SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Prevention and treatment of oxidative stress-induced damage to preserve reproductive and cardiac function

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Introduction

One of the important elements of cellular homeostasis is the balance of agents with reductive and oxidative properties to maintain normal function. If the redox balance is upset and molecules with oxidative properties become predominant, can lead to the development of oxidative stress. The harmful effect of oxidative stress on tissues is well documented and is the central theme of many previous studies [Mancini; Matin]. Cells use different defense mechanisms to maintain their homeostasis, against oxidative stress.

In my thesis, I investigate the response of two very important organs to oxidative stress and the possibilities of protection against them in animal experimental models.

In our first series of experiments (I.), we induced oxidative stress in the testes by injection of isoproterenol (ISO), and then examined the effects of regular, moderate swimming training, both in the semen and in the testicular tissue [Osváth].

In the second study, we examined the protective effect of paclitaxel in cardiac muscle tissue, after the injection of isoproterenol, which was used as a damaging agent [Matusovits].

I. Relationship between male fertility and physical activity

Although fertilization is essential for the survival of all species, the underlying signalling pathways and biochemical mechanisms remain only partially understood. In our first series of experiments, we examined the effect of moderate physical exercise in animal experiments as one of the options for protection against induced oxidative stress [Osváth]. In the humans, sperms are particularly sensitive to changes in their environment. It is well documented that, increased production of reactive oxygen species results in a state of oxidative stress, with a cascade of events leading to damage lipids, proteins and deoxyribonucleic acid (DNA). All of these can lead to subfertility, infertility and/or early termination of pregnancy [Mancini].

In the review of literature in the thesis, we will go through the conditions that cause oxidative stress, their known effects on male fertility, and the positive effects of exercise at the population and individual level.

In the present study, we used isoproterenol, a non-selective β-adrenergic receptor agonist, to induce tissue damage through transient ischemia [Szabó; Jimenez; Díaz-

Muñoz]. Our research group previously confirmed that the underlying mechanisms of tissue damage are due to an imbalance of oxidant/antioxidant homeostasis and accelerated inflammatory processes [Díaz-Muñoz]. We also proved that the damage caused by isoproterenol was successfully restored by regular, moderate exercise in estrogen hormone-deficient female rats [Szabó]. In addition to the modulation of processes mediated by estrogen, a decrease in the endogenous testosterone level can be associated with a reduced antioxidant capacity and an elevated level of inflammatory parameters [Varga].

II. Paclitaxel and its effects

Another series of experiments (II.) was also carried out on the topic of oxidative stress in relation to another extremely important organ, the heart [Matusovits].

Today, paclitaxel-coated stents and balloons are frequently used in clinical practice to reduce the high rate of post-interventional restenosis observed in procedures using standard uncoated intracorporeal devices [Spargias; Steiner].

Although there is a large body of research on the oncological and vascular use of paclitaxel, due to the lack of adequate data, it is difficult to draw conclusions about its cellular effects in the myocardium. In our study, we aimed to analyze the potential cardioprotective properties of paclitaxel in rats exposed to isoproterenol-induced cardiac injury.

Objectives

Examination I

The aim of our **first** study was to investigate the hypothesized beneficial effects of moderate-intensity physical exercise (swimming) on the antioxidant status and inflammatory parameters of the testicles, the testosterone concentration, and the binding capacity to hyaluronic acid, thus the fertility of the individual.

Examination II

In our **second** study, similar to the previous one, the aim was to investigate the isoproterenol-induced oxidative damage and the mechanisms that protect against it, using paclitaxel.

Our hypothesis in this study was that paclitaxel administration protects the heart by suppressing oxidative and inflammatory processes such as TNF-α and NF-κB proinflammatory signaling pathways and stimulating the expression of protective molecules such as HO-1 and superoxide-dismutase (SOD).

Materials and methods

We used male (Harlan-)Wistar rats in both of our studies. During the tests, we kept the number of rats to the minimum necessary to perform the experiments. The sample size was determined based on similar previous experiments and experimental work carried out in our laboratory and by calculating the resource equation.

