do not affect the activity of the HDAC enzyme (Figure 7). Furthermore, the observed differences in activity were not statistically significant.

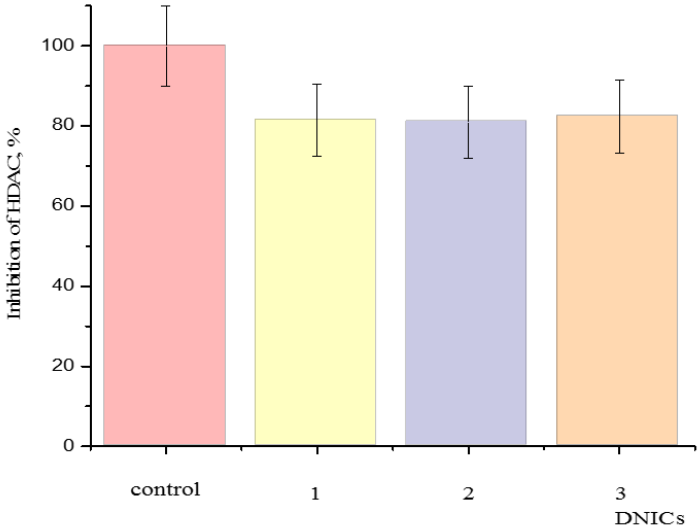

Figure 7. The effect of DNICs on the activity of histone deacetylase: 1- DNIC#3, 2- DNIC#4, 5- DNIC#6. M ± m, n = 3, *- p≤ 0.05.

3.5. Effect of DNICs on the activity of PARP-1.

In order to identify molecular targets of DNICs action, the effect of DNICs on the activity of recombinant human PARP-1 was studied. We analyzed the effect of six DNICs (#1, 2, 3, 4, 6, 7) on the rate of the auto-poly(ADP-ribosylation of hrPARP-1. Analysis of the reaction rate of hrPARP-1 auto modification using immunochemical dot-blot analysis of PAR showed that all analyzed DNICs (at a concentration of 100 μM) had a significant inhibitory effect (80-85 %) on the activity of recombinant hrPARP-1 (Figure 8A). Residual activity of hrPARP-1 varied, depending on the tested DNICs, in a small range - from 15 to 20% (Figure 8B). At the same time, the classical inhibitor of PARP-1, 3-AB, suppressed PARP-1 activity by 98 %.

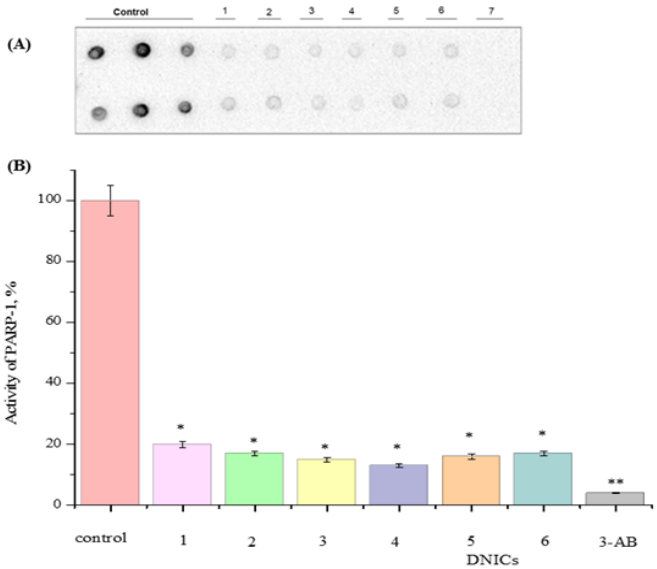

Figure 8. Effect of DNICs on the rate of auto-poly(ADP-ribosyl)ation of recombinant human PARP-1: (A)-chemiluminescence imaging of PAR after dot-blot analysis; (B) - the results of a quantitative analysis of the obtained image (chemiluminescent image): 1- DNIC#1, 2- DNIC#2, 3- DNIC#3, 4- DNIC#4, 5- DNIC#6, 6 – DNIC#7, 3-AB (3-aminobenzamide). M ± m, n = 3, *p < 0.05, ** - p ≤ 0.01.

Thus, the results obtained showed that the DNICs effectively inhibited the activity of the human recombinant PARP-1. Therefore, they have the potential for the treatment of CVD.

4. Conclusions

Previously, some of the authors of this work developed new synthetic NO donors and water-soluble cationic mono- and binuclear DNICs. By themselves, as donors of the vasodilating NO molecule, these complexes can undoubtedly be considered pharmacological substances with a potential cardioprotective effect. In this study, we tried to find out if they are able to modulate somehow the catalytic activity of a number of enzymes known to mediate the pathogenesis of CVD.

