Original Paper

The Neuroprotective Role of Alpha Thioctic Acid and Vitamin B Complex in Diabetic Neuropathy - an Experimental Study

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ABSTRACT: Worldwide, approximately 463 million people are estimated to suffer from a form of diabetes mellitus, with diabetic neuropathy being one of its most common complication. Using streptozotocin to induce diabetes in C57BL/6J mice, we assess the neuroprotective role of alpha thioctic acid and vitamin B complex in diabetic neuropathy. In order to highlight the peripheral nerve changes produced by diabetes, we performed an electroneurographic recording of the animals and compared the amplitude of the compound muscle action potential (CMAP). Treatment with alpha thioctic acid (A), or vitamin B complex (B), or A+B caused a smaller decrease in CMAP amplitude than if these therapies had not been applied. On the other hand, we found that in group A+B a smaller decrease of CMAP amplitude was observed compared to the control group (6 weeks after the onset of diabetes $p<0.0001$). Also, separate treatment with alpha thioctic acid alone caused a smaller decrease in CMAP amplitude compared to the control group (6 weeks after the onset of diabetes mellitus $p<0.0436$), but also separate treatment with vitamin B complex alone resulted in a smaller decrease of CMAP amplitude compared to the Control group (6 weeks after the onset of diabetes $p<0.0070$). The combined therapy with alpha thioctic acid and vitamin B complex has a greater effect in preventing axonal degeneration in diabetic neuropathy than the single therapy only with alpha thioctic acid or only with vitamin B complex.

KEYWORDS: *Alpha thioctic acid, vitamin B complex, diabetes mellitus, diabetic neuropathy, axonal degeneration.*

Introduction

Worldwide, approximately 463 million people are estimated to suffer from a form of diabetes mellitus in 2019. By the year 2045 this number will increase to approximately 700 million patients [1-3].

The high cost associated with this disease is not just due to a direct medical treatment but also the costs associated with diabetic complications.

The increase of the patients suffering from diabetes worldwide will be accompanied by the increase in complications of this disease [4].

Polyneuropathy, a frequent complication of diabetes, affects more than half of the patients suffering from diabetes mellitus [1,4].

One of the most common complication of diabetes is diabetic neuropathy [4].

The clinical problem with diabetic neuropathy the fact that it represents a heterogeneous group of afflictions that impact different parts of the nervous system [5].

Several mechanisms that impact the nervous system have been described starting from direct axonal degeneration caused by the accumulation of acylcarnitines (derived from acetyl-CoA) and ending with an oxidative stress approach that show a high production of reactive oxygen species (ROS) that lead to damage of Schwann cells and glycation of many structural and functional proteins [6-8].

Unsurprisingly, due to the complex pathophysiology of diabetic neuropathy, it's therapy has undergone numerous changes among the years and multiple therapies have been designed to target different pathogenesis aspects of this disease.

The morbidity and mortality in patients suffering from diabetes mellitus will be reduced by early recognition and proper treatment of diabetic neuropathy [5,6].

Considering that hyperglycemia is involved in the pathophysiology of diabetic neuropathy, the most effective treatment for diabetic neuropathy should be glycemic control [4].

However, this has been shown to be difficult to achieve in clinical practice, and although it is the most important part of the therapy of the diabetic neuropathy [5,6], multiple promising therapies are currently the target of experimental studies or they are already in clinical trials.

In the present paper we aim to experimentally quantify the neuroprotective role of alpha thioctic acid and vitamin B complex in a mouse model of diabetes, analyzing the functional changes associated with experimental diabetic neuropathy.

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Material and Methods

Animals and experimental groups

The study was performed on 8-10 weeks wild-type C57BL/6J mice in order to evaluate the clinical and electrophysiological impact of diabetes neuropathy.

In total, 30 mice were used for this study, randomly divided into five groups:

(a) one that was only injected with saline, considered to be the Sham (N=6),

(b) another in which diabetes was induced but not treatment was given (N=6),

(c) a third one that received alpha thioctic acid as treatment (N=6),

(d) a forth one that received a vitamin B complex as treatment and a last one (N=6 (e) that received both alpha thioctic acid and a vitamin B complex as treatment (N=6).

Before the beginning of the experimental procedures, the animals were housed in a controlled 12h/12h of light/dark cycle with free access to food and water.

Two days prior to the start of all procedures, animals were taken to the behavior room where they were kept until the end of all procedures.

The weights for each animal was recorded before group placement.

All experiments have been carried out in the Animal Facility of the University of Medicine and Pharmacy of Craiova in strict compliance with the recommendations of European and Romania guidelines for the welfare of experimental animals.

Diabetes induction and electroneurography recordings

Diabetes was induced using streptozotocin (STZ).

A single intraperitoneal injection of 150mg/kg STZ was made in all animals except the Sham group where saline was injected. Electrophysiological recordings and glucose testing were performed once every two weeks.

Before determining glucose levels and physiological recordings a light anesthesia was induced by injecting 100µl solution of Ketamine (50mg/ml) and Xylazine (7mg/ml) intraperitoneally.

Prior to blood glucose determination food was taken from the animals one night before, with tests being done in the first part of the morning.

Glucose levels were recorded using venous blood taken from the tail.

A portable Counture plus one (Ascensia Diabetes care, Switzerland) glucose strip machine was used to determine blood glucose levels.

Electrophysiological monitoring was performed using a Neuro MEB4 (Neurosoft, Russia) electromyography device.

The depth of anesthesia was tested by pinch reflex.

Bepantene (Bayer, Germany) was placed on the eyes of the animals during all procedures. For recording we used 4 monopolar needle electrodes (SEI EMG, Italy).

Two electrodes were used to perform nerve stimulation and the other two to record nerve impulses.

The recording bandwidth was set between 20Hz and 10kHz, with a sweep speed setting at 1ms/division and a 5mV/division sensitivity.

For each animal, three stimuli were applied, each with a duration of 0.2ms, and a current intensity 5mA (first stimulation) and 6mA for the next two, if the motor amplitude recorded din not increase.

In two cases a stimulation of 7mA was used to reach maximal compound motor action potential (CMAP).

The final analyzed parameters for CMAP were calculated as averages of the maximal CMAP produced by the last two stimuli (Figure 1).

Before STZ injection glucose levels and electroneurography were recorded for all animals in order to establish a baseline.

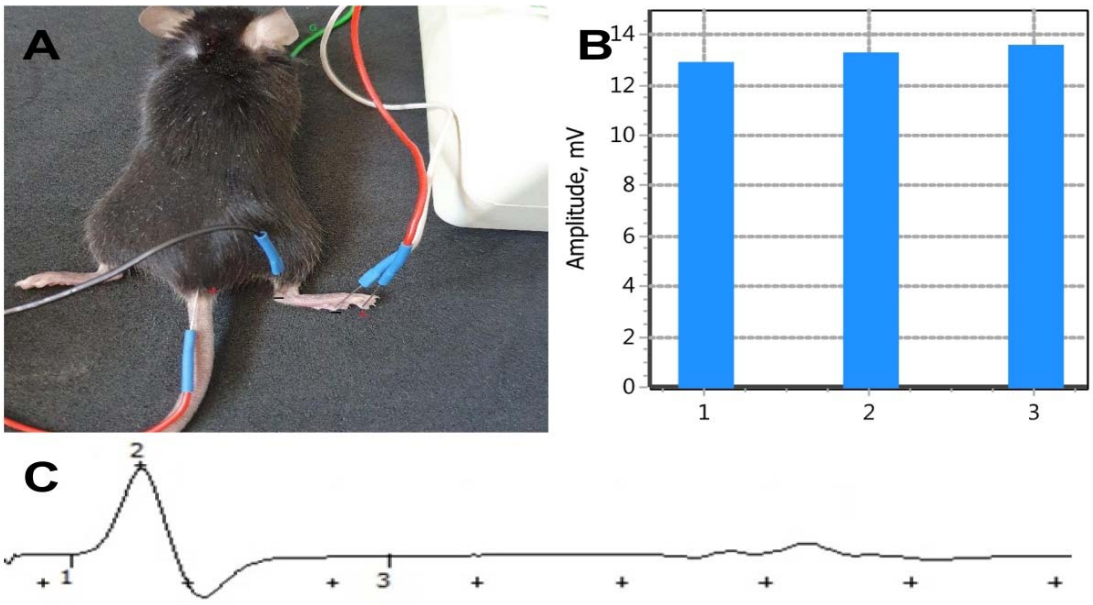

Figure 1. Experimental electroneurography recordings.
(A) The used setup allowed for multiple recordings with various amplitudes. (B) Due to the small movement generated by stimulating muscles slight variations can be seen on (C) the recorded signals.

Clinical evaluation and treatment

Weight was measured once every two weeks, for all animals included in the study.

Once the presence of diabetes was confirmed by the presence of high glucose, treated animals started to receive intraperitoneal treatment, 5 days a week for 6 weeks.

One group of animals was treated with 100mg/kg body weight of acid alpha thioctic (Thiossen, Germany) (Group A).

The second treated group (Group B) received a vitamin B complex as follows: thiamychlorohydrochloride-B1 100mg/kg body weight and pyridoxynhydrochloride-B6 50mg/kg body weight (MILGAMMA, Germany).

To quantify if the two treatments will have a cumulative effect both acid alpha thioctic and a vitamin B complex was given to the last treated group (Group A+B).

In order to avoid acute water intoxication of the animals, we decided to initially administer the dose of alpha thioctic acid (approx. 200µl per animal) and then, at an interval of at least 6 hours, the dose of vitamin B complex (approx. 100µl per animal).

Data analysis

Statistical analysis was performed using GraphPad Prism software (Version 7.0) and Microsoft Excel.

Statistical significance was determined by one-way ANOVA with Tukey's post hoc test for multiple group comparisons.

All results are given as the means±SD. Statistical significance is displayed as follows: ∗p<0:05, ∗∗p<0:01, and ∗∗∗p<0:001.

Results

As glucose levels increase independent of neuroprotective therapy

After induction of diabetes, all animals included in our study were visually inspected daily.

Blood glucose level of all diabetes animals did not vary depending on the therapy used.

It should be noted that a few days after the administration of streptozotocin, all diabetes animals, showed plasma glucose levels close to 600mg/dl (Figure 2B), without any significant differences in terms of glucose levels between groups ($p>0.05$).

Weight loss was observed in all animals that received with streptozotocin (Figure 2A) ($p<0.000$).

Experimental electroneurography can be used to quickly assess diabetes neuropathy

Electrophysiological recordings have detected changes over the 6 week monitoring period (Figure 3).

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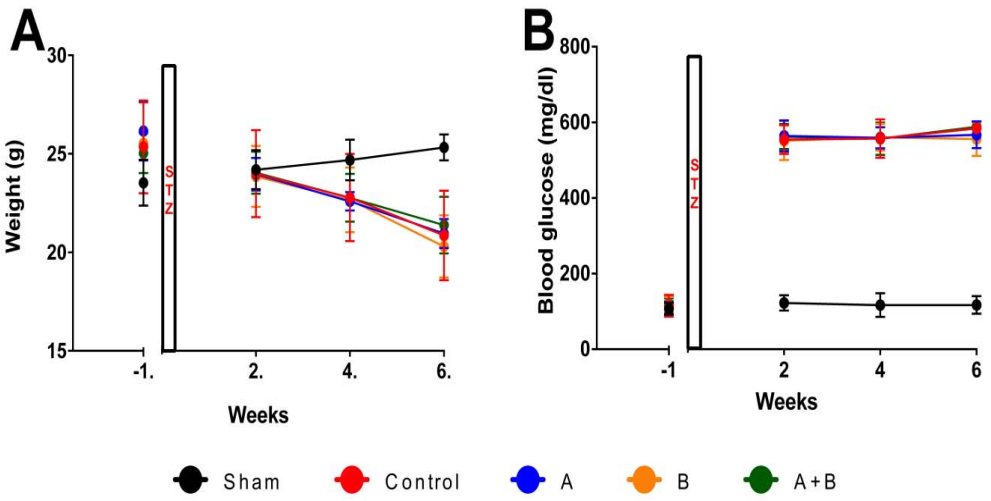

Figure 2. Biological evaluation of animals, (A) show a gain in the body weight of sham animals compared to the constant weight loss suffered by the animals in which diabetes was induced. (B) this seems to perfectly correlate with the really high levels of plasma glucose recorded in these animals.