Examination I:

Setting the study groups

At the beginning of the study, the rats were divided into five groups as follows:

(1) Absolute controls (CTRL); (2) Isoproterenol-treated (ISO); (3) Pre-treatment swimming training + isoproterenol (PRE+ISO); (4) ISO + swimming training after treatment (ISO+POST), (5) Swimming before treatment + isoproterenol treatment + swimming after treatment (PRE+ISO+POST)

In our study, we used a maximum of 9 rats per group (total number of animals n = 45). The testicular morphological changes were induced by an injection of isoproterenol 1.0 mg/kg, which was diluted in 1 ml of physiological saline solution and injected under the skin. The appropriate dose of isoproterenol was set in our previous studies, and in preliminary experiments we also made sure that the dose used was not high, but sufficient to cause myocardial damage [Chigurupati]. During the study, we made every effort to minimize stress factors for the animals.

Exercise protocol

The experimental animals were trained individually in a pool filled with water for 5 days a week for 3 weeks [Cheng]. Animals in the PRE+ISO group were trained for 3 weeks before the isoproterenol injection, while the animals in the ISO+ POST group were trained for 3 weeks after the isoproterenol injection. The rats in the PRE+ISO+POST group were trained for 3 weeks before and after isoproterenol treatment. At the end of the entire experimental period, blood, semen, and testicular tissue samples were collected, on which the following tests were performed:

- *Serum testosterone levels were determined* from a blood sample; the result was measured by chemiluminescence immunoassay and expressed as ng/dl.

- *Semen collection*: Artificial ejaculation was achieved with electroejaculation: Whole semen samples were used for the biochemical measurements and the hyaluronic acid binding test.

- *Hyaluronic acid binding test* (HBA®): The functional evaluation of sperm cells was carried out with HBA® tests, which assesses motile sperm cells.

- Proinflammatory cytokines: determination of *TNF-α and IL-6 concentration*: The concentration of proinflammatory cytokines TNF-α, IL-6 was determined using the ELISA method from a testicular tissue sample.

- Determination of *reduced, oxidized, and total glutathione levels* (GSH, GSSG; GSH+GSSG): Total glutathione content (GSH+GSSG), indicating the antioxidant status of the testes, was measured from the semen and testicular tissue samples.

Total glutathione content was expressed as nmol/mg protein content. The measurement of GSH+GSSG content is based on the biochemical reaction (catalytic effect) of GSH or GSSG in the reduction of DTNB with a mixture of glutathione reductase and NADPH.

- Measurement of the *myeloperoxidase* (MPO) enzyme activity: after the proper preparation, tissue samples of the rat testicles were examined spectrophotometrically. The *myeloperoxidase* activity of sperm and testes was expressed in μU/mg protein content.

- *Protein analysis*: The diluted testicular and sperm samples were examined spectrophotometrically using a commercially available protein determination kit. The protein level was expressed in mg protein/ml.

- *Statistical analysis*: Statistical calculations were performed with the software GraphPad Prism for Windows. All data are presented as group mean ± standard deviation (SD). The Shapiro-Wilk normality test was used to estimate the Gaussian distribution. The analysis was then performed using a one-way analysis of variance (ANOVA) test, followed by Tukey's multiple comparison post-test, post hoc analysis (if F reached $p<0.05$ and the normality test was passed). Statistical analyzes were performed only for experiments where the size of each group (n) was at least 5. Probability values (p) of less than 0.05 were considered significantly different, and the exact p-values are presented for each group in the Results chapter, on scatter plots.

Examination II.

The following materials and methods have been used in the study to investigate isoproterenol-induced myocardial damage and the enzymes and signaling pathways involved in its reduction:

- *Setting the study groups*: After acclimatization to the experimental conditions, the Wistar rats were divided into 4 groups (CTRL, ISO, PAC, ISO+PAC). Intraperitoneally (IP) saline (CTRL) or 1 mg/kg isoproterenol (ISO) was administered. Blood samples were taken 20 hours later from the saphenous vein for lactate dehydrogenase (LDH) and myoglobin levels determination to confirm myocardial damage. Three weeks after isoproterenol treatment, paclitaxel (PAC) (10 mg/kg/day) was administered orally for 5 days [49] to both control rats (PAC) and isoproterenol-treated rats (ISO+PAC).

