UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL SCHOOL

PhD THESIS
-ABSTRACT-

THE NEUROPROTECTIVE ROLE OF THIOCTIC ACID AND VITAMIN B COMPLEX IN DIABETIC NEUROPATHY

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CONTENT

1. INTRODUCTION

The theme of this study was detached from the requirements of medical practice in the field of nutrition and metabolic diseases, taking into account the incidence and the effects of diabetes together with its complications in pathology and its growth in the coming decades.

The most common complication of diabetic disease is neuropathy (approximately 50% of patients with diabetes, develop damages of the peripheral nervous system).

This is the main motivation for elucidating the aspects of pathophysiology of the diabetic neuropathy, thus understanding the mechanisms of production, outlining new approaches in preventing and treating this complication, promoting the establishment of a beneficial status in the life of patients suffering from diabetes.

Therapies used today fail to provide effective control of this complex diabetic complication, so we focused our study on the treatment by testing the

effects of combined administration of elements known to be beneficial in treating neuropathy, but the potentiation of their simultaneous application has not yet been highlighted, aspect studied by us both experimentally and clinically.

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2. CURRENT STATE OF KNOWLEDGE

In Chapter 2.1 we structured 3 subchapters: definition, diagnosis and classification of diabetic neuropathies, epidemiological aspects and clinical features. All these aspects being supported by the cited bibliography.

Chapter 2.2 presents classic and recent notions about altered processes in the pericarion and axonal extensions, as well as the impairment of insulin signaling, risk factors for diabetic neuropathy and its production mechanisms.

Chapter 2.3 addresses the therapy of diabetic neuropathy - first-line therapy (tricyclic antidepressants and selective serotonin and norepinephrine reuptake inhibitors), secondary and third-line therapy.

3. PERSONAL CONTRIBUTION

3.1. WORKING HYPOTHESIS AND GENERAL OBJECTIVES

The main objective of this thesis was to evaluate the neuroprotective role of thioctic acid and vitamin B complex in diabetic neuropathy.

Analyzed individually, each of the two therapies has proven its neuroprotective capacity in diabetic neuropathy, this can be seen in various experimental or observational studies, but the effect of their concomitant use has not been analyzed. Therefore, we proposed in this paper to conduct both an experimental study, using an animal model of diabetic neuropathy, and a clinical study, in which we examined the effect of the concomitant use of thioctic acid (alpha lipoic acid) and vitamin B complex. in diabetic neuropathy, to highlight a possible mutual potentiation of them.

The personal contribution of this paper is represented, on the one hand, by the experimental study on an animal model of diabetic neuropathy and, on the other hand, by a clinical study, observational-descriptive, performed on a representative group of patients with diabetes.

In order to concretize the main objective through the experimental study, we considered under the direct coordination of the mentor and the members of the guidance commission, the following secondary objectives:

- creating a complete database, with all models of diabetic neuropathy on experimental animals;
- creating a group of animals, to which diabetes will be induced and selecting a control group;
- extension of the histopathological and morphometric knowledge, which intervene in the pathogenesis of diabetic neuropathy and trying to better understand its mechanisms;
- identification of new biomarkers for neuropathy in experimental animals, in which diabetes was induced;

- identifying and defining the morphological parameters that characterize the peripheral nervous system, in order to achieve correlations with the pathogenesis of diabetic neuropathy;
- testing the combined effect of alpha thioctic acid and vitamin B complex in diabetic neuropathy;
- electrophysiological evaluation of the animals included in the study;
- highlighting the effects of the therapies tested by ameliorating or by unfavorable changes of the electrophysiological parameters followed.

Also, in order to achieve the main objective through the clinical study, under the direct coordination of the mentor and the members of the guidance commission, we took into account the following secondary objectives:

- compiling a database of patients diagnosed with diabetes who have diabetic neuropathy;
- outlining the clinical characteristics for the patients included in the study;
- creating groups of patients that will be treated with thioctic acid, or vitamin B complex, or with combined therapy and also a control group, which will not be subjected to these therapies;
- identification of possible prognostic and therapeutic targets considering the structural and functional alterations of the peripheral nervous system induced by diabetes.

3.2. RESEARCH METHODOLOGY

3.2.1. Approaching the topic

In this subchapter I described the approach taken in the investigation process, in which I described the orientation and theoretical justification of the chosen research topic.

3.2.2 Material and method

3.2.2.1.1. Induction of diabetes.

I performed the study in the Animal Facility of the University of Medicine and Pharmacy of Craiova. I requested and obtained the approval of the University and Scientific Ethics and Deontology Commission within UMF Craiova.

