STUDY ON MEDICAL INTERACTIONS IN METABOLIC SYNDROME

PhD THESIS ABSTRACT

Scientific Coordinator,
Prof. Elena Cătălina LUPUȘORU, PhD

PhD Student,
Ana Roxana RUSU (GĂNCEANU-RUSU)

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The thesis includes 126 pages, 57 figures, 67 tables and 412 citation references. For editing the abstract, a limited number of figures and tables was selected preserving their numbering in the thesis.

Keywords: metabolic syndrome, cardiovascular diseases, immuno-biochemical markers, pharmacological therapy, adverse effects.
INTRODUCTION

Metabolic syndrome (MS) is a progressive disease involving a wide range of disorders with specific metabolic abnormalities and constituting a risk factor for cardiovascular disease (CV), where insulin resistance and visceral adiposity play an essential role.

The association between MS and the increased risk of multiple chronic conditions (CV, arthritis, chronic kidney disease, schizophrenia, neoplasms) has been reported for several decades. The lack of coherence in the clinical definition of MS and component limit values implies complex efforts to accurately capture its impact and identify prevention strategies. Using the definition developed by the International Diabetes Federation (IDF), MS prevalence in the US is estimated at >30%; however, using the criteria for the adult treatment group, the prevalence is estimated at around 22%.

The mechanism by which MS causes imbalances in the body is not entirely clear at this time, but some suggest that it is also due to the systemic oxidative stress caused by obesity. A proposed mechanism by which obesity produces oxidative stress is mitochondrial and peroxisomal oxidation of fatty acids, which in turn can generate other oxidation reactions.

Clinical identification and management of patients are important in order to begin the correct administration of the treatment and to reduce the risk of subsequent illness. Pharmacological treatment should be considered in patients whose risk factors are not adequately reduced in the application of preventive measures and lifestyle changes.
CHAPTER IV
PERSONAL CONTRIBUTION

In the experimental studies carried out within this PhD thesis, we want to achieve results that could open the perspective of a better predictability regarding the control of the blood pressure values, in the MS treated with associations between ACE and NSAIDs, but also of the haematological, biochemical, immunological modifications on markers of oxidative stress and inflammation, in MS rats subjected to stress due to physical strain. The results obtained can provide useful information to ensure the optimization of current treatment regimens.

THE OBJECTIVES OF THE DOCTORAL STUDIES

The general objective of the study

The inconsistency of existing data in the literature on the pharmacodynamics effects of ACE associations with NSAIDs in laboratory animals has led to the study of the effects of such associations on blood pressure values, biochemical status, immunological status and oxidative stress in experimentally challenged MS rats exposed to stress.

Specific objectives

The specific objectives proposed to achieve the overall objective of this experimental study were as follows:
- Experimental research on the effects of Enalapril with Indometacin, Ketoprofen or Nimesulide on blood pressure values in cholesterol-induced MS rats;
- Impact evaluations from the combination of Enalapril with Indometacin, Ketoprofen or Nimesulide on haematological profile (leukocyte formula), biochemical (cholesterol, lipid profile, cortisol), immunologic (phagocytosis capacity of peripheral blood neutrophil polymorphonuclear), MS-induced rat cholesterol;
- Experimental investigation of the effects of Enalapril with Indometacin, Ketoprofen or Nimesulide on markers of inflammation (interleukin: IL-1β, IL-6, TNF-α) and oxidative stress (superoxide dismutase, malondialdehyde, glutathione peroxidase) MS-induced cholesterol diet;
- Experimental investigation of the effects of combined administration of Enalapril with Indometacin, Ketoprofen or Nimesulide on locomotor activity and physical exercise resistance to the rat treadmill test (forced locomotion test);
- Making and highlighting changes in clinical-paraclinical investigations in patients with metabolic syndrome;
- Assessing the correlations between biochemical markers and specific medication in patients diagnosed with metabolic syndrome.
METHOD OF STUDY

Experimental research

For experiments, albino Wistar rats (weighing between 150-200g) were assigned from the “Grigore T. Popa” University of Medicine and Pharmacy, Iași.

For induction of dyslipidemia, all animals were subjected to cholesterol diet (0.2 g / kg body weight / day, 4 weeks).

The animals were distributed in 9 batches (6 rats) and received the following substances, single-shot, intraperitoneal injection, following the protocol:

**Lot M1 (witness1):** 0.8% NaCl (Normal Salt, NS) - 0,5ml/100g body;
**Lot M2 (witness 2):** cholesterol diet
**Lot ENP:** Enalapril – 1 mg / kg body weight / day;
**Lot IND:** Indomethacin – 1 mg / kg body weight / day;
**Lot KET:** Ketoprofen – 3 mg / kg body weight / day;
**Lot NMS:** Nimesulid – 1,5 mg / kg body weight / day;
**Lot ENP+IND:** Enalapril – 1 mg / kg body weight / day + Indomethacin – 1 mg / kg body weight / day;
**Lot ENP+KET:** Enalapril – 1 mg / kg body weight / day + Ketoprofen – 3 mg / kg body weight / day;
**Lot ENP+NMS:** Enalapril – 1 mg / kg body weight / day + Nimesulid – 1,5 mg / kg body weight / day.