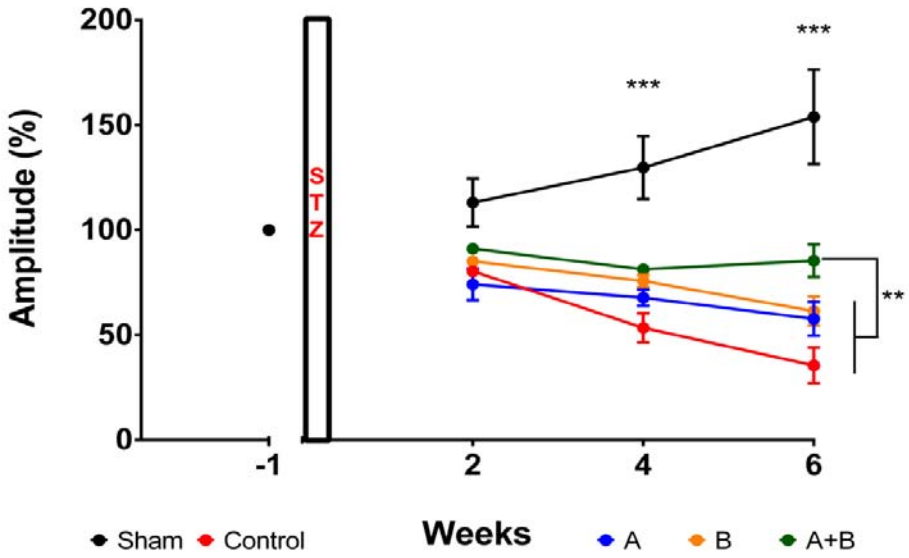

Figure 3. Electroneurographical variation on CMAP amplitudes. There is a progressive increase in amplitude recorded for the Sham group, while after STZ administration, all animals had a slow CMAP decrease with controls having almost 50% of the initial CMAP amplitude, after 6 weeks. Starting from week 4, marked differences can be recorded between normal and diabetes animals can be observed (p<0.01). Although, alpha thioctic acid or vitamin B complex can seem to make a difference compared to controls (p<0.01), the association of the two seem to have the greatest benefit.

There are changes in EMG parameters, especially in terms of CMAP amplitude, with a decrease in the control group and an increase in the sham group.

Decreasing the amplitude of CMAP means axonal degeneration and we in this study we tried to see if the treatment with vitamin B complex and alpha thioctic acid prevents this during the evolution of diabetic neuropathy.

We found that in group A+B (the group that was treated with both alpha thioctic acid and vitamin B complex) a smaller decrease in CMAP amplitude was observed compared to the control group (6 weeks after the onset of diabetes $p<0.0001$).

Also, separate treatment with alpha thioctic acid alone caused a smaller decrease in CMAP amplitude compared to the Control group (6 weeks after the onset of diabetes mellitus $p<0.0436$) and, also, separate treatment with vitamin B complex alone caused a smaller decrease in CMAP amplitude compared to the Control group (6 weeks after the onset of diabetes $p<0.0070$).

Thus, we observed that the combined therapy (A+B) has a greater protective effect than each therapy taken separately (only alpha-thioctic acid or only vitamin B complex).

The results of the multiple comparisons performed by the ANOVA test regarding the CMAP amplitude are presented in Table 1.

Table 1. The results of multiple comparisons performed by the ANOVA test regarding the amplitude of CMAP.

Tukey's multiple comparisons test	Adjusted P Value Week 2	Adjusted P Value Week 4	Adjusted P Value Week 6
Sham vs. Control	0,0038	0,0003	0,0001
Sham vs. A	0,0116	0,0014	0,0022
Sham vs. B	0,0086	0,0025	0,0017
Sham vs. A+B	0,0255	0,0034	0,0012
Control vs. A	0,3871	0,0838	0,0436
Control vs. B	0,0064	0,0067	0,0070
Control vs. A+B	0,0013	0,0015	<0,0001
A vs. B	0,1290	0,0615	0,3062
A vs. A+B	0,0106	0,0031	0,0256
B vs. A+B	0,0238	0,0075	0,0196

Discussion

Although taken separately, the two therapies have proven their neuroprotective effect in diabetic neuropathy in various experimental or observational studies, so far, the effect of their concomitant use has never been analyzed.

Therefore, we decided to conduct an experimental study, using an animal model of diabetic neuropathy, in which we examined the effect of concomitant use of alpha thioctic acid and vitamin B complex in diabetic neuropathy.

It should be noted that we have previously evaluated the combined effect of the two therapies compared to alpha thioctic acid alone, and we found an improvement in the clinical features [9].

Experimental studies have shown that alpha thioctic acid prevents lipid peroxidation, restores glutathione levels, increases blood flow, increases the activity of antioxidant enzymes and glucose uptake [10-12].

The efficacy of alpha thioctic acid therapy was also demonstrated in the multicenter, double blind, randomized, placebo controlled ALADIN III trial, in the SYDNEY2 trial, but also in the largest and longest trial, Nathan I, a randomized, double blind, multicenter, parallel group trial [13-16].

Despite all these data, the US FDA has not yet approved the widespread use of this compound in the treatment of diabetic neuropathy [4].

In recent decades, the role of vitamin B complex (B1, B6 and B12) in the therapy of diabetic neuropathy has been studied.

It has been shown that the use of vitamin B complex has a role in accelerating nerve regeneration and in the recovery of nerve function, especially in the acute phase of the injury [17].

Regarding B-complex vitamins, it should be noted that there is only one short-term (6-week), placebo controlled, double blind, phase III clinical trial that showed a borderline difference between the use of benfotiamine (which increases intracellular B1-thiamine levels) and placebo (P=0.055) [18,19].

Conclusion

Combined therapy with alpha thioctic acid and vitamin B complex has a greater effect in preventing axonal degeneration in diabetic neuropathy than single therapy with alpha thioctic acid alone or vitamin B complex alone.

Conflict of interests

None to declare.

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