

Experimental Diabetic Neuropathy: Electrophysiological Changes & Antioxidant Supplementations

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Abstract: Diabetic neuropathy is the one of the serious secondary complications of diabetes mellitus in which an alteration or a damage in nerve cells is observed. The nerve disorders can be developed at any time, but the possibility of the development increases with the longer duration of diabetes. The damage can be occurred in the sensory, motor, and autonomic nervous system, therefore the damage is observed in the innervated organs and systems. As a result, diabetic neuropathy is the most common cause of non-traumatic amputations and autonomic failure. In their lifetime, diabetic patients with diabetic neuropathy have a 15% incidence of undergoing one or more amputations.

Due to the similarities of the presented pathologies to the human diseases and its the easiness, diabetes induced animal models are widely used in studies. Since diabetes and therefore diabetic neuropathy is a worldwide problem, it is necessary to examine the effects of diabetes mellitus on the neural system. The recorded compound action potentials revealed that diabetes is the reason of the significant increase in time to peak, rheobase and chronaxie values. Furthermore, the maximum depolarization, area, kinetics and the conduction velocities of both the fast and slow nerve fibers were found to be decreased. In addition to the decline of the conduction velocities, a shift from faster fibers to the slower ones was observed.

Since the oxidant agents are unavoidable, the aim should be to minimize it as much as possible. For minimization, the antioxidant agents are crucial. It is also shown that beyond the inhibition of the oxidation agents, some of them also restore the damaged nerves.

Currently to avoid diabetic neuropathy it is suggested to keep the glucose levels as close as possible to the normal values. If it is necessary pain therapy can be used to minimize the pain. The promising results of the animal studies show that the treatment strategies should be renewed by including the antioxidants to the daily diet to the diabetic patients.

Keywords: Antioxidants, Conduction velocity distribution, Diabetic neuropathy, STZ.

INTRODUCTION

A metabolic disease, which is defined by hyperglycemia resulting from defects in insulin secretion and/or insulin action named as Diabetes mellitus (DM). Evolution of DM ranges not only from the autoimmune destruction of the β -cells of the pancreas with subsequent insulin deficiency (type I) but also from the abnormalities that result in a resistance to insulin action (type II).

Deficient insulin action results from an inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points within the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.

The basis of the abnormalities seen in carbohydrate, fat, and protein metabolism in DM is

mainly due to deficient action of insulin on target tissues. The persistent hyperglycemia is associated with long-term tissue damages, dysfunctions, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels.

Among the others Diabetic neuropathies (DNP), the family of nerve disorders, are caused by DM. Since the damages in the nerves can be seen throughout the body, patients with DNP lead to numbness, pain and weakness in the extremities [1]. Although patients with DM can develop nerve problems at any time, but the longer they have DM, the greater the risk can be. The highest rate of DNP was seen among the ones who have had the disease for at least 25 years [2]. Indeed, DNP also appears to be more common among the patients who have had problems; (i) in controlling their blood glucose levels, (ii) in those with high levels of blood fat and blood pressure and (iii) over the age of 40. As a result, DNP is the most common cause of non-traumatic amputations and autonomic failures [3]. In their lifetime, diabetic patients with DNP have a 15% incidence of undergoing one or more amputations [4].

The formation of free radicals or oxidants is a well-established physiological event in aerobic cells.

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Increased oxidation within the cells convenes by enzymatic and nonenzymatic resources by removing the oxidizing species which was collectively known as antioxidant defense system. An imbalance between oxidants and antioxidants, the two terms of the equation defines oxidative stress (OS), and the consequent damages to cell molecules constitutes the basic tenet of several pathophysiological states, such as neurodegeneration, cancer, mutagenesis, cardiovascular diseases and aging.

Majority of the researches dealing with the OS have concluded that it is the primarily cause and/or the major contributor to the etiology of severe pathologies with serious public health implications such as DM and its complications [5-7].

The aim of this review is to provide up-to-date information on the DNP from the point of electrophysiology. It is also our target to summarize the antioxidant treatment strategies in animal models of DM.