The left ventricles of each rat were harvested for biological measurements 24 hours after the last oral dose of paclitaxel. Left ventricular samples were pulverized in liquid nitrogen and stored at -80◦C until the analysis.

The following tests were then performed on the myocardial tissues:

- Determination of the *concentration of HO-1, NF-kB and TNF-α* in the heart: The pulverized ventricular sample was homogenized in ice-cold phosphate buffer, and after centrifugation further ELISA and protein analyzes were performed.

The data were determined at an optical density of 450 nm with a microplate reader.

Mean ± SD	Control	ISO	PAC	ISO + PAC
MPO (μU/mg of protein)	16,511 ± 5944	27,697 ± 4979	16,967 ± 3453	17,260 ± 2979
HO-1activity (nmol bilirubin/h/mg protein)	0.431 ± 0.0629	0.035 ± 0.0259	0.36 ± 0.1346	0.242 ± 0.05391
HO-1 (pg/mg of protein)	491.1 ± 58.87	237.8 ± 42.52	467 ± 50.29	381.4 ± 79.82
Total Glutathione (nmol/mg of protein)	3.894 ± 0.5405	1.758 ± 0.5383	3.718 ± 0.7018	2.762 ± 0.7259
SOD (ng/mL)	141.9 ± 3.1	64.6 ± 4.624	132 ± 9.95	109.1 ± 14.96
TNFα (pg/mg protein)	9.33 ± 1.395	15.63 ± 2.089	10.13 ± 1.103	12.16 ± 1.542
NFκB (pg/mg of protein)	189.4 ± 83.26	411.8 ± 42.69	119.6 ± 29.52	185.7 ± 47.8

MPO: myeloperoxidase, HO-1: heme oxygenase-1, SOD: superoxide dismutase, TNF-α: tumor necrosis factor-alpha, NF-kB: nuclear factor kappa-B

- Assessment of the *heart's total glutathione* (GSH+GSSG) level: After preparing the buffers and adding the reagents, DTNB formation was detected. GSH levels were expressed as nM/milligrams of protein.
- Investigation of *superoxide dismutase* (SOD) in the myocardial tissue: The SOD activity of

the heart muscle was measured using a specific kit (Abcam, ab65354). Results were expressed in ng/mL.

- M*yeloperoxidase activity* measurement of the myocardial tissue: After the appropriate procedure, myeloperoxidase activity was detected from the pulverized chambers at 490 nm and expressed in μU/mg of protein.

- Determination of *heme oxygenase activity* of the heart: Heme oxygenase-1 activity was determined as the amount of bilirubin produced per hour (in nmol) and given per milligram of protein.

- *Statistical analysis*: Statistical analysis was also performed with the GraphPad Prism software. Probability values (p) less than 0.05 were considered significant and marked with an asterisk on the graphs.

Results

Examination I.

- Changes in *serum testosterone* levels: Serum testosterone levels were measured at the end of the experimental period in all groups and the serum concentration of testosterone significantly decreased due to isoproterenol compared to the control group. Pre-treatment swimming (PRE) or post-treatment swimming (POST) separately produced significant increases in testosterone compared to the untrained ISO group. Moreover, the combination of pre- and post-treatment swimming (PRE+ISO+POST) resulted in androgen levels twice as high as either pre-treatment or post-treatment exercise.

- *Hyaluronic acid binding test* (HBA®) results were significantly reduced in animals treated with isoproterenol compared to the control group, while 3 weeks of swimming training, either before or after isoproterenol injection, or in combination, significantly increased HBA values.