Cardiovascular disease results not only from overload or injury to the heart but also from a complex interplay between genetic, neurohormonal, inflammatory, and biochemical changes affecting myocardial cells, interstitial heart cells, or both. Important mediators and indicators of these processes are some intra- and extracellular enzymes, including MMP, MPO, renin, PARP. The aggravation of pathological disorders is usually accompanied by increased activity of these enzymes in the tissues. It was shown that the use of inhibitors of MMP, MOP, renin, PARP had a pronounced therapeutic effect in modeling CVD in animals.

It has been shown that MMP activity increases with the appearance and progression of CVD. The level of MMPs and their systems of induction and activation increase with pathological phenomena of CVD, such as heart failure, atherosclerosis, and heart infarction [54,55]. It is known that under the conditions of myocardial infarction, the MMP level significantly increases within a few hours after the infarction as a result of local activation of cytokines and infiltration of inflammatory cells [56,57]. MMPs play an important role in maintaining the structure and function of blood vessels but are also involved in pathological processes such as tissue remodeling of the cardiovascular system, formation, and destabilization of atherosclerotic plaques [58,59]. Thus, given the important role of MMP in cardiovascular disease, the search for new MMP inhibitors is essential for the treatment of cardiovascular disease.

In this study, we showed that DNICs effectively inhibited MMP. The results of our study showed that of the seven studied DNICs, DNICs#3 and #6 were the most effective MMP inhibitors. Matrix metalloproteinases are a family of calcium (Ca^{2+}) - and zinc (Zn^{2+}) - dependent endopeptidases involved in both physiological and pathological processes in the human body [60,61]. Since DNICs are known to be nitric oxide donors, it is likely that the resulting NO induces the release of zinc from MMP active sites by N-nitrosylation of histidines that coordinate Zn^{2+}. As an alternative mechanism, it can be assumed that NO is able to inhibit MMP by direct interaction with the Zn^{2+} active site and thus competitively inhibit substrate hydrolysis.

The results obtained show that myeloperoxidase is also a molecular target of cationic DNICs. DNIC#3 and #4 effectively inhibited the activity MPO. Comparative evaluation of the action of two compounds showed that DNIC#4 is more an effective inhibitor of enzyme activity than DNIC#3. Evaluating the data obtained from a practical point of view, we can conclude that DNIC#4 is a promising candidate for further pharmacological studies. MPO activation under the action DNIC#7 indicates its therapeutic potential for treating immunodeficiency states. NO is known to modulate the peroxidase activity of MPO with the help of at least two different mechanisms [62,63]. At high concentrations of NO, reversibly bind-fused with Fe(III) in the active center of MPO and formed contains nitrosyl complex MPO–Fe(II)–NO, which

leads to inhibition of the enzyme activity. However, at low NO concentrations, a significant increase in the catalytic activity of MPO occurs since the stage that limits the rate of Fe(II) catalysis in MPO-Fe(III) is accelerated [62]. Based on the foregoing, we assume that DNICs 1-6 act according to the first mechanism. However, in the case of DNIC#7, we assume that DNIC#7 when dissolved, it forms a small amount of NO, which causes MPO activation by the second mechanism of action. In addition, compared to DNICs #1-6, compound DNIC#7 has a structure that differs from that DNICs#1–6 because in the composition of this compound BF_4^- anion is present. It is known that MPO catalyzes the reactions of hydrogen oxides and with other halides (Br^-, I^-), as well as pseudohalides (S^-, CN^-) [62]. Since BF_4^- (like Br^-) is extremely reactive with non-metals and the strongest oxidizing agents, we assume that MPO catalyzes the reactions of hydrogen oxide also with BF_4^-, which leads to an increased reduction of the enzymatic activity of MPO.

The renin-angiotensin-aldosterone system (RAAS) is a complex endocrine system that regulates electrolyte levels, circulating plasma volume, vascular tone, and blood pressure in healthy individuals. The RAAS is involved in developing and progressing cardiovascular diseases, particularly atherosclerosis, hypertension, ischemia, and heart failure [64-66]. In the body, renin acts on the blood glycoprotein angiotensinogen, specifically hydrolyzing the peptide bond between leucine and valine. This produces an inactive decapeptide (angiotensin I), which is converted enzymatically (under the action of an angiotensin-converting enzyme) into the active hormone angiotensin II (hypertension, or angiotonin), which constricts blood vessels and stimulates the secretion of aldosterone by the adrenal glands [65,66]. These two effects lead to an increase in blood pressure. Currently, RAAS has been identified as a therapeutic target for the treatment of heart failure, and various pharmacological agents have been developed that act at specific stages of the RAAS cascade, including angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, aldosterone antagonists, direct renin inhibitors, and aldosterone synthase inhibitors [67,68]. Since it is known that NO regulates the tone of small and medium blood vessels, and vasodilation [69-71], we were interested in studying the effect of DNICs on renin activity. In this work, we have shown that DNICs (at concentration $2x10^{-4}$M) inhibit renin activity by 50%. These data indicate that DNICs have the potential to design new drugs for the treatment of hypertension and coronary heart disease.

Several authors currently suggest that PARP inhibitors can be successfully used in the treatment of several cardiovascular diseases accompanied by acute or chronic inflammation and cardiac surgery and transplantology [72-75]. Previously, it was shown that the PARP inhibitor INO-1001 could be a potential cardioprotector in the complex therapy of a number of cardiovascular pathologies [76]. The pharmacokinetics and pharmacodynamics of this inhibitor have also been studied in patients with myocardial infarction [77,78]. In this work, we studied the influence of DNICs (#1-6) on the catalytic activity of recombinant poly(ADP-ribose)-human polymerase (hrPARP-1). It was shown that all DNICs have a pronounced inhibitory effect on the activity of hrPARP-1. The maximum inhibitory effect of all DNICs on hrPARP-1 was manifested at a concentration of 100 μM. Based on the results obtained, it can be concluded that all DNICs considered in this work contain a universal pharmacophore that determines the PARP-1 inhibitory effect of DNICs. We assume that treatment with hrPARP-1 by DNICs leads to reversible S-nitrosylation of Cys residues, involved in the formation of coordination bonds with Zn^{2+} in zinc fingers of the DNA-binding domain of the enzyme. The

use of PARP inhibitors can be very effective in treating many pathologies, primarily cardiovascular diseases.

Thus, the results of our study showed that DNICs are inhibitors of such enzymes as matrix metalloproteinase, myeloperoxidase, renin, and poly(ADP-ribose) polymerase, which are indicators of cardiovascular diseases. All this makes it possible to consider DNICs as promising candidates for developing new drugs with a cardioprotective effect. Based on this, it can be concluded that these DNICs have therapeutic potential for treating cardiovascular diseases.

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Conflicts of Interest

The authors declare no conflict of interest. The funders had no role in the study's design, in the collection, analyses, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

References

1. WHO reveals leading causes of death and disability worldwide: 2000-2019 // *WHO newsletter*. News release **2020**.
2. Fan, W.; He, Y.; Guan, X.; Gu, W.; Wu, Z.; Zhu, X.; Huang, F.; He, H. Involvement of the nitric oxide in melatonin-mediated protection against injury. *Life Sciences* **2018**, *200*, 142-147, https://doi.org/10.1016/j.lfs.2018.03.035.
3. Fan, W.; Liu, Q.; Zhu, X.; Wu, Z.; Li, D.; Huang, F.; He, H. Regulatory effects of anesthetics on nitric oxide. *Life Sciences* **2016**, *151*, 76-85, https://doi.org/10.1016/j.lfs.2016.02.094.
4. Magierowski, M.; Magierowska, K.; Kwiecien, S.; Brzozowski, T. Gaseous mediators nitric oxide and hydrogen sulfide in the mechanism of gastrointestinal integrity, protection and ulcer healing. *Molecules* **2015**, *20*, 9099-9123, https://doi.org/10.3390/molecules20059099.
5. Gori, T. Exogenous NO therapy for the treatment and prevention of atherosclerosis. *Int J Mol Sci* **2020**, *21*, 2703, https://doi.org/10.3390/ijms21082703.
6. Cavet, M.E.; Vittitow, J.L.; Impagnatiello, F.; Ongini, E.; Bastia, E. Nitric oxide (NO): an emerging target for the treatment of glaucoma. *Invest Ophthalmol Vis Sci* **2014**, *55*, 5005-5015, https://doi.org/10.1167/iovs.14-14515
7. Wimalawansa, S.J. Nitric oxide: new evidence for novel therapeutic indications. *Expert Opin Pharmacother* **2008**, *9*, 1935-1954, https://doi.org/10.1517/14656566.9.11.1935.
8. Pattillo, C.B.; Bir, S.; Rajaram, V.; Kevil, C.G. Inorganic nitrite and chronic tissue ischaemia: a novel therapeutic modality for peripheral vascular diseases. *Cardiovasc Res* **2011**, *89*, 533-541, https://doi.org/10.1093/cvr/cvq297.
9. Cho, S.L.; Liao, C.J.; Lu, T.T. Synthetic methodology for preparation of dinitrosyl iron complexes. *J Biol Inorg Chem* **2019**, *24*, 495-515, https://doi.org/10.1007/s00775-019-01668-z.

10. Vanin, A.F. Physico-Chemistry of Dinitrosyl Iron Complexes as a Determinant of Their Biological Activity. *Int J Mol Sci* **2021**, *22*, 10356, https://doi.org/10.3390/ijms221910356.
11. Pectol, D.C.; Khan, S.; Chupik, R.B.; Elsabahy, M.; Wooley, K.L.; Darensbourg, M.Y.; Lim, S.M. Toward the optimization of dinitrosyl iron complexes as therapeutics for smooth muscle cells. *Mol Pharm* **2019**, *16*, 3178-3187. https://doi.org/10.1021/acs.molpharmaceut.9b00389
12. Carvalho, E.M.; Ridnour, L.A.; Júnior, F.S.G.; Cabral, P.H.B.; do Nascimento, N.R.F.; Wink, D.A.; Franco, D.W.; de Medeiros, M.J.C.; de Lima Pontes, D.; Longhinotti, E.; de Freitas Paulo, T.; Bernardes-Génisson, V.; Chauvin, R.; Sousa, E.H.S.; Lopes, L.G.F. A divergent mode of activation of a nitrosyl iron complex with unusual antiangiogenic activity. *J Inorg Biochem* **2020**, *210*, 111133, https://doi.org/10.1016/j.jinorgbio.
13. Aldoshin, S.M., Sanina, N.A. Fundamental sciences for medicine: Biophysical medical technologies; Grigorieva, A.I., Vladimirova, Yu.A., Eds.; Moscow, *MAKS Press* **2015**, *1*.
14. Aldoshin, S.M.; Bozhenko, K.V.; Utenyshev, A.N.; Sanina, N.A.; Emel'yanova, N.S. Formation of supramolecular synthons in the crystalline structure of the dinitrosyl iron complexes with aliphatic thiourea ligands. *J Mol Model* **2020**, *26*, 330, https://doi.org/10.1007/s00894-020-04594-z.
15. Vanin, A.F.; Sanina, N.A.; Serezhenkov, V.A.; Burbaev, D.Sh.; Lozinsky, V.I.; Aldoshin, S.M. Dinitrosyl-iron complexes with thiol-containing ligands: spatial and electronic structures. *Nitric Oxide* **2007**, *16*, 82-93, https://doi.org/10.1016/j.niox.2006.07.0052.
16. Karakas, M.; Koenig, W. Myeloperoxidase production by macrophage and risk of atherosclerosis. *Curr Atheroscler Rep* **2012**, *14*, 277-283, https://doi.org/10.1007/s11883-012-0242-3.
17. Zeng, L.; Mathew, A.V.; Byun, J.; Atkins, K.B.; Brosius, F.C. 3rd, Pennathur, S. Myeloperoxidase-derived oxidants damage artery wall proteins in an animal model of chronic kidney disease-accelerated atherosclerosis. *J Biol Chem* **2018**, *293*, 7238-7249, https://doi.org/10.1074/jbc.RA117.000559.
18. Maiocchi, S.L.; Ku, J.; Thai, T.; Chan, E.; Rees, M.D.; Thomas, S.R. Myeloperoxidase: A versatile mediator of endothelial dysfunction and therapeutic target during cardiovascular disease. *Pharmacol Ther* **2021**, *221*, 107711, https://doi.org/10.1016/j.pharmthera.2020.107711.
19. Hatachi, G.; Tsuchiya, T.; Miyazaki, T.; Matsumoto, K.; Yamasaki, N.; Okita, N.; Nanashima, A.; Higami, Y.; Nagayasu, T. The poly(adenosine diphosphate-ribose) polymerase inhibitor PJ34 reduces pulmonary ischemia-reperfusion injury in rats. *Transplantation* **2014**, *98*, 618-624, https://doi.org/10.1097/TP.0000000000000305.
20. Korkmaz-Icöz, S.; Radovits, T.; Loganathan, S.; Li, S.; Ruppert, M.; Benke, K.; Brlecic, P.; Szabó, C.; Karck, M.; Szabó, G. Prolonging hypothermic ischaemic cardiac and vascular storage by inhibiting the activation of the nuclear enzyme poly(adenosine diphosphate-ribose) polymerase. *Eur J Cardiothorac Surg* **2017**, *51*, 829-835, https://doi.org/10.1093/ejcts/ezw426.
21. Sahin, K.; Durdagi, S. Identifying new piperazine-based PARP1 inhibitors using text mining and integrated molecular modeling approaches. *J Biomol Struct Dyn* **2021**, *39*, 681-690, https://doi.org/10.1080/07391102.2020.1715262.
22. Hirohata, S.; Inagaki, J.; Ohtsuki, T. Diverse Functions of a Disintegrin and Metalloproteinase with Thrombospondin Motif-1. *Yakugaku Zasshi* **2017**, *137*, 811-814, https://doi.org/10.1248/yakushi.16-00236-4.
23. Güneş, M.F.; Akpinar, M.B.; Cömertoğlu, I.; Akyol, S.; Demirçelik, B.; Gürel, Ö.M.; Aynekin, B.; Erdemli, H.K.; Ateş, M.; Eryonucu, B.; Demircan, K. The Investigation of a Disintegrin and Metalloproteinase with ThromboSpondin Motifs (ADAMTS) 1, 5 and 16 in Thoracic Aortic Aneurysms and Dissections. *Clin Lab* **2016**, *62*, 425-433.
24. Atale, N.; Mishra, C.B.; Kohli, S.; Mongre, R.K.; Prakash, A.; Kumari, S.; Yadav, U.C.S.; Jeon, R.; Rani, V. Anti-inflammatory Effects of S. cumini Seed Extract on Gelatinase-B (MMP-9) Regulation against Hyperglycemic Cardiomyocyte Stress. *Oxid Med Cell Longev* **2021**, *2021*, 8839479, https://doi.org/10.1155/2021/8839479.
25. Hu, Q.; Ecker, M. Overview of MMP-13 as a Promising Target for the Treatment of Osteoarthritis. *Int J Mol Sci* **2021**, *22*, 1742, https://doi.org/10.3390/ijms22041742.
26. Nandi, S.S.; Katsurada, K.; Sharma, N.M.; Anderson, D.R.; Mahata, S.K.; Patel, K.P. MMP9 inhibition increases autophagic flux in chronic heart failure. *Am J Physiol Heart Circ Physiol* **2020**, *319*, H1414-H1437, https://doi.org/10.1152/ajpheart.00032.2020.
27. Macchiavello, S.; Fardella, C.; Baudrand, R. Actualización en el manejo clínico de la hipertensión hiporreninémica [Update in the clinical management of low renin hypertension]. *Rev Med Chil* **2019**, *147*, 490-498, https://doi.org/10.4067/S0034-98872019000400490.

28. Baudrand, R.; Vaidya, A. The Low-Renin Hypertension Phenotype: Genetics and the Role of the Mineralocorticoid Receptor. *Int J Mol Sci* **2018**, *19*, 546, https://doi.org/10.3390/ijms19020546.
29. Lu, Y.T.; Fan, P.; Zhang, D.; Zhang, Y.; Meng, X.; Zhang, Q.Y.; Zhao, L.; Yang, K.Q.; Zhou, X.L. Overview of Monogenic Forms of Hypertension Combined With Hypokalemia. *Front Pediatr* **2021**, *8*, 543309, https://doi.org/10.3389/fped.2020.543309.
30. Tapia-Castillo, A.; Carvajal, C.A.; López-Cortés, X.; Vecchiola, A.; Fardella, C.E. Novel metabolomic profile of subjects with non-classic apparent mineralocorticoid excess. *Sci Rep* **2021**, *11*, 17156, https://doi.org/10.1038/s41598-021-96628-6.
31. Bagchi, R.A.; Weeks, K.L. Histone deacetylases in cardiovascular and metabolic diseases. *J Mol Cell Cardiol* **2019**, *130*, 151-159, https://doi.org/10.1016/j.yjmcc.2019.04.003.
32. Chun, P. Therapeutic effects of histone deacetylase inhibitors on heart disease. *Arch Pharm Res* **2020**, *43*, 1276-1296, https://doi.org/10.1007/s12272-020-01297-0.
33. Sanina, N.A.; Aldoshin, S.M.; Shmatko, N.Yu.; Korchagin, D.V.; Shilov, G.V.; Knyazkina, E.V.; Ovanesyan, N.S.; Kulikov, A.V. Nitrosyl iron complexes with enhanced NO donating ability: synthesis, structure and properties of a new type of salt with the DNIC cations $[Fe(SC(NH_2)_2)_2(NO)_2]^+$. *New Journal of Chemistry* **2015**, *39*, 1022–1030, https://doi.org/10.1039/C4NJ01693A.
34. Sanina, N.A.; Manzhos, R.A.; Emel'yanovaa, N.S.; Kupchinskaya, E.N.; Krivenko, A.G.; Aldoshin, S.M. Redox reactions of cationic nitrosyl iron complexes with thiourea and its aliphatic derivatives: The experiment and DFT investigation. *Journal of Molecular Structure* **2019**, *1181*, 253-260, https://doi.org/10.1016/j.molstruc.2018.12.112.
35. Shmatko, N.Yu.; Korchagin, D.V.; Shilov, G.V.; Ovanesyan, N.S.; Kulikov, A.V.; Sanina, N.A.; Aldoshin, S.M. The cationic dinitrosyl iron complexes family with thiocarbamide derivatives: Synthesis, structure and properties in the solid state. *Polyhedron* **2017**, *137*, 72–80, https://doi.org/10.1016/j.poly.2017.08.006.
36. Shmatko, N.Yu.; Korchagin, D.V.; Shilov, G.V.; Sanina, N.A.; Aldoshin S.M. Molecular and crystal structure of a cationic dinitrosyl iron complex with 1, 3-dimethylthiourea. *J Struct Chem* **2017**, *58*, 353-355, https://doi.org/10.1134/S0022476617020172.
37. Emel'yanova, N.S.; Shmatko, N.Yu.; Sanina, N.A.; Aldoshin, S.M. Quantum-chemical study of the Fe(NO)2 fragment in the cation of mononuclear nitrosyl iron complex $[Fe(SC(NH_2)_2)_2(NO)_2]Cl \cdot H_2O$. *Computational & theoretical chemistry* **2015**, *1060*, 1–9, https://doi.org/10.1016/j.comptc.2015.02.004.
38. Bradford, M.M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry* **1976**, *72*, 248–254, https://doi.org/10.1006/abio.1976.9999.
39. Abu-Soud, H.M.; Hazen, S.L. Nitric oxide modulates the catalytic activity of myeloperoxidase. *J Biol Chem* **2000**, *275*, 5425–5430, https://doi.org/10.1074/jbc.275.8.5425.
40. Akhadov, Sh.V.; Ruzbanova, G.R.; Molchanova, G.S.; Talalaeva, T.G.; Khoreva, S.N. The role and target level of individual components of the renin-angiotensin-aldosterone system in the development of cardiovascular complications in patients with arterial hypertension. *Russian Journal of Cardiology* **2009**, *14*, 26-32.
41. Chappell, M.C. Biochemical evaluation of the renin-angiotensin system: the good, bad, and absolute? *Am J Physiol Heart Circ Physiol* **2016**, *310*,H137-152, https://doi.org/10.1152/ajpheart.00618.2015.
42. Yang, S.; Wang, Z.; Guo, M.; Du, M.; Wen, X.; Geng, L.; Yu, F.; Liu, L.; Li, Y.; Feng, L.; Zhou, T. UPLC - MS-Based Serum Metabolomics Reveals Potential Biomarkers of Ang II-Induced Hypertension in Mice. *Front Cardiovasc Med* **2021**, *8*, 683859, https://doi.org/10.3389/fcvm.2021.683859.
43. Sutanto, H.; Dobrev, D.; Heijman, J. Angiotensin Receptor-Neprilysin Inhibitor (ARNI) and Cardiac Arrhythmias. *Int J Mol Sci* **2021**, *22*, 8994, https://doi.org/10.3390/ijms22168994.
44. Hartman, R.E.; Rao, P.S.S.; Churchwell, M.D.; Lewis, S.J. Novel therapeutic agents for the treatment of diabetic kidney disease. *Expert Opin Investig Drugs* **2020**, *29*, 1277-1293, https://doi.org/10.1080/13543784.2020.1811231.
45. Corti, R.; Burnett, J.C.Jr.; Rouleau, J.L.; Ruschitzka, F.; Lüscher, T.F. Vasopeptidase inhibitors: a new therapeutic concept in cardiovascular disease? *Circulation* **2001**, *104*, 1856-1862, https://doi.org/10.1161/hc4001.097191.
46. Marques, A.A.M.; da Silva, C.H.F.; de Souza, P.; de Almeida, C.L.B.; Cechinel-Filho, V.; Lourenço, E.L.B.; Gasparotto Junior, A. Nitric oxide and Ca2+ -activated high-conductance K+ channels mediate nothofagin-induced endothelium-dependent vasodilation in the perfused rat kidney. *Chem Biol Interact* **2020**, *327*, 109182, https://doi.org/10.1016/j.cbi.2020.109182.

47. Liu, T.; Zhang, M.; Mukosera, G.T.; Borchardt, D.; Li, Q.; Tipple, T.E.; Ishtiaq Ahmed, A.S.; Power, G.G.; Blood, A.B. L-NAME releases nitric oxide and potentiates subsequent nitroglycerin-mediated vasodilation. *Redox Biol* **2019**, *26*, 101238, https://doi.org/10.1016/j.redox.2019.101238.
48. Jin, L.; Conklin, D.J. A novel evaluation of endothelial dysfunction ex vivo: "Teaching an Old Drug a New Trick". *Physiol Rep* **2021**, *9*, e15120, https://doi.org/10.14814/phy2.15120
49. Gallinari, P.; Di Marco, S.; Jones, P.; Pallaoro, M.; Steinkühler, C. HDACs, histone deacetylation and gene transcription: from molecular biology to cancer therapeutics. *Cell Res* **2007**, *17*, 195-211, https://doi.org/10.1038/sj.cr.7310149.
50. Luque-Martin, R.; Van den Bossche, J.; Furze, R.C.; Neele, A.E.; van der Velden, S.; Gijbels, M.J.J.; van Roomen, C.P.P.A.; Bernard, S.G.; de Jonge, W.J.; Rioja, I.; Prinjha, R.K.; Lewis, H.D.; Mander, P.K.; de Winther, M.P.J. Targeting histone deacetylases in myeloid cells inhibits their maturation and inflammatory function with limited effects on atherosclerosis. *Front. Pharmacol* **2019**, *10*, 1242, https://doi.org/10.3389/fphar.2019.01242.
51. Bagchi, R.A.; Weeks, K.L. Histone deacetylases in cardiovascular and metabolic diseases. *J Mol Cell Cardiol* **2019**, *130*, 151-159, https://doi.org/10.1016/j.yjmcc.2019.04.003.
52. Shanmugam, G.; Rakshit, S.; Sarkar, K. HDAC inhibitors: Targets for tumor therapy, immune modulation and lung diseases. *Transl Oncol* **2022**, *16*, 101312, https://doi.org/10.1016/j.tranon.2021.101312.
53. Hamminger, P.; Rica, R.; Ellmeier, W. Histone deacetylases as targets in autoimmune and autoinflammatory diseases. *Adv Immunol* **2020**, *147*, 1-59, https://doi.org/10.1016/bs.ai.2020.06.001.
54. Kremastiotis, G.; Handa, I.; Jackson, C.; George, S.; Johnson, J. Disparate effects of MMP and TIMP modulation on coronary atherosclerosis and associated myocardial fibrosis. *Sci Rep* **2021**, *11*, 23081, https://doi.org/10.1038/s41598-021-02508-4.
55. Głogowska-Ligus, J.; Dąbek, J.; Piechota, M.; Gallert-Kopyto, W.; Lepich, T.; Korzeń, D.; Gąsior, Z. Can the expression of the metalloproteinase 9 gene and its inhibitor be considered as markers of heart failure? *Minerva Cardiol Angiol* **2021**, *69*, 172-177, https://doi.org/10.23736/S2724-5683.20.05202-0.
56. Sanchez, J.; Le Jan, S.; Muller, C.; François, C.; Renard, Y.; Durlach, A.; Bernard, P.; Reguiai, Z.; Antonicelli, F. Matrix remodelling and MMP expression/activation are associated with hidradenitis suppurativa skin inflammation. *Exp Dermatol* **2019**, *28*, 593-600, https://doi.org/10.1111/exd.13919.
57. Du, H.; Zhang, X.; Zeng, Y.; Huang, X.; Chen, H.; Wang, S.; Wu, J.; Li, Q.; Zhu, W.; Li, H.; Liu, T.; Yu, Q.; Wu, Y.; Jie, L. A Novel Phytochemical, DIM, Inhibits Proliferation, Migration, Invasion and TNF-α Induced Inflammatory Cytokine Production of Synovial Fibroblasts From Rheumatoid Arthritis Patients by Targeting MAPK and AKT/mTOR Signal Pathway. *Front Immunol* **2019**, *10*, 1620, https://doi.org/10.3389/fimmu.2019.01620.
58. Stasinopoulou, M.; Kadoglou, N.P.E.; Christodoulou, E.; Paronis, E.; Kostomitsopoulos, N.G.; Valsami, G.; Liapis, C.D.; Kakisis, J. Statins' Withdrawal Induces Atherosclerotic Plaque Destabilization in Animal Model-A "Rebound" Stimulation of Inflammation. *J Cardiovasc Pharmacol Ther* **2019**, *24*, 377-386, https://doi.org/10.1177/1074248419838499.
59. Golforoush, P.; Yellon, D.M.; Davidson, S.M. Mouse models of atherosclerosis and their suitability for the study of myocardial infarction. *Basic Res Cardiol* **2020**, *115*, 73, https://doi.org/10.1007/s00395-020-00829-5.
60. Verma, R.P.; Hansch, C. Matrix metalloproteinases (MMPs): Chemical-biological functions and (Q)SARs. *Bioorganic Med Chem* **2007**, *15*, 2223-2268, https://doi.org/10.1016/j.bmc.2007.01.011.
61. Bassiouni, W.; Ali, M.A.M.; Schulz, R. Multifunctional intracellular matrix metalloproteinases: implications in disease. *FEBS J* **2021**, *288*, 7162-7182, https://doi.org/10.1111/febs.15701.
62. Davies, M.J. Myeloperoxidase-derived oxidation: mechanisms of biological damage and its prevention. *J Clin Biochem Nutr* **2011**, *48*, 8-19, https://doi.org/10.3164/jcbn.11-006FR.
63. Chen, S.; Chen, H.; Du, Q.; Shen, J. Targeting Myeloperoxidase (MPO) Mediated Oxidative Stress and Inflammation for Reducing Brain Ischemia Injury: Potential Application of Natural Compounds. *Front Physiol* **2020**, *11*, 433, https://doi.org/10.3389/fphys.2020.00433.
64. Kaesler, N.; Babler, A.; Floege, J.; Kramann, R. Cardiac Remodeling in Chronic Kidney Disease. *Toxins (Basel)* **2020**, *12*, 161, https://doi.org/10.3390/toxins12030161
65. Putra, B.E.; Jonny; Soewandi, A.H. Renal Intra-Arterial Heparin Flushing for Blood Pressure Control in Refractory Hypertension: A New Method to Salvage the Unsalvageable Renal Artery Stenosis. *Indian J Nephrol* **2019**, *29*, 200-203, https://doi.org/10.4103/ijn.IJN_194_18.