In order to study diabetic neuropathy we induced diabetes mellitus in C57BL / 6 mice by intraperitoneal injection of a body dose of 150 mg / kg streptozotocin (STZ).

3.2.2.1.2. Experimental groups.

The experiments were performed on 30 C57BL / 6 mice, male and female, aged between 8 and 10 weeks, weighing between 21 and 28 grams.

The animals were randomly divided into five distinct lots, as follows:

1. Sham Group. Only 0.9% NaCl was injected into this group of animals, without streptozotocin being injected, so without diabetes.
2. Control group. This group of animals was injected with stretozotocin, 150 mg / kg body weight, intraperitoneally, single administration, at 8-10 weeks of age. The group was not given any therapy

3. Therapeutic dose of alpha thioctic acid group. In this group of animals, after inducing diabetes by injecting intraperitoneal STZ and confirming the presence of diabetes, the therapeutic protocol was applied by intraperitoneal administration of 100 mg / kg body weight alpha thioctic acid.
4. Therapeutic dose group of vitamin B complex. In this group of animals, after induction of diabetes, the therapeutic protocol was applied. by intraperitoneal administration of a vitamin B complex, as follows: thiamychlorohydrochloride-B1 100 mg / kg body weight and pyridoxynhydrochloride-B6 50 mg / kg body weight.
5. Therapeutic dose group of alpha thioctic acid and vitamin B complex. In this group of animals, after induction of diabetes, the therapeutic protocol was applied, both by intraperitoneal administration of alpha thioctic acid (see the therapeutic protocol described in no. 3), but also of the vitamin B complex (see the therapeutic protocol described in no. 4).

3.2.2.1.3. Assessment of functional biomarkers of diabetic neuropathy.

Functional biomarkers induced by diabetic neuropathy were analyzed by electrophysiological monitoring, respectively by recording the compound motor action potential (CMAP).

3.2.2.1.4. Assessment of structural biomarkers of diabetic neuropathy.

To assess the structural biomarkers of diabetic neuropathy, we analyzed, from a histological point of view, the sciatic nerve, both in mice with diabetes and in mice without this disease.

3.2.2.2. Design and type of clinical trial.

Another objective of this study was to evaluate classical therapies, represented by adequate glycemic control and lifestyle change versus classical therapies combined with new antioxidant therapies in diabetic neuropathy.

In this regard, we conducted an observational, prospective study, between October 2017 and December 2019, which included 188 patients with diabetic neuropathy.

3.2.2.3 Statistical data processing.

The numerous values obtained, both in the experimental study and in the clinical study, were statistically processed, in order to analyze certain characteristics of diabetic neuropathy, as well as, the way they may evolve over time. For data analysis we used Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and Graph Pad Prism statistical analysis program (version 6 or newer, GraphPad Software, La Jolla, CA, USA). To compare the averages of the different groups studied, as appropriate, we used the following statistical methods: T Student test. ANOVA test and Pearson correlation test. In all cases where we calculated the value of $P < 0.05$, we considered that there is a statistically significant difference between the compared means from the various groups.

3.3. RESULTS

3.3.1. Experimental study

3.3.1.1. Characteristic parameters followed

The glycemic values obtained before the administration of streptozotocin, in order to cause diabetes, but also in the following weeks are presented in Figure 3.3. We note that the diabetes induction protocol, used in our study, was characterized by a natable increase of the plasma glucose levels around approx. 600 mg / dl, this value being maintained at this level throughout our study. At the time of inclusion in the study, the animals weighed as follows: sham group 23.53 ± 1.16 g, control group 25.35 ± 2.35 g, alpha thioctic acid treated group 26.15 ± 1.49 g, the group treated with vitamin B complex 25.53 ± 2.06 g and the group treated with both alpha thioctic acid and vitamin B complex 25.03 ± 2.07 g. These data are shown in Figure 3.4.

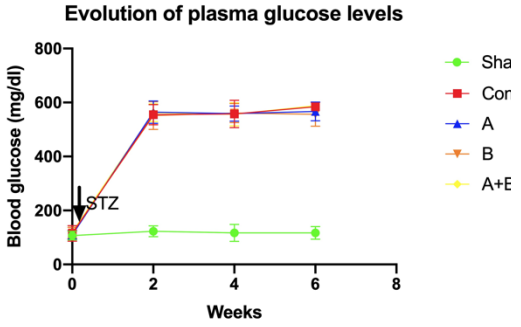

Figure 3.3. Plasma glucose levels in the STZ groups of animals and, also, in the Sham group, which did not receive STZ. A high mean plasma glucose level was observed 2 weeks after STZ, 4 weeks and 6 weeks. There were no statistically significant differences between the groups in terms of plasma glucose levels depending on the therapy applied.