The animals were brought the day before for accommodation, being kept under optimum laboratory conditions (constant temperature of 21 ° C ± 2 ° C, relative humidity of 50-70% and alternating illumination mode (light / dark ratio = 12 hours / 12 hours) with specific granular feed and water *ad libitum.*
Experimental procedures:

Effort Test:
The physical exercise capacity analysis after administration of the test substances was performed using the Treadmill Test (stress test or forced locomotion test) of the rat over a 10-minute interval. This experimental model is used to evaluate the motor function and effort resistance of laboratory animals.

The device consists of a plexiglass box (14 cm x 36 cm x 33 cm) in which there is a drive belt driven by a powerful engine that has the ability to transmit different rotational speeds. Collected data is viewed on a display and the device is connected to a computer to collect the results.

The conveyor belt is made of a special material that ensures good motion performance under intense use conditions.

Methodology: The animal is positioned at the end of the treadmill (in the grid area) and allowed to move continuously, the speed being adjustable (over 150 cm / min) according to species ratio (300 cm / min in the rat). The moving conveyor can be tilted (with variations between -25 and +25 degrees) to increase the effort to which the animal is moving on the ramp.

The displacement area has a suitable width for the animal to be able to coordinate its displacement, thus allowing a precise assessment of the effort resistance, fatigue moment, without motor coordination disturbances. Also, when the animal wears and stops, electric shocks of constant intensity (from 0 to 2 mA) can be applied through the grille in the form of currents flowing through its body.
This experimental model is part of the classical behavioral test group, used to evaluate the rat motor function and its ability to withstand sustained physical effort. At the same time, the exploration of the motor coordination and resistance of the laboratory animal under stress-induced by forced labor.

A retrospective study was carried out at the Third Medical Clinic of the Emergency County Clinical Hospital "Sf. Spiridon ", Iași. Of the initial 250 monitored patients, 143 patients returned to the clinic for reassessment after 12 months of treatment. In order to perform this study, the patient's clinical observation sheet was used as a working instrument, with the elaboration of special fiches on: clinical, biochemical and therapeutic aspects.

RESULTS

IV.1. EXPERIMENTAL RESEARCH

This behavioral test, by which the animal is subjected to forced physical effort, motivated by a penalty, is considered an experimental model of stress, being physiologically very different from the physical exercise, even very intense, by man.

By measuring the distance traveled by the animal in running on the conveyor, the capacity for resistance to physical effort is assessed within the assigned time interval.

Administration of Enalapril produced an increase in the distance traveled by the animal on the conveyor but statistically insignificant compared to the control group. The treatment with the NSAIDs tested resulted in a
decrease in the distance traveled, statistically significant (* p < 0.05) for IND and NMS, compared to the control (fig.IV.2).

**Fig. IV.2.** Effects of ENP, IND, KET and NMS on the distance traveled by rats in the effort test. The values are the mean ± standard deviation (DS) of the mean distance traveled (m) for 6 rats. * p < 0.05 relative to the control

In fig. IV.4, it can be seen that the majority of shocks were administered to group 3 receiving *Indometacin* and the distance traveled by the experimental animals was the smallest. The same situation was found in groups 5 and 6, where *Nimesulide* and *Enalapril* were administered in combination with *Indometacin*.

In the conveyor belt test the experimental animals experienced electrical shocks, while the distance traveled and the maximum number of shocks were measured. The use of *Enalapril* was accompanied by a decrease in the number of electrical shocks applied to the animal on the conveyor, but without statistical significance, compared to witness lot.
Fig. IV.4. The batch relationship of the distance traveled to the number of shocks administered in the stress test

In this experimental model, reducing the amount of time to apply electrical shocks or reducing the number of electrical impulses applied to the animal to forcibly continue the movement means an effect of increasing the stress resistance produced by the test substance.

In contrast, prolonging the length of time to apply electrical shocks or increasing the number of electrical shocks needed to drive the animal's motor activity is the expression of a decrease in physical exercise resistance produced by the investigated substance.

Effort test results indicate a longer duration of shock in rats requiring a higher number of electrical stimuli (fig. IV.7).
IV.1.7.2. Analysis of correlations between the components of oxidative stress and inflammatory syndrome with metabolic syndrome components in rats subjected to physical stress

➢ **Evaluation of changes in serum cortisol values in rats with cholesterol diet**

After the stress test of Wistar rats, there was a significant increase in serum cortisol in all the groups receiving the cholesterol diet, but a value close to that of the control group was obtained in rats given *Enalapril*.

The most prominent increase in serum cortisol values under experimentally induced forced effort was found in the control group with cholesterol diet (fig. IV.27).

NSAID treatment reduced the level of plasma cortisol but insignificantly statistically compared to the control group receiving cholesterol diet, during the stress test.

The association of *Enalapril* with the studied NSAIDs decreased serum cortisol values but statistically
insignificant compared to both the control group without cholesterol diet and the control group with cholesterol diet, under stress conditions. The most pronounced effect was found for the combination of Enalapril + Ketoprofen (fig. IV.27).

Fig. IV.27. Changes produced by test substances on cortisol values in rats with cholesterol diet (a. Batch 1-6 b. Groups 4-9)

- **Evaluation of changes in SOD activity in rats with cholesterol diet**

In animals with physiological serum and cholesterol diet, subjected to effort stress, the decrease in SOD values was noted compared to the control cholesterol group, which is consistent with the data in the literature on activity variations SOD in forced labor. Treatment with Enalapril resulted in an increase in SOD activity, statistically significant from the group receiving the cholesterol diet and underwent the test of the conveyor belt.

Administration of NSAID did not conclusively alter the activity of SOD in the batch of serum and cholesterol diet under forced labor conditions of the rat. The use of the combination between Enalapril and the
NSAIDs studied did not result in significant variations in the determined levels of SOD, compared to the control cholesterol test group, in the rat exertion test (fig. IV.28).

![SOD (U/mg protein) values in rats with cholesterol diet and stress](image)

![SOD (U/mg protein) values in rats with cholesterol diet and stress](image)

**a.**

**b.**

**Fig. IV.28.** Changes produced by test substances on SOD activity in rats with cholesterol diet (a. Batches 1-6; b. Batches 4-9)

- **evaluating changes in MDA activity in rats with cholesterol diet**

At control animals receiving the cholesterol diet, there was a mild, insignificant increase in MDA, compared to the group with saline but without cholesterol. In control animals receiving a cholesterol diet, there was a slight, statistically insignificant increase in MDA, compared to the physiological saline group but without cholesterol diet.

Administration of NSAID did not conclusively alter the MDA activity against the serum with saline and cholesterol diet under forced labor conditions of the rat.

The use of the combination between ENP and the NSAIDs studied did not result in significant variations in MDA levels from the control cholesterol control group in the exercise test (FIG. IV.29).
IV.2. PHARMACOEPIDEMIOLOGICAL RESEARCH

IV.2.7.3. Changes in specific markers during treatment of metabolic syndrome

At patients treated with beta blockers (111 cases and 77.6% of the patients studied), improvements in marker levels involved in MS diagnosis were noted, with significant decreases observed for blood glucose and cardiometabolic risk factor.

Of all the evaluated parameters, abdominal circumference (AC) and HDL-cholesterol had the smallest changes, with a decrease of just 3 cm between the two AC evaluations and an increase of only 3 mg / dl of HDL-cholesterol, changes statistically insignificant (p> 0.05).

In patients treated with ACE, significant decreases in BP were observed compared to ARBs, while blood glucose and cardiometabolic risk factors decreased more in those treated with ARBs (p = 0.040 vs. 0.049).
In addition to improvements in renal function, inflammatory markers, total cholesterol and LDL-cholesterol, a statistically significant (p = 0.001) decrease in liver transaminases (AST, ALT and GGT) was also obtained.

Patients receiving Acetylsalicylic Acid may also have a decrease in inflammatory markers even if the dose of drug administered has more antiplatelet properties than anti-inflammatory.

The same situation was observed in patients receiving Clopidogrel. Thus, statistically significant changes in blood glucose and cardiometabolic risk were observed (p ≤ 0.05), while the remaining parameters evaluated in MS were statistically significant changes.

**IV.2.7.4.2. Correlations between administered medication and adverse effects**

The association of adverse effects with the change in heart rate in re-evaluated patients is the only statistically confirmed. Thus, patients with dyspepsia, myalgia and xerostomia have experienced an improvement in heart rate since the last evaluation.

In the present study, there was no relationship of dependence between ACE, ARBs and statins and the occurrence of adverse effects, the results being statistically insignificant.

The results of adverse events occurring after the use of beta blockers show that only 12.5 mg of Carvedilol has the least side effects (vertigo and xerostomia) compared to Bisoprolol 5 mg and Nebivolol 5 mg.
IV.2.7.4.4. Interaction of medication with ischemic heart disease and neurological disorders

In the present study, the results of the non-parametric test indicate the association of platelet antiaggregants with ischemic cardiomyopathy and beta-blockers with neurological disorders, the results obtained being statistically significant.