NERVE CONDUCTION STUDIES

In principle, nerve conduction velocity (CV) measurements are carried out by testing how fast the electrical signals move through a nerve. Although the technique is so familiar, briefly it was performed by using two electrodes (stimulating and recording electrodes) with a known distance. Following the stimulation, compound action potentials (CAP) were recorded together with their latencies (time differences between the electrodes). The CV is then calculated simply by the dividing of distance between the electrodes to the latencies. Besides the easiness of the application the information obtained from the signal analysis of these records are quite valuable.

DM INDUCED CV CHANGES

Majority of the *in vitro* CV recording studies were carried on the experimentally induced diabetic animal models. Not only its easiness but also the similarities with its secondary pathologies, those seen in human, made them frequently used for the research purposes.

The measured parameters from the *in-vitro* CAP recordings were summarized in Table 1. Four weeks of exposure to high blood glucose (≥ 400 mg/dL) resulted in a significant decrease in the total area, the maximum depolarization (MD) point of CAP recordings and an increase in the time required to reach the MD (TP). The amount of the CAP area is in relation with the number

of actively contributing single fiber action potentials. Period of DM somehow results a decrease in the number of the active contributions of the neurons. As it was estimated, the excitability of neurons was significantly reduced during DM, which was reflected by a significant increase in measured rheobase and chronaxie values [8].

Latency measurements can be carried out from the artifact either to the start of CAP or to the MD. Former gives information about the speed of the fastest neurons while the later give information about the intermediately conducting neurons. Indeed, this type of measurements provides not only the speed but also an idea about the velocity contribution percentages of the active nerve fibers. Model studies have shown that DM suppressed the actively contributing nerve fibers of both the fast and the intermediate groups compared to their age matched control group values. This result leads us a morphological damage and/or modifications in ion channel activities in CV changes.

The decision of the change whether it's a morphological or a functional can be done (at least to certain point) by the CV distribution measurements mostly with the aid of collision technique [9]. In model studies, contributions of the nerve fibers were the shifted towards the relatively slower conducting side which then results in a decrease in the activity of the fastest groups. Changes in the CV due morphological changes with progression of DM can also be verified by the other model studies [10, 11].

OXIDATIVE STRESS AND ANTIOXIDANT SUPPLEMENTATION

In principle, both the degree of oxidative stress and the success of the antioxidant treatments mainly depend on the exposure time period and application protocol (time, dose and type of the antioxidant) respectively. Among the tested antioxidants the ones that have the capability of reducing the blood glucose, either through with insulin defects or not, seems to have more potent. Those are which also a power agents in reducing the oxidative stress markers of the nerve tissue.

Lipid peroxidation measurement is an entrenched method to assess the extent of oxidative damage by free radicals. The results of many research agree that it was increased by hyperglycemia-induced glucose auto-oxidation and glycation of the proteins [6, 12-16]. The mechanism oriented studies have shown that glucose

Table 1: Changes in CAP Parameters During DM and Antioxidant Treatments. In the Table: TP: Time Required to Reach to the Peak; MD: Maximum Depolarization Value; \pm dV/dt: Up and Down Kinetics of CAP; Latency: Measure to AP and Peak of CAP; CVD: Conduction Velocity Distribution. \uparrow Refers to Increase, \downarrow Refers Decrease, \leftrightarrow Refers Normalization to Control Values Whereas NC Means no Change

Measured Parameter	DM	Tested Antioxidants		
		Selenium (7 μ mol/kg/day) 4 Weeks	Coenzyme Q ₁₀ (10 mg/kg/day) 2 Weeks	α -Lipoic Acid (100 mg/kg/day) 2 Weeks
Rheobase	\uparrow	\leftrightarrow	\leftrightarrow	NC
Chronaxie	\uparrow	\leftrightarrow	\downarrow	NC
TP	\uparrow	\downarrow	NC	NC
MD	\downarrow	\leftrightarrow	NC	NC
+dV/dt	\downarrow	\uparrow	\uparrow	NC
-dV/dt	\downarrow	\uparrow	\uparrow	NC
Latency				
AP	\downarrow	\leftrightarrow	\uparrow	\uparrow
Peak	\downarrow	\leftrightarrow	NC	NC
CAP Area	\downarrow	\leftrightarrow	\uparrow	\uparrow
CVD shift	slower	\leftrightarrow	higher	higher