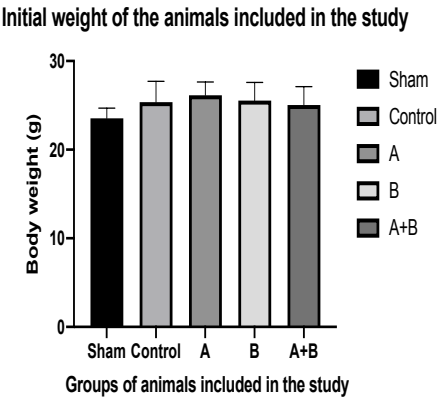

Figure 3.4. The initial weight of the animals included in the study according to the various therapeutic protocols.

From an electrophysiological point of view, we analyzed several parameters of the compound motor action potential (CMAP). These were:

- CMAP amplitude (mV)
- CMAP latency at onset (ms)
- Peak CMAP latency (ms)
- CMAP area (mVxms)
- CMAP duration (ms)

Regarding the amplitude of CMAP we noticed that at the inclusion in the study there was a variation of the initial amplitude in the absence of STZ administration, there are variations between different groups of animals, as shown in Figure 3.8. For this reason, we statistically analyzed the percentage variation of the CMAP amplitude (Figure 3.9).

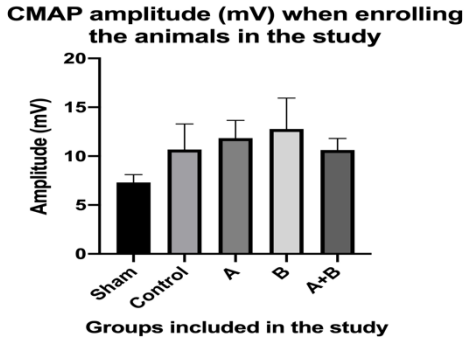

Figure 3.8. CMAP amplitude (mV) when enrolling the animals in the study. It is observed that the initial average amplitude differs when enrolling the animals in the study, that conditioned us to analyze in the study their percentage variations and not the absolute variations.

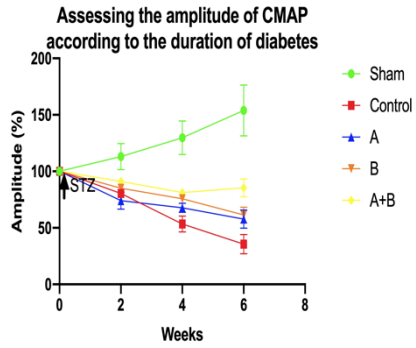

Figure 3.9. The variation of the percentage amplitude of CMAP for the different groups of animals included in our study.

To highlight the evolution of compound motor action potential (CMAP) in mice without diabetes (sham group) or in the presence of diabetes (but in the absence of any therapy), we exemplified in Figure A 3.28 both the amplitude of CMAP and electroneuromyography at inclusion in the study and in weeks 2, 4 and 6 after the enrollment. We analyzed (Figure 3.40) the microscopic images and found that in the groups of animals with diabetes the integrated optical density of myelin decreased (control = 76 ± 38, A = 93 ± 45, B = 84 ± 37, A + B = 103 ± 49), compared to the group of animals without diabetes (sham = 187 ± 49). It should be noted that treatment with alpha lipoic acid and/or vitamin B complex slowed down this process.

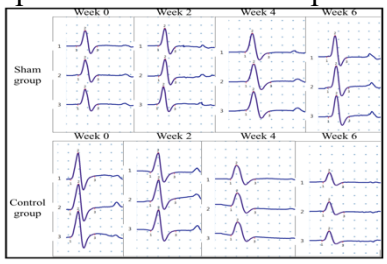

Figure 3.29. The evolution of CMAP in the sham group (without diabetes) and in the control group (with diabetes). There is a significant decrease in amplitude and an increase in latency in the group with diabetes.

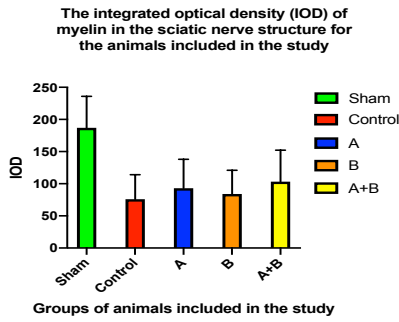

Figure 3.40.

3.3.2. Clnical study

The aim of our study was to evaluate the classical therapies represented by adequate glycemic control and lifestyle changes versus classical therapies, combined with new antioxidant therapies in diabetic neuropathy.

Depending on the MNSI score one year after inclusion in the study, each patient was included in the "responders" group if the MNSI dropped below 7 or in the "non-responders" group if the MNSI did not dropped below 7. Based on this criteria, 34.04% (n = 64) of the patients were "responders" to the classical therapy, while 16.49% (n = 31) of the patients were "non-responders"

to the classical therapy. On the other hand, a higher number of patients were "responders" to combined therapy (n = 73, 38.83%) and only 21.51% (n = 20) were not "responders" to combined therapy (p = 0.101, OR = 0.56, 95% CI 0.2967 - 1.106 and reciprocal OR = 1.76, 95% CI 0.9206 - 3.370). These data are illustrated in Figure 3.41.

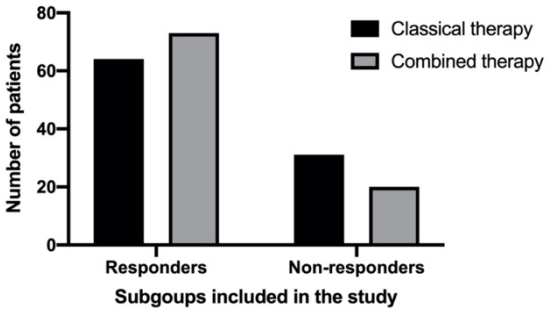

Figure 3.41.

5. DISCUSSIONS AND CONCLUSIONS

Following the experimental study, we concluded:

1) The diabetes induction protocol used in our study was characterized by a marked increase in plasma glucose levels around approx. 600 mg / dl, this value remaining at the same level throughout our study.
2) In all groups of animals with induced diabetes mellitus, there was a marked decrease in body weight and this was not influenced by any therapeutic protocol used.
3) From an electrophysiological point of view, in the absence of diabetes, the amplitude of CMAP increases with age (6 weeks after inclusion in the study registering a percentage increase of approximately 50%), while in the presence of diabetes, the amplitude of CMAP decreases considerably (in the control group, 6 weeks after inclusion in the study there is a percentage decrease of approximately 40%). Treatment with alpha thioctic acid and vitamin B complex decreased the rate of this process in animals with diabetes (a decrease of only 10-20%).
4) CMAP latency until the onset of response, increases significantly in the group of animals with diabetes mellitus (an increase of approximately 0.6 ms at 6 weeks after induction), for which no therapeutic protocol was applied, while in the groups of animals without diabetes (sham) or with diabetes, but who received therapies with thioctic acid and / or vitamin B complex, there was a less pronounced increase in this parameter at 6 weeks after the induction of diabetes (approximately 0.2-0.3 ms).
5) Regarding the duration of CAMP, there was an increase in animals without diabetes (on average 0.8 ms), while in animals with diabetes there was a decrease, a decrease attenuated by thioctic acid treatment protocols and by vitamin B complex (0.4 ms compared to 0.2 ms).

6) Between the weight of the animals included in the study and the amplitude of CMAP, a positive correlation is observed between these parameters, the higher the weight of the animals, the greater the amplitude of CMAP (R = 0.8247, 95% confidence interval 0.6608-0 , 9136, P = 0.0001).
7) From the point of view of histopathological analysis we found that in the groups of animals with diabetes the integrated optical density of myelin decreased (control = 76 ± 38, A = 93 ± 45, B = 84 ± 37, A + B = 103 ± 49), compared to the group of animals without diabetes (sham = 187 ± 49).

Following the clinical study, we concluded:

1) There is an increase in response to combined therapy after one year of treatment and this has been associated with short duration of diabetic neuropathy, insulin treatment, low glycated hemoglobin, absence of dyslipidemia or associated cardiovascular disease. Thus, combined therapy is an alternative to reduce side effects and increase efficacy.
2) Combined therapy with thioctic acid and vitamin B complex has a greater effect in stopping axonal degeneration in diabetic neuropathy than the therapy with thioctic acid alone or vitamin B complex alone.

As a final conclusion we can say that the combined therapy with thioctic acid and vitamin B complex is an effective option, without significant risks, which can be used in current medical practice. Thus, the main objective of the study was met also by achieving the secondary objectives. The hypothesis evaluated in our study have a significant character of originality, with applicability in the medical practice.

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