- ***Pro-Inflammatory cytokine tests***

- Determination of *TNF-α and IL-6 concentration* in the semen

As expected, the level of TNF-α in semen was significantly higher after the administration of isoproterenol compared to the control group, and similar results were also obtained for the concentration of IL-6. However, three weeks of regular swimming significantly attenuated these increases in pro-inflammatory cytokines in both the PRE+ISO and POST+ISO groups. Combined swimming training before and after treatment (PRE+ISO+POST) had the most significant beneficial effect on the concentrations of TNF-α and IL-6 compared to the isoproterenol treatment group without training.

All observed changes were statistically significant compared to the ISO and CTRL groups.

- Measurement of *testicular TNF-α and IL-6 concentration*

In the isoproterenol-treated group, a significant increase in IL-6 concentration was observed in the testes compared to the control animals. Swimming before or after isoproterenol administration attenuated the adverse effect of isoproterenol, resulting in a significant decrease in testicular IL-6 concentration compared to isoproterenol-treated and control groups. The combined training consisting of swimming before and after the treatment proved to be the most effective in terms of reducing the elevated IL-6 concentration induced by isoproterenol.

Similar to the change in IL-6 concentration, isoproterenol treatment significantly increased the level of TNF-α in the testis compared to the control group. Swimming training was again effective in reducing TNF-α levels in the PRE+ISO and POST+ISO groups, and especially in the PRE+ISO+POST groups.

- Determination of *myeloperoxidase activity*

Like the changes observed in pro-inflammatory cytokine levels in semen and testes, MPO activity was significantly higher in isoproterenol-treated animals than in the controls. It was again revealed that the inflammatory processes related to the testicles and semen are alleviated by regular physical activity: in the PRE+ISO and POST+ISO groups, a significant decrease was observed in MPO activity compared to the isoproterenol-treated and control groups. Furthermore, swimming before and after the treatment (PRE+ISO+POST) caused the greatest decrease in MPO activity compared to the group treated only with isoproterenol.

- Evaluation of *testis and semen glutathione* (GSH + GSSG) content

Compared to control animals, the level of GSH was significantly reduced in both the semen and the testis; however, regular swimming before or after isoproterenol treatment significantly improved the antioxidant status of testes and semen. Again, combined pre- and post-treatment training resulted in the greatest improvement in GSH values: changes were significant compared to both the CTRL and ISO groups.

Examination II. results

The results of our second series of experiments in evaluating the myocardial tissue were as follows:

- *Heme oxygenase-1 (HO-1) protein expression*

HO-1 protein concentration was significantly reduced by isoproterenol treatment, while paclitaxel alone did not affect HO-1 protein expression in normal rat hearts. When paclitaxel was co-administered with isoproterenol, HO-1 concentrations did not differ from control levels.

- *Heme oxygenase (HO) protein activity*

The activity of heme oxygenase was also significantly reduced by isoproterenol treatment, while paclitaxel alone did not affect the activity of the enzyme in rat hearts. When paclitaxel was co-administered with isoproterenol, HO activity was not statistically different from isoproterenol-suppressed activity.

- *Superoxide dismutase (SOD) concentration*

SOD-protein concentration was significantly decreased in the group treated with isoproterenol alone, while paclitaxel alone did not affect protein expression in control hearts. When paclitaxel was given together with isoproterenol, the SOD concentration returned to the control level and there was no statistically significant difference between the groups.

- *Total glutathione content*

Total glutathione levels decreased with isoproterenol administration, but remained at control levels when paclitaxel alone was administered. The combined treatment with isoproterenol and paclitaxel significantly increased the total glutathione level compared to the group treated with isoproterenol alone, without any difference compared to the control.

- *Myeloperoxidase activity*

The myeloperoxidase activity increased in the group treated with isoproterenol, which returned to the control level with the co-administration of paclitaxel. Paclitaxel alone had no effect on myeloperoxidase activity.

- *NF-κB protein expression*

Isoproterenol significantly increased the concentration of NF-κB, while paclitaxel alone had no effect on its expression. When paclitaxel was co-administered with isoproterenol, NF-κB levels were restored and were not significantly different from control.

- *TNF-α protein expression*

TNF-α protein concentration was significantly increased in the group treated with isoproterenol alone, while paclitaxel alone did not affect protein expression in control hearts. When co-administered with paclitaxel, the level of TNF-α was reduced compared to the administration of isoproterenol alone and was not significantly different from the control.