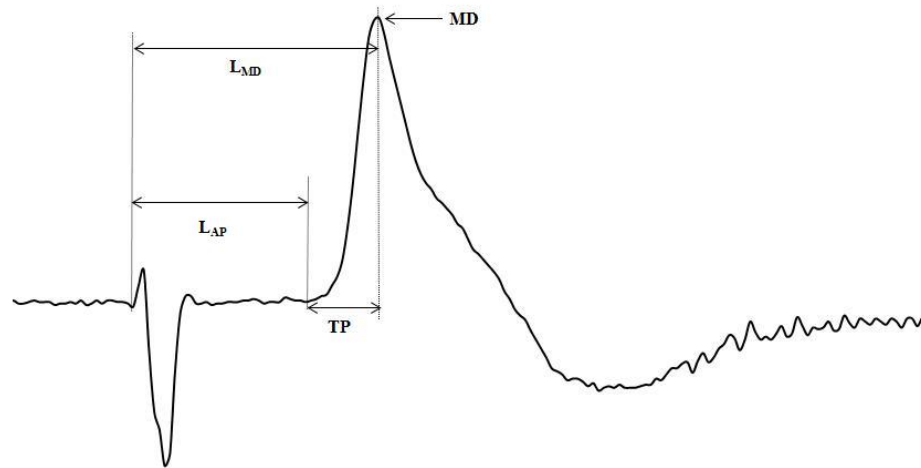


Figure 1: Sample trace of CAP recorded from the distal end of sciatic nerve from a rat. Vertically positioned dash lines represents the time line periods for the corresponding parameter. Arrow shows the point of measurement. The abbreviations; MD: Maximum depolarization value; TP: time required to reach the MD point; Latencies used for the two CV measurements LAP: for the calculation of the fastest group of CVs; and LMD: for intermediate ones.

autoxidation and interaction of advanced glycation end products with their receptors provide the main contributions to the free radical generation. Indeed, amadori products have also produced a down-regulation of some of the antioxidant defense enzymes [18].

Antioxidant supplementations on the other hand alleviate the DM induced functional defects that were seen in peripheral nerves via reducing the oxidative

stress. Depending on the dose and choice of the antioxidant treatments many of the seen electrophysiological abnormalities in the nerve of diabetic rats has been restored nearly to its healthy status.

Along with the tested antioxidants, our experiences have shown that selenium (Se) supplementation is the most powerful agent [8, 9], which has been long known as an essential trace element with antioxidant

properties. Indeed, Se has shown an insulin mimetic effect in streptozotocin induced model studies [15, 16]. The antioxidant enzymes such as glutathione peroxidase (family of an enzyme with peroxidase activity) and thioredoxin reductase (group of enzymes that reduce thioredoxins) also include Se in their structure [17].

Antioxidant supplementation to the diabetic animals blocks and/or restores the DM-induced functional remodelling in the nerve tissue [9, 19-25]. From the electrophysiological point of view, promising results for the clinical applications obtained by these supplementations are summarized in Table 1.

CONCLUSIONS AND REMARKS

Besides the fundamental importance of the oxygen, in an evolutionary manner probably the only disadvantage of the aerobic respiration is the oxidation. Since it is almost impossible to prevent the formation of these naturally occurring agents we have to supply antioxidants through our diets. In addition to their natural occurrence, their presence can be augmented and cause a stress during disease cases such as DNP.

The animal model of DM is chosen widely for the research purposes not only of its easiness but also pathological similarities that was seen in man. The tested antioxidants in these animal models provide promising results on the DM induced both the functional and the structural alterations. Depending on the dose and the exposure time many of the DM induced electrophysiological changes can be normalized by the antioxidant treatments such as CVs and their distributions.

Besides the presence of these charming effects, the clinical treatment strategies are frequently exclude antioxidant included treatments. The integration of the antioxidants to DM treatments in the patients is suggested and topic oriented further researches are needed.

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