From the analysis of the distribution of ischemic cardiomyopathy cases in relation to the administered medication, it follows that: 52% of patients treated with Acetylsalicylic Acid (Aspenter®), 83% of patients treated with Aspirin Cardio, 59% of patients treated with Clopidogrel and 27% of patients treated with Triflusal (Platrox) have ischemic cardiopathy.

The analysis of the distribution of cases of neurological affection in relation to the medication administered (fig. II.59) shows that: 70% of the patients treated with Bisoprolol, 55% of the patients treated with Carvedilol, all patients treated with Metoprolol and 77% Nebivolol also suffers from neurological damage.

From ACE, it can be seen that Perindopril is commonly associated with the occurrence of adverse effects, between statins Atorvastatin, and between ARBs, Telmisartan and Candesartan.

MS symptoms are not immediate and direct (cause-effect), but they are associated and interconnected, so that although the damage is obvious, it is quite difficult to determine with certainty the moment when the whole process begins to take place.

MS requires more in-depth studies before its definition of "syndrome" is fully justified and before its clinical utility is adequately defined.
In the future, the increase in the number of people with MS will bring other requirements to the health system. Maintaining an independent and active lifestyle for as long as possible is a crucial factor for the quality of life in the aging process. These findings underline the importance of ongoing efforts to identify and treat MS as soon as possible to prevent atherosclerotic disease.

Long-term additional population studies are needed in both men and women to clarify the key factors to be included in the MS definition, the relative importance of the various MS components, and the associations of risk factors that are most useful in predicting outcomes disease.

Considering the nonspecific presence of the inflammatory process and associated markers, there is a definite need to continue research into the validation of a specific panel of markers as a pathognomonic of the MS for appropriate diagnostic and prognostic use in laboratory medicine.

With this perspective, it is imminent to consider that MS, a group of risk factors for cardiometabolic disorders, becomes a serious pathogenic entity in an endemic proportion. This fatal occurrence can be attributed mainly to our genetic creation, which was originally intended for better survival in a poor environment, but was eventually influenced and modified epigenetically to become a complex phenotype in the modern environment, where a completely different lifestyle, with access to excess food, prevails.

Significantly, as a major change in the MS pathology paradigm, recent evidence suggesting visceral adiposity has been able to explain the link between
metabolic changes and inflammatory pathologies involving cytokines and adipokines.

MS is indeed a major global problem that predisposes and represents the initial phase for more severe pathologies, thus requiring special attention and implementation of cardiovascular prevention programs at national level.

CHAPTER VI
CONCLUSIONS

- **Enalapril** exhibited a beneficial effect on the decrease in total cholesterol, LDL-cholesterol, and triglycerides in cholesterol diet rats;

- The administration of ACE and / or NSAIDs significantly improves the process of chronic inflammation in rats with metabolic syndrome induced experimentally through cholesterol diet for the time period of the experiments;

- Treatment with **Enalapril** produced increased locomotor behavior and increased resistance to exercise and protective effects on oxidative stress in rats subjected to forced exercise in the conveyor belt test;

- Administration of **Indometacin**, **Ketoprofen** and **Nimesulide** had the effect of lowering the resistance of laboratory animals to effort in the forced locomotion test, with the most pronounced effects being produced by **Indometacin**;

- The combination of **Enalapril** with **Indometacin**, **Ketoprofen** and **Nimesulide**, respectively, slightly
improved the resistance of the animals to the effort, compared with the single-agent administration of non-steroidal anti-inflammatory drugs;

- NSAIDs have reduced the antihypertensive effect of *Enalapril* by lowering the resistance of laboratory animals to forced physical effort;

- Hypertensive patients have a pro-inflammatory and procoagulant status as well as elevated levels of uric acid, which implies an increased risk of cardiovascular disease;

- Statin treatment has diminished associated inflammatory processes in patients with metabolic syndrome;

- The use of *Telmisartan* moderately decreased plasma levels of uric acid in the patients enrolled in the study;

- *Perindopril* therapy reduced serum C-reactive protein levels in patients with metabolic syndrome;

- *Acetylsalicylic Acid*, in platelet-derived antiaggregant doses, and *Clopidogrel* have led to a decrease in elevated blood glucose levels in patients with metabolic syndrome;

- Treatment with ACE, ARBs and statins did not produce neurological adverse effects or specific clinical manifestations of coronary ischaemia;
The association between beta-blockers and statins has been accompanied by changes in myocardial ischemia, and the use of anti-platelet medication has produced neurological manifestations.

SELECTIVE BIBLIOGRAPHY


