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Implications of oxidative stress in the pathogenesis of diabetic neuropathy

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Abstract

Diabetes mellitus is characterized by the excess of oxygen free radicals production and/or the decrease of antioxidant defense capacity. The intensity of oxidative stress is correlated with the appearance of complications of diabetes (diabetic neuropathy). This study was to assess the endogenous antioxidant defense capacity, the metabolic balance, respectively, nerve conduction speed in a group of patients with type II diabetes after two months of treatment with lipoic acid.

Key words: Reactive oxygen species, oxidative stress, diabetic neuropathy, lipoic acid.

Introduction

Free radicals (FR) are chemical species possessing an unpaired electron and may be considered highly active molecular fragments[1].

Reactive species cause oxidation of biomolecules and lead to tissue injury, namely cell death. Establishing the involvement of free radicals in the pathogenesis of various diseases is very difficult due to very short life time of these species.

Cells have developed their own antioxidant defense system to prevent excess of free radical formation or limit their destructive effect. This system includes enzymes that decompose peroxides; proteins seize transition metals or compounds as "scavengers" of free radicals. Antioxidants (AO) are the first target of FR action being oxidized or converted into other less harmful FR, and therefore they are destroyed for the protection of biological molecules (DNA, enzymes). Therefore, AO regeneration is important by transfer reactions or metabolic coupling reactions [2].

Antioxidants are classified as non-enzymatic antioxidants (natural or synthetic) which in turn can be hydrosoluble or liposoluble and enzymatic antioxidants. Another classification of AO refers to the existence of AO endogenous and exogenous (used as therapeutic agents).

Enzymatic, endogenous antioxidants: Enzymatic, endogenous antioxidants are protective systems that work in certain stages of O_2 activation. They are metabolically interconnected to ensure maximum efficiency: antioxidant enzymes have the highest response rates (superoxide dismutase $2x10^9$ $M^{-1}s^{-1}$) and are found in all aerobic cells in appