THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Investigating Preventive Strategies Against Doxorubicin-induced Myocardial Damage in a Rat Model

by Mária Lódi

Supervisor: Dániel Czuriga, MD, PhD

UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF KÁLMÁN LAKI

DEBRECEN, 2021

Investigating Preventive Strategies Against Doxorubicin-induced Myocardial Damage in a Rat Model

By Mária Lódi, Msc

Supervisor: Dániel Czuriga, MD, PhD

Kálmán Laki Doctoral School, University of Debrecen

Head of the **Examination Committee**:	Csongor Kiss, MD, PhD, DSc
Members of the Examination Committee:	István Balázs Tóth, PhD
	Henriette Gavallér, MD, PhD

The Examination takes place online at 10:00 AM, June 30, 2021.

Head of the **Defense Committee**:	Csongor Kiss, MD, PhD, DSc
Reviewers:	Csilla András, MD, PhD
	Balázs Sax, MD, PhD
Members of the Defense Committee:	István Balázs Tóth, PhD
	Henriette Gavallér, MD, PhD

The PhD Defense takes place online at 11:00 AM, June 30, 2021.

Publicity is guaranteed during the online Defense. If you are willing to participate, please indicate via e-mail to lodi.maria@med.unideb.hu until 4:00 PM on June 29th, 2021.

1. Scientific background

1.1. General features and molecular effects of doxorubicin

Doxorubicin (DOX) is an anthracycline antibiotic agent built on a tetracycline base and connecting amino acids. Shortly after its discovery, this drug became a promising agent in oncotherapy; however, its cumulative, dose-dependent, and cardiotoxic side effects were also revealed.

The main effect of DOX is the same in malignancies and cardiomyocytes. DOX can induce reactive oxygen species (ROS) production in many different ways. Firstly, DOX forms a complex with the DNA double strain leading to the inhibition of the topoisomerase IIβ enzyme. This inhibition results in the break of the DNA double strain. This process starts the activation of the p53 protein, leading to mitochondrial dysfunction and ROS production in the cells. As another possible source of ROS, the semikinon ring of DOX can react with the Fe^{3+} ions, leading to ROS production. At the end of the process, lipid peroxidation and/or protein carbonylation lead to apoptosis.

As a further cardiotoxic effect, DOX can cause abnormalities in the Ca^{2+} homeostasis of the cardiomyocytes and can decrease the value of antioxidant enzymes in the myocardium, thus also leading to apoptosis. Under electron microscope, DOX-induced cardiomyopathy shows well-known signs of damage: the myofilaments are degraded, Z disc deformation can be revealed, and the distortion of the sarcoplasmic reticulum as well as chromatin damage appear in the nucleus.

1.2. The therapeutic usage of doxorubicin, acute and chronic side effects

Despite the cardiotoxic side effects of DOX, it is still widely used in clinical practice for the treatment of a wide range of oncological malignancies. To prevent cardiomyopathy caused by DOX, the cumulative dose of the drug was limited to a maximum of 400 mg/m^2,

where the development of cardiomyopathy is about 3–5%. If a dosage increase is necessary, this percentage can significantly range up.

Heart failure is not the only side effect of DOX. Right after the administration of DOX, ECG changes may appear, which are reversible. As a further effect, early, late-stage, or late-onset cardiomyopathy may develop, the latter 15 years after treatment. Some studies revealed irreversible changes in the myocardium right after the administration of DOX, which may clinically manifest years after oncotherapy. This fact underlines the importance of the early diagnosis of DOX-induced cardiomyopathy, because in this case the cardioprotective therapy may attenuate the symptoms, while the treatment of an advanced staged heart failure may be difficult.

1.3. Preventive strategies against doxorubicin cardiotoxicity

Despite intensive research efforts in the field, DOX-induced cardiomyopathy remains an unresolved problem in clinical practice. In the past few years, several cardio-oncological guidelines attempted to provide instructions for the prevention of DOX-induced heart failure. Communication between cardiologists and oncologists is the first step to reveal the possible risks for the development of heart failure due to chemotherapy. As another preventive method, the optimisation of DOX dosage from a bolus injection to continuous infusion every 3 weeks may decrease the side effects of the agent. Analogues of DOX could be another possibility for prevention, but none of these alternatives have been able to decrease cardiotoxicity yet. As of today, only the liposomal, pegylated formation of DOX could reach a higher concentration in the tumorous region without having any other side effects in the gastrointestinal tract or the heart. Thus, this formulation is only used in patients with high cardiovascular risk in the clinical practice and only as a monotherapy or in a limited number of chemotherapeutic protocols.

Supportive therapies administrated along with chemotherapy were the next step in the prevention process. Vitamin C and E were investigated in numerous animal experiments with varying results. On the one hand, the ion-chelator dexrazoxane was able to prevent DOX-induced cardiotoxicity in animal as well as human studies. On the other hand, the limitation of the use of this drug is its myelotoxic side effect. Medications used in cardiology were also investigated: probucol in animal experimental settings, β-blockers, ACE inhibitors, statins, or angiotensin receptor blockers in clinical studies. These investigations did not lead to unequivocal results. This is why it was important to set up an animal experimental model, where we could investigate the *in vivo* parameters of the animals as well as the tissues with several *in vitro* assays. A guide to develop an appropriate animal experimental model for later experiments was recently published. According to this, the following parameters should be outlined when planning new animal experimental protocols: 1) a high dosage of DOX may cause organ damage not only in the heart, 2) to successfully model the conditions of human oncotherapy, DOX should be administered intravenously, 3) a long follow-up period is necessary after chemotherapy to investigate late-onset cardiomyopathy, 4) the administration of cardioprotective therapy should be prophylactic and 5) the gender of the animals should also be considered, depending on the nature of the experiments.

2. The aim of the study

In our experiments, we wished to model the cardiotoxic side effects of the human DOX chemotherapy protocol and investigate whether prophylactic heart failure medication in combination may have beneficial results compared to that applied only at a later stage. Next, we also investigated the effects of individually applied, prophylactic heart failure drugs in the same model.

3. Materials and methods

3.1. Animal experiments to identify the optimal timing of combined heart failure medications

Twelve-week-old Wistar rats were divided into 4 groups. The negative control (CON) group received saline, while the other animals had 6 cycles of DOX intravenously into the tail vein in a light sedation from the 8th experimental day, on every 3rd day. A prophylactic combination of heart failure medication (2.5 mg/kg bisoprolol, 2 mg/kg perindopril, 6.25 mg/kg eplerenone) was administrated in a vehicle (mucilago hydroxyethilcellulosi) one week before DOX chemotherapy to the PRE group. The dosage of the drugs was uptitrated during the first week to reach the end concentration. Vehicle was administered to CON and D-CON during the whole experiment, and until day 51 to the POST group, too. From the 52nd experimental day the POST group received the same heart failure medication as the PRE animals until the final day of the experiment, also uptitrated for the maximal dosage in the course of one week. Echocardiography was performed on days 0, 51, and 80 in deep anaesthesia, while blood pressure and heart rates were measured on days 0, 7, and 39, in an awake state. After the last echocardiographic measurement, the animals were sacrificed, the tissues were frozen in liquid nitrogen and stored at –70°C.

3.2. Animal experiments to identify the effects of prophylactic, individually applied heart failure medications

Animals of the same age and species used in our previous setup were divided into 5 groups. DOX administration, echocardiography, blood pressure, and heart rate measurements were performed as described earlier. CON and D-CON animals received only vehicle, while BB (2.5 mg/kg bisoprolol), ACEI (2 mg/kg perindopril) and AA (6.25 mg/kg eplerenon) treatments were started prophylactic 1 week before DOX administration until the end of the

experiment; the drugs were uptitrated for 1 week to the maximal dosage. After the last echocardiographic measurement, the animals were sacrificed, the tissues were frozen in liquid nitrogen and stored at –70°C.

3.3. Histology

A piece from the left ventricle was embedded into Shandon^{TM} Cryomatrix and 15 μm thick sections were cut with cryostat. The nuclei were stained with Mayer's hemalum, while picrosirius red staining revealed fibrotic tissue areas. After washing with isopropyl alcohol, sections were dehydrated, mounted with DPX and investigated under an Olympus BX-50 microscope. Representative pictures were taken and analysed using the ImageJ program.

3.4. TUNEL assay

Cryostat sections were fixed with formalin, postfixed with ethanol and acetic acid, then boiled in citrate buffer. Sections were incubated with Tdt, and the nuclei were stained with DAPI. After mounting with Mowiol, the slides were investigated under a Zeiss Axioskop microscope.

3.5. Electron microscopy

Tissue processing was performed using a modified protocol. One piece of the left ventricle free wall was fixed, then washed in phosphate buffer and post-fixed with osmium-tetroxid. After dehydration, the tissues were embedded into DURCUPAN^{TM} ACM resin and sections of 50 nm were made with ultra-microtome. After contrasting with uranil-acetate and Reynold's lead citrate, ultrathin sections were examined under electron microscope and representative pictures were taken. Densitometry was performed using the ImageJ program.

3.6. Isolated, skinned cardiomyocyte measurements

After mechanical isolation and Triton-based skinning, single cardiomyocytes were mounted between an electromagnetic motor and a force transducer. Measurements were performed at 15°C and at a 2.2 μm sarcomere length. Repeated activating and relaxing cycles were evoked in solutions with decreasing Ca^{2+} concentrations to measure the Ca^{2+} activated active forces, the Ca^{2+} independent passive forces and to define the Ca^{2+} sensitivity of the probes. The rate constant of the force redevelopment ($k_{tr,max}$) was measured in the activating solution.

3.7. Determining the oxidative status of contractile proteins

Tissue samples were isolated in the same way as in the case of the isolated, skinned cardiomyocyte measurements. Positive controls were prepared using hypo and Fenton reagents. The samples were then solubilised in a special sample buffer, and vortexed for 1 hour. The protein concentration was measured with the help of a dot blot-based method using BSA standard solutions. Then protein concentration of the samples was set to 3 mg/mL and derivatised with dinitrophenyl-hydrazine. 10% SDS polyacrylamide gels were run and the proteins were blotted onto a nitrocellulose membrane. The blocking and the incubation of antibodies were performed according to the protocol of the company. Protein bands were revealed with a Westernbright ECL kit and normalised to the amount of total proteins. The total protein amount was determined using a super-sensitive membrane staining kit.

3.8. Determining the phosphorylation status of contractile proteins

The heart samples were isolated with the same method used in the experiments for determining the oxidative status, then diluted with 1× Laemmli buffer and vortexed. Protein

concentrations were measured as described above, then the samples were diluted to 50 mg/mL. 10% SDS polyacrylamide gels were run and stained with ProQ™ Diamond gel staining according to the protocol of the company. The protein bands were detected, then the gels were stained with Coomassie to determine the total protein amount for normalisation.

3.9. Using Western immunoblot to identify mitochondrial proteins

Samples of the left ventricular free wall were isolated in an isolation solution, sonicated and centrifugated. 5× sample buffer and β-mercaptoethanol were added to the samples, which then were boiled. After measuring the protein concentrations, 50 µg proteins were loaded onto 8% SDS polyacrylamide gels. After running them, the proteins were blotted onto nitrocellulose membranes and incubated with the following antibodies: anti acetil-coenzyme A carboxylase (ACC) anti phospho-ACC, anti-peroxisome proliferator activated receptor-gamma coactivator 1 α (PGC1α) and anti-Forkhead box protein O1 (FoxO1). After washing, the membranes were incubated with secondary antibodies and after washing, the protein bands were detected. After detection, β-actin labelling was performed on the membranes for normalisation.

3.10. Using Western immunoblot to identify the caspase-3 protein

Deep-frozen left ventricular samples were lysed, then the protein concentration was measured. 35 µg proteins were loaded onto 12% TGX Stain-Free™ acrylamide gels and 4-20% Mini PROTEAN® TGX™ Precast Protein Gels. After blotting, the membranes were blocked and incubated with anti-caspase-3 primary antibody. After incubating with a secondary antibody, the protein bands were detected and normalised to the total protein amount.

3.11. Data analysis and statistics

The measurements of isolated, skinned cardiomyocytes were recorded and analysed with a LabVIEW analysing software package (Myo). The registered data points were plotted using Origin 6.0. Protein bands detected with Western immunoblot were analysed using ImageJ and MagicPlot programs. During our experiments, multiple measurements per animals were performed giving a mean value, which represents the final values (except body weight measurements, body mass index, and strain measurements).

4. Results

4.1. Animal experiments to identify the optimal timing of combined heart failure medications

The survival of the D-CON animals was significantly lower compared to CON, while PRE treatment resulted in a better survival rate. After starting heart failure treatment in the POST group, no more worsening in the originally declining survival rate was observed. The body weight increased continuously in the CON animals, while in the other groups no change could be found in this parameter compared to the beginning of the study. Until the 39th day of the study (which is the day until we were able to use the animals' tails for blood pressure and heart rate measurements), the D-CON and POST animals were exposed to the same conditions, thus blood pressure and heart rate measurement data were pooled. This pooled group had higher heart rate values compared to CON, while the blood pressure was lower in the PRE animals compared to CON. The heart rate values of the PRE group were also lower compared to CON and D-CON+POST.

The last echocardiography revealed a decreased ejection fraction (EF) in the D-CON animals, while the PRE treatment could contribute to the preservation of the EF. The POST treatment had no effect on the DOX-induced EF drop. The diastolic parameter, isovolumetric relaxation time (IVRT) was increased in all of the DOX-treated groups

despite the applied treatments. The longitudinal, systolic strain rate parameter showed decreased values in the DOX-receiving groups, whereas the PRE attenuated this decrease compared to D-CON.

During our histological experiments, we observed a higher rate of fibrotic area in the DOX-treated animals, which could not be affected by any of the treatments. The number and the area of the capillaries in the heart sections showed a slight decrease in the D-CON group, which was not significant compared to other groups. At the ultrastructural level, signs of typical DOX cardiomyopathy could be observed in D-CON animals. In contrast with this finding, the PRE treatment could prevent most of these changes, while the POST animals showed the same pattern observed in D-CON. The damaged myocardium represented a lower value during densitometry analysis (as in D-CON and POST), while seemingly healthy tissues had a higher value (as in CON and PRE).

We found no significant difference between the groups in terms of Ca^{2+} sensitivity, in Ca^{2+} activated active or Ca^{2+} independent passive forces during our mechanical measurements in isolated, skinned cardiomyocytes. The rate constant of the force redevelopment of the cellular preparations was significantly decreased in all of the DOX-treated groups.

The TUNEL assay showed increased rates of apoptotic nuclei in the D-CON group compared to other groups, while the PRE and POST treatments could successfully prevent cardiomyocyte apoptosis. The relative amount of caspase-3 protein was increased in the D-CON and POST groups, while it was preserved in PRE animals.

Our Western immunoblot experiments revealed lower values of the PGC1α protein in the DOX-treated groups despite the applied treatments, while difference in the relative amount of pACC/ACC ratio was non-significant between the groups. During our investigation on the phosphorylation status of the contractile proteins, we found a

statistically marginal increase in a band running at the height of the phospho-troponin I on the gels from the D-CON group compared to CON. The oxidative status of contractile proteins did not show any differences between the animals.

4.2. Animal experiments to identify the effects of prophylactic, individually applied heart failure medications

Decreased values of systolic and diastolic blood pressure could be observed in ACEI receiving animals compared to all other groups, while DOX treatment increased the systolic blood pressure in the D-CON animals. The heart rates of BB receiving animals were significantly lower than in other groups.

The body weight of the CON group developed the same way as in our earlier experiments, while the weight of the animals receiving DOX treatment did not change. The survival of the D-CON animals was also significantly lower compared to CON. The prophylactically applied treatments could induce an increase in animal survival, which was statistically not significant at this number of animals. The lung wet/dry ratios were significantly lower in the BB and ACEI receiving animals compared to D-CON.

We found decreased EF values during our echocardiographic measurements, while the BB treatment could prevent the changes of EF. We observed a significant drop in the EF of ACEI receiving animals compared to baseline, but the end-point EF value did not significantly change from that of the CON group. The IVRT was increased in all of the DOX-treated groups, corresponding to the finding of our earlier experiments. The diastolic strain rate E was decreased in the D-CON animals compared to baseline values, while its preservation could be seen in the BB and ACEI groups.

Histology revealed increased fibrotic area in the D-CON and AA animals, while the BB and ACEI groups had lower levels of fibrosis. We could not find any differences between the groups in the capillary area of the myocardial tissues. The typical signs of DOX

cardiomyopathy could be observed in myocardial ultrastructure of the D-CON animals, while the prophylactic treatments could preserve this damage of the myocardium. In line with this finding, decreased densitometry values could be found in the D-CON group compared to all other groups. Cardiomyocyte diameters were measured at the nuclei level, where increased hypertrophy could be observed in the AA animals, while the ACEI group showed significantly decreased values.

An increased ratio of TUNEL positive nuclei could be found in the D-CON animals. This change could be prevented by all of the prophylactic treatments, while the increase in the relative amount of caspase-3 protein could only be prevented by the BB and ACEI medications.

Measurements in isolated, skinned cardiomyocytes did not show any differences between the groups in terms of Ca^{2+} sensitivity, while the $k_{tr,max}$ values were lower in all of the DOX-treated groups. The active force of the isolated cardiomyocytes was decreased in the AA animals compared to CON.

Western immunoblot showed lower relative values of the PGC1α protein in all of the DOX-treated groups, while we found no significant differences in values of the FoxO1 protein or in the ratio of pACC/ACC between the groups.

5. Discussion

DOX-induced heart failure is still an intensively researched topic, lacking evidence for prevention. Preventive therapies are mainly applied in patients with a high cardiovascular risk, although low-risk patients may also benefit from cardioprotective treatments. For the widespread use of cardiovascular medications in oncological patients, we first need to further investigate DOX-induced cardiomyopathy and the possible effects of cardiovascular medications in this disease both at *in vivo* and *in vitro* levels.

During our animal experiments, we could successfully model human oncotherapeutic protocols in accordance with the points of the guideline: a low dosage of DOX was injected intravenously in several cycles; long-term follow up period was used after the chemotherapy to model late-onset cardiomyopathy; cardiovascular medications were administrated through oral gavage; we investigated multiple timings and setups of the cardiovascular treatments.

All of the cardiovascular medications applied by us have IA recommendations in clinical guidelines for patients with a reduced EF, as they improved mortality and the rate of hospitalisation. For this reason, not only oncological patients with high cardiovascular risk may benefit from the application of these drugs, but lower risk patients too. A meta-analysis confirms this theory as well: the decrease in the relative risk of DOX-induced cardiomyopathy development can be 70% in the case of β-blocker and 90% in case of ACEI application.

Several clinical studies aimed at examining the effect of combining prophylactically applied cardiovascular medications. One of these was the combination of carvedilol and enalapril, where the mortality and heart failure end-points were significantly decreased in the prophylactic patient group. In line with this finding, our PRE treatment could increase the survival rates of animals and prevent DOX-induced decrease in EF. Although this EF decrease was not yet significant at the interim echocardiographic measurement, at our second investigation, the difference between the baseline and follow-up results showed significant changes. We should note the “positive selection” of our experiment, where the deceased animals could have even worse cardiac parameters compared to those who survived until the end of the study. From this aspect, our significant results are even more impressive.

Studies investigating the effects of carvedilol and enalapril applied in a single dose had varying results. In our model, the BB therapy prevented the decrease in EF, attenuated fibrotic remodelling, preserved the ultrastructure of the myocardium and decreased the amount of apoptosis. In contrast, in the ACEI group a significant drop could be observed in the EF compared to the baseline, however the treatment could protect the heart from increased apoptotic activity and pronounced fibrosis compared to the D-CON animals.

Spironolactone, a drug from the AA group, could previously successfully prevent DOX-cardiomyopathy in animal and human experiments. At the same time, another drug from this group, eplerenone had discrepant results in animal experiments. Although this medication could increase the survival rates of animals in our model, it could not prevent the decrease in EF and increase in myocardial fibrosis. The DOX-induced increase in the diastolic parameter, IVRT could not be prevented by any of the treatments, while the DOX-induced decrease in diastolic strain rate could be substantially preserved, mostly by the prophylactic application of BB and ACEI.

In our study, the POST group represented the clinical practice where heart failure medications are applied only after the emergence of manifest cardiomyopathy. Although the survival in this group was prevented after the initiation of the treatment, the *in vitro* parameters did not show any differences between the D-CON and POST animals. These results reveal the importance of the early timing of heart failure medications. In line with this observation, an animal experiment showed increased fibrosis 1 week after DOX application in rabbits, despite the fact that no significant differences could be observed in the echocardiographic parameters. In this study, authors found differences in the expression and phosphorylation state of the titin protein. At the same time, no changes could be observed during isolated, skinned cardiomyocyte experiments, which finding is in line with most of our results from the mechanical measurements. However, we observed lower values

in the $k_{tr,max}$ in all of the DOX-treated animals, which suggests the disruption of the actin-myosin cross-bridge cycle.

The main effect of DOX is ROS production, which affects not only the tumour cells, but the cardiomyocytes as well. Cardiomyocytes are more vulnerable to oxidative stress, thus specific mitochondrial and other ultrastructural changes can be observed in the myocardium after DOX administration. In our study, DOX-induced ultrastructural damage of the myocardium could be prevented by all prophylactic treatments, but not by late-applied therapy.

Our biochemical experiments showed decreased values of the PGC1α protein in all DOX-exposed groups, and this result was not affected by any of the treatments. This protein plays an important role in cellular energy homeostasis and mitochondrial biogenesis. Our results suggest that none of the applied cardioprotective treatments had an effect on the antitumour activity of DOX. Next to ROS production, DOX can also increase the levels of the caspase-3 protein, which is a key apoptotic enzyme, and its increase could also be observed during our experiments. In accordance, increased fibrotic area was also present in the D-CON animals, which could be attenuated with the BB and ACEI treatments. These results show that beside the beneficial haemodynamic effects of BB and ACEI, these agents also exert their protective effects on the molecular level.

Our experiments revealed that individually applied BB and ACEI treatments and their combination have several beneficial effects in DOX-induced cardiomyopathy. Before the introduction of our findings into clinical practice, future clinical trials are necessary.

6. Summary

Doxorubicin- (DOX) induced cardiomyopathy is still an unresolved clinical problem in oncological patients. Although several animal experiments and clinical trials have investigated possible approaches to the treatment of DOX cardiotoxicity, current guidelines include no substantive preventive strategy for oncological patients at a low risk of cardiovascular disease.

In the course of our experiments, we developed a rat model which closely mimicked human oncotherapeutic protocols. Thus, the effects of single or combined heart failure medications (BB, ACEI, AA) for preventing DOX-induced cardiomyopathy could be examined, with special respect to the timing of treatments. According to our results, the prophylactic application of combined heart failure therapy prevented the DOX-induced reduction of EF, damage in the myocardial ultrastructure, as well as cardiomyocyte apoptosis, which eventually led to the better survival of the animals. In contrast, combination therapy applied only at a later stage did not exert these beneficial effects. When examining the effects of the individual components of prophylactic therapy, the best effect on EF could be achieved by BB treatment, while the higher apoptotic activity could be partially reduced by both BB or ACEI treatments. At the same time, the myocardial ultrastructure was successfully conserved by all three drugs. Thus, the highest survival rate could be observed in the BB and ACEI groups.

Taken together, our results suggest that both bisoprolol and perindopril, or preferably the combination of these drugs could potentially attenuate DOX cardiotoxicity when commenced before chemotherapy.

7. List of publications

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

Registry number: DEENK/214/2021.PL
Subject: PhD Publication List

Candidate: Mária Lódi
Doctoral School: Kálmán Laki Doctoral School
MTMT ID: 10057390

List of publications related to the dissertation

1. **Lódi, M.**, Bánhegyi, V., Bódi, B., Gyöngyösi, A., Kovács, Á., Árokszállási, A., Hamdani, N., Fagyas, M., Édes, I., Csanádi, Z., Czuriga, I., Kisvárday, Z., Lekli, I., Bai, P., Tóth, A., Papp, Z., Czuriga, D.: Prophylactic, single-drug cardioprotection in a comparative, experimental study of doxorubicin-induced cardiomyopathy.
   J. Transl. Med. 18 (1), 1-48, 2020.
   IF: 4.124 (2019)
2. **Lódi, M.**, Priksz, D., Fülöp, G. Á., Bódi, B., Gyöngyösi, A., Nagy, L. N., Kovács, Á., Kertész, A. B., Kocsis, J., Édes, I., Csanádi, Z., Czuriga, I., Kisvárday, Z., Juhász, B., Lekli, I., Bai, P., Tóth, A., Papp, Z., Czuriga, D.: Advantages of prophylactic versus conventionally scheduled heart failure therapy in an experimental model of doxorubicin-induced cardiomyopathy.
   J. Transl. Med. 17, 1-16, 2019.
   DOI: http://dx.doi.org/10.1186/s12967-019-1978-0
   IF: 4.124

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

List of other publications

3. Reil, J. C., Reil, G. H., Kovács, Á., Sequeira, V., Waddingham, M. T., **Lódi, M.**, Herwig, M., Ghaderi, S., Kreusser, M. M., Papp, Z., Voigt, N., Dobrev, D., Meyhöfer, S., Langer, H. F., Maier, L. S., Linz, D., Mügge, A., Hohl, M., Steendijk, P., Hamdani, N.: CaMKII Activity Contributes to Homeometric Autoregulation of the Heart: a Novel Mechanism for the Anrep Effect.
J. Physiol.-London. 598 (15), 3129-3153, 2020.
DOI: http://dx.doi.org/10.1113/JP279607
IF: 4.547 (2019)

4. Kolijn, D., Pabel, S., Tian, Y., **Lódi, M.**, Herwig, M., Carrizzo, A., Zhazykbayeva, S., Kovács, Á., Fülöp, G. Á., Falcao-Pires, I., Reusch, P. H., Linthout, S. V., Papp, Z., Heerebeek, L. v., Vecchione, C., Maier, L. S., Ciccarelli, M., Tschöpe, C., Mügge, A., Bagi, Z., Sossalla, S., Hamdani, N.: Empagliflozin Improves Endothelial and Cardiomyocyte Function in Human Heart Failure With Preserved Ejection Fraction via Reduced Pro-Inflammatory-Oxidative Pathways and Protein Kinase Gα Oxidation.
Cardiovasc. Res. 2020, 1-13, 2020.
DOI: http://dx.doi.org/10.1093/cvr/cvaa123
IF: 8.168 (2019)