Discussion

I. The relationship between male fertility and exercise

Our first study shows that isoproterenol-induced oxidative stress promotes sterile orchitis and hormonal imbalance, characterized by low testosterone concentration and a decrease in the antioxidant capacity of the testes, these processes ultimately lead to a decrease in the mature/immature sperm ratio. These adverse changes may be associated with a decrease in male fertility.

Our findings on the negative effects of oxidative stress on the reproductive system are consistent with several studies that suggest that oxidative stress causes testicular dysfunction. These studies also suggest that oxidative stress in the testicular milieu is associated with DNA damage and abnormal sperm production, which explains the increasing frequency of reduced male fertility [Kumar; Karna; Turner]. Moreover, according to studies, oxidative stress factors play a causal role in the development of sperm abnormalities and testicular dysfunction [Agarwal; Cocuzza].

From these studies it is clear that the isoproterenol-induced oxidative stress and the consequent reduced blood supply to the testicles, along with the decrease in testosterone secretion, reduces the hyaluronic acid binding of sperm, as we demonstrated in our present experiment. In addition, in our rat model, we found significantly lower glutathione levels both in the testis and in the semen of animals treated with isoproterenol, which suggests that isoproterenol-induced testicular damage destroyed the tissue's antioxidant protection. The reduced testicular glutathione level may also play a role in the impairment of testosterone biosynthesis after isoproterenol treatment.

Our experiments also shown that isoproterenol-induced oxidative stress generates an inflammatory process through the 'up-regulation' of MPO/TNF-α/IL-6, which may also contribute to reduced testosterone concentrations and reduced proportion of mature sperm counts, leading to subfertility or infertility.

In our study, we observed increased myeloperoxidase activity after isoproterenol treatment, suggesting that the oxidative stress caused by isoproterenol in the testicular tissues is associated with neutrophil accumulation. However, in our rat model, the negative effects of isoproterenol were alleviated by moderate swimming. In the present study, we investigated the effect of moderate swimming before and/or after treatment to protect against the inflammatory response induced by isoproterenol and reduced capacity of the GSH-mediated antioxidant system.

To the best of our knowledge, our study is the first to clarify whether moderate swimming exercise has a protective role against oxidative stress-induced testicular inflammation in a rat model.

Our results show that either pre-treatment or post-treatment swimming alone is effective against isoproterenol-induced adverse effects; however, the most significant benefit was in the combined 'pre- and post-treatment swimming' group, indicating that moderate physical activity (swimming) has a strong potential to improve isoproterenol-induced inflammation and shifts in antioxidant capacity. Specifically, moderate-intensity swimming improved the antioxidant status by enhancing the reduced activity of the endogenous antioxidant enzyme, glutathione, and successfully reversed the increased myeloperoxidase activity in the testes and semen.

To further support our hypothesis, we also measured the serum concentration of pro-inflammatory cytokines. Analysis of testicular tissue and semen from male rats showed that moderate-intensity swimming was associated with a reduction in IL-6 and TNF-α levels, further confirming that regular physical activity can reduce testicular inflammation. These observations are consistent with the results of glutathione activity, serum testosterone, and semen HBA analysis.

Based on these previous results and the results of the present study, we hypothesize that moderate-intensity swimming can inhibit pro-inflammatory cytokines by reducing oxidative stress, which can promote testosterone synthesis by increasing anti-inflammatory cytokines.

In our study, we used isoproterenol injection, which resulted high oxidative stress and high inflammatory response of the testicular tissues in male rats. This is supported by increased levels of MPO, IL-6 and TNF-α, as well as decreased levels of GSH. In addition to molecular and biochemical changes in the testicular tissue, profound changes also appear in the semen.

We found that the percentage of mature sperms with hyaluronic acid binding ability improved significantly as a result of exercise.

The present study suggests that disruption of the antioxidant defense system and elevated inflammation have adverse effects on the male reproductive system, which may lead to testicular damage and adverse changes in semen. However, moderate-intensity swimming can have a significant protective effect against testicular oxidative stress and inflammation and can improve the mature/immature sperm

ratio.

II. Paclitaxel and its effects

The main finding of our study with isoproterenol and paclitaxel was that paclitaxel protected the myocardium against isoproterenol-induced damage by reducing the levels of inflammatory cytokines and increasing the concentration of antioxidant and anti-inflammatory molecules. Administration of isoproterenol led to a significant increase in the level of the inflammatory molecules NF-κB, TNF-α and MPO, while depleting or inhibiting the activity of the antioxidant and anti-inflammatory molecules HO-1, SOD and GSH in the left ventricular tissue. On the other hand, when administered in combination with isoproterenol, paclitaxel restored the levels of these molecules towards control levels.

Our study suggests that local toxicity can be prevented with the oral administration of paclitaxel [Kim]. Limitations of the current study include the lack of morphological indicators of cardiac damage or measurement of microtubular structures. However, it is well known that isoproterenol induces structural and functional changes in rat heart muscle similar to those observed in humans [Althunibat; Chen].

Conclusions

I. The relationship between male fertility and exercise

Summarizing our first study, 1mg/kg isoproterenol injection reduced the production of the antioxidant GSH and 'up-regulated' the MPO-IL6-TNF-α inflammatory pathway in the testes and sperm, leading to stress-induced testicular damage. This is the first evidence that moderate-intensity physical activity (swimming) can effectively alleviate the negative effects of isoproterenol-induced inflammation and antioxidant capacity damage on the male reproductive system, so it can be used as a preventive and/or therapeutic strategy against adverse effects such as oxidative tissue damage, to counteract inflammatory processes, reduced testosterone biosynthesis and unfavorable mature/immature sperm ratio.

Our results provide insights into the biochemical background of the beneficial effects of moderate swimming, which may serve as an effective approach to improve male fertility.

Results of this study also supported by the results of our own clinical studies, according to which a lower BMI indicates a greater ability to fertilize, since regular exercise is an important cornerstone of body weight control.

II. Paclitaxel and its effects

Our second study, investigating the protective effect of paclitaxel on the myocardium, proved that oral administration of paclitaxel effectively maintains the expression of important antioxidant and anti-inflammatory molecules, HO-1, SOD and GSH, and suppresses TNF-α, MPO and NF-κB production, those are involved in oxidative and inflammatory processes during myocardial damage. In addition, a major component of this cellular defense appears to be the expression of HO-1, which can reduce ROS, TNF-α, and thereby NF-κB signaling in the myocardium. [Kumazawa; Issan; Lawrence] Future studies should also analyze the levels of other cytokines, such as members of the interleukin family (e.g. IL-1, IL-6), as these have been shown to play a role in various inflammatory states of the heart [80;81], specifically blocking IL-1 reduced doxorubicin-induced damage in the myocardium, which may indicate a potential target in the isoproterenol-damaged model [Székely]. It would be useful to further investigate the expression of the transcription factor Nrf2 in the same model to clarify whether paclitaxel has a direct effect on the *de novo* synthesis of antioxidant and anti-inflammatory molecules.

Summary

Maintaining the cellular homeostasis is prerequisite for cell survival, the most important method for this is the maintenance of the redox balance, the mechanisms against oxidative (or reductive) stress. Oxidative stress is suffered by all tissues of the body and implicated in the pathomechanism of many diseases as the ultimate cell-damaging factor. The signalling pathways involved in oxidative stress are only partially understood, and the intracellular mechanisms that mediate the process are also not fully understood.

In our study, we investigated the reversal of the damaging effects of oxidative stress. In the reproductive system, oxidative stress mostly affects the polyunsaturated fatty acid components of cell membranes and induces inflammatory processes, well characterized by the level of pro-inflammatory cytokines. Oxidative stress was induced by the intraperitoneal administration of isoproterenol, and then the experimental animals participated in moderate-intensity training (swimming) according to the protocol described above. After the three-week training period, blood, testicular tissue and semen were collected. At the end of the experimental period, we measured serum testosterone levels, sperm hyaluronic acid binding and total glutathione content, as well as myeloperoxidase activity, Tumor Necrosis Factor alfa and Interleukin-6 concentrations in the testis and semen.

Serum testosterone levels, hyaluronic acid binding and glutathione content of semen decreased significantly, while the concentration of myeloperoxidase, Tumor Necrosis Factor-alfa and interleukin-6 in the testis and semen increased after isoproterenol treatment compared to the control group.

Our results clearly show that regular, moderate-intensity swimming effectively alleviated the negative effects of oxidative stress.

The results of our research may serve as the first evidence that regular swimming provides protection against the harmful, oxidative effects induced by isoproterenol on the inflammatory parameters of the male reproductive system, so based on our animal model we can conclude that it is a form of exercise that can be reasonably recommended in clinical practice for those suffering from oxidative stress to restore or prevent testicular function. It would also be useful to examine these results in a human population, of course without the administration of isoproterenol, in patients with testicular oxidative stress due to smoking or varicocele.

In our second study, we also examined the intracellular mechanisms of protection

against isoproterenol as a damaging, oxidative stress-causing agent after paclitaxel administration. Paclitaxel is a drug frequently used in cardiology to cover intravascular devices to prevent restenosis. Its effect is highly dose-dependent, which has been proven in previous studies.

In our experiment, we investigated the preservation of isoproterenol-damaged myocardial function with the administration of paclitaxel orally. Our results showed that heme oxygenase-1 protein concentration, heme oxygenase activity, superoxide dismutase protein concentration, and total glutathione decreased significantly after isoproterenol treatment.

When paclitaxel co-administered with isoproterenol, the concentration of heme oxygenase-1, superoxide dismutase and total glutathione did not differ from control levels. Myeloperoxidase activity, Nuclear Factor kappa B concentration and Tumor Necrosis Factor-alpha protein concentration were significantly increased in only in the group receiving isoproterenol, while the level of these molecules was restored when it was used together with paclitaxel.

Our study demonstrated that the use of paclitaxel protected the myocardial tissue from the damaging effects of isoproterenol on oxidative stress, also demonstrated that oral paclitaxel suppresses oxidative and inflammatory processes and this suppression is achieved through the stimulation of protective enzymes such as superoxide dismutase and heme oxygenase.

Key words

isoproterenol, exercise, swimming, oxidative stress, inflammatory cytokines, male fertility, HBA, paclitaxel, myocardial damage

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Candidate: Péter Osváth
Doctoral School: Doctoral School of Nutrition and Food Sciences
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List of publications related to the dissertation

1. Matusovits, D., Murlasits, Z., Kupai, K., Baráth, Z., Kang, H. L., **Osváth, P.**, Szűcs, M., Priksz, D., Juhász, B., Radák, Z., Várkonyi, T., Pávó, I. J., Pósa, A.: Paclitaxel Protects against Isoproterenol-Induced Damage in Rat Myocardium: its Heme-Oxygenase Mediated Role in Cardiovascular Research.
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DOI: http://dx.doi.org/10.3390/antiox12051129
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2. **Osváth, P.**, Szűcs, M., Börzsei, D., Szabó, R., Lesi, Z. N., Turcsán, Z., Veszelka, M., Sebestyén, J., Juhász, B., Priksz, D., Varga, C., Pósa, A.: Andrological Aspects of Exercise: moderate Swimming Protects against Isoproterenol Induced Testis and Semen Abnormalities in Rats.
Antioxidants. 11 (3), 1-14, 2022.
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List of other publications

3. Szűcs, M., **Osváth, P.**, Jakab, A., Varga, D., Varga, B., Juhász, B.: Hyaluronan bound mature sperm count (HB-MaSC) is a more informative indicator of fertility than conventional sperm parameters: correlations with Body Mass Index (BMI).
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7. Berczi, C., **Osváth, P.**, Tóth, Á., Flaskó, T.: Retroperitoneális tumor terhességben.
Uroonkológia. *9* (3), 68-70, 2012.

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