66. Zhang, Z.Y.; Qian, L.L.; Wang, R.X. Molecular mechanisms underlying renin-angiotensin-aldosterone system mediated regulation of BK channels. *Front Physiol* **2017**, *8*, 698, https://doi.org/10.3389/fphys.2017.00698.
67. Beusekamp, J.C.; Tromp, J.; Cleland, J.G.F.; Givertz, M.M.; Metra, M.; O'Connor, C.M.; Teerlink, J.R.; Ponikowski, P.; Ouwerkerk, W.; van Veldhuisen, D.J.; Voors, A.A.; van der Meer, P. Hyperkalemia and Treatment With RAAS Inhibitors During Acute Heart Failure Hospitalizations and Their Association With Mortality. *JACC Heart Fail* **2019**, *7*, 970-979, https://doi.org/10.1016/j.jchf.2019.07.010.
68. Murohara, T. Role of RAAS inhibitors for the treatment of heart failure. Nih*on Rinsho* **2012**, *70*, 1577-1581.
69. De Lima, R.G.; Silva, B.R.; da Silva, R.S.; Bendhack, L.M. Ruthenium complexes as NO donors for vascular relaxation induction. *Molecules* **2014**, *19*, 9628-9654, https://doi.org/10.3390/molecules19079628.
70. Palatini, P.; Grassi, G. Nondipping status and impaired nitroglycerin-mediated vasodilation in resistant hypertension: evidence and mechanisms. *J Hypertens* **2015**, *33*, 1533-1535, https://doi.org/10.1097/HJH.0000000000000641.
71. Münzel, T.; Steven, S.; Daiber, A. Organic nitrates: update on mechanisms underlying vasodilation, tolerance and endothelial dysfunction. *Vascul Pharmacol* **2014**, *63*, 105-113, https://doi.org/10.1016/j.vph.2014.09.002.
72. Henning, R.J.; Bourgeois, M.; Harbison, R.D. Poly(ADP-ribose) Polymerase (PARP) and PARP Inhibitors: Mechanisms of Action and Role in Cardiovascular Disorders. *Cardiovasc Toxicol* **2018**, *18*, 493-506, https://doi.org/10.1007/s12012-018-9462-2.
73. Maluchenko, N.V.; Feofanov, A.V.; Studitsky, V.M. PARP-1-Associated Pathological Processes: Inhibition by Natural Polyphenols. *Int J Mol Sci* **2021**, *22*, 11441, https://doi.org/10.3390/ijms222111441.
74. Pazzaglia. S.; Pioli, C. Multifaceted Role of PARP-1 in DNA Repair and Inflammation: Pathological and Therapeutic Implications in Cancer and Non-Cancer Diseases. *Cells* **2019**, *9*, 41, https://doi.org/10.3390/cells9010041.
75. Pacher, P.; Szabó, C. Role of poly(ADP-ribose) polymerase 1 (PARP-1) in cardiovascular diseases: the therapeutic potential of PARP inhibitors. *Cardiovasc Drug Rev* **2007**, *25*, 235-260, https://doi.org/10.1111/j.1527-3466.2007.00018.x.
76. Radovits, T.; Seres, L.; Gero, D.; Berger, I.; Szabó, C.; Karck, M.; Szabó, G. Single dose treatment with PARP-inhibitor INO-1001 improves aging-associated cardiac and vascular dysfunction. *Exp Gerontol* **2007**, *42*, 676-685, https://doi.org/10.1016/j.exger.2007.01.013.
77. Roesner, J.P.; Mersmann, J.; Bergt, S.; Bohnenberg, K.; Barthuber, C.; Szabo, C.; Nöldge-Schomburg, G.E.; Zacharowski K. Therapeutic injection of PARP inhibitor INO-1001 preserves cardiac function in porcine myocardial ischemia and reperfusion without reducing infarct size. *Shock* **2010**, *33*, 507-512, https://doi.org/10.1097/SHK.0b013e3181c4fb08.
78. Morrow, D.A.; Brickman, C.M.; Murphy, S.A.; Baran, K.; Krakover, R.; Dauerman, H.; Kumar, S.; Slomowitz, N.; Grip, L.; McCabe, C.H.; Salzman, A.L. A randomized, placebo-controlled trial to evaluate the tolerability, safety, pharmacokinetics, and pharmacodynamics of a potent inhibitor of poly(ADPribose) polymerase (INO-1001) in patients with ST-elevation myocardial infarction undergoing primary percutaneous coronary intervention: results of the TIMI 37 trial. *J Thromb Thrombolysis* **2009**, *27*, 359–364, https://doi.org/10.1007/s11239-008-0230-1.