5. Kolijn, D., Kovács, Á., Herwig, M., **Lódi, M.**, Sieme, M., Alhaj, A., Sandner, P., Papp, Z., Reusch, P. H., Haldenwang, P., Falcao-Pires, I., Linke, W. A., Jaquet, K., Linthout, S. V., Mügge, A., Tschöpe, C., Hamdani, N.: Enhanced Cardiomyocyte Function in Hypertensive Rats With Diastolic Dysfunction and Human Heart Failure Patients After Acute Treatment With Soluble Guanylyl Cyclase (sGC) Activator.
Front. Physiol. 11, 1-21, 2020.
DOI: http://dx.doi.org/10.3389/fphys.2020.00345
IF: 3.367 (2019)

6. Herwig, M., Kolijn, D., **Lódi, M.**, Hölper, S., Kovács, Á., Papp, Z., Jaquet, K., Haldenwang, P., Dos Remedios, C., Reusch, P. H., Mügge, A., Krüger, M., Fielitz, J., Linke, W. A., Hamdani, N.: Modulation of Titin-Based Stiffness in Hypertrophic Cardiomyopathy via Protein Kinase D.
Front. Physiol. 11, 2020.
DOI: http://dx.doi.org/10.3389/fphys.2020.00240
IF: 3.367 (2019)

7. Nagy, L., Gődény, I., Nánási, P. P. i., Leskó, Á., Balogh, L., Bánhegyi, V., Bódi, B., Csípő, T., Csongrádi, A., Fülöp, G. Á., Kovács, Á., **Lódi, M.**, Papp, Z.: A szív pozitív inotróp támogatása a miozin-aktivátor hatású omecamtiv-mecarbil segítségével.
Cardiol. Hung. 47 (1), 69-76, 2017.

8. Nagy, A. C., **Lódi, M.**, Balogh, I., Czuriga, D., Kocsis, J.: Az onkokardiológiai ellátás új irányelvei.
Magyar Onkol. 61 (3), 219-227, 2017.

UNIVERSITY AND NATIONAL LIBRARY
UNIVERSITY OF DEBRECEN
H-4002 Egyetem tér 1, Debrecen
Phone: +3652/410-443, email: publikaciok@lib.unideb.hu

9. Gulácsi-Bárdos, P., Szokol, M., **Lódi, M.**, Czuriga, D., Czuriga, I., Édes, I., Nagy, A. C., Sármán, B.: Ischaemiás szívbetegség és tumoros betegségek együttes előfordulása: kérdések és problémák.
Orvosi Hetilap. 158 (43), 1691-1697, 2017.
DOI: http://dx.doi.org/10.1556/650.2017.30897
IF: 0.322

Total IF of journals (all publications): 28,019
Total IF of journals (publications related to the dissertation): 8,248

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

22 April, 2021

DEBRECENI EGYETEM
Egyetemi és Nemzeti Könyvtár

8. Acknowledgements

First of all, I would like to thank my supervisor, Dr. Dániel Czuriga, for his support, professional guidance, and patronage over the years. The experiments we performed together, the evaluation of the results, and the following brainstorming will always be great memories of mine.

I am very grateful to Professor Zoltán Papp, Head of the Division of Clinical Physiology, for allowing me to do research in the division, improving our work and results with his opinions and critical revisions, and teaching me to do my experiments even more thoroughly.

I would also like to thank Professor Attila Tóth, who always contributed new ideas to our results from a novel perspective, thus highlighting new opportunities in our project.

I am also indebted to Nazha Hamdani, in whose lab I gained a lot of useful insights.

Special thanks to all our collaborators who participated in the completion of our work and the successful publication of the results by contributing their methods and useful comments.

Finally, I would like to thank my parents and my partner. Without their loving support, I would not have been able to complete my PhD research.

This research was supported by the GINOP-2.3.2-15-2016-00043 project (co-financed by the European Union and the European Regional Development Fund) and the EFOP-3.6.2-16-2017-00006 project (co-financed by the European Union and the European Social Fund). Project no. TKP2020-NKA-04 has been implemented with the support provided by the National Research, Development and Innovation Fund of Hungary, financed in the framework of the 2020-4.1.1-TKP2020 funding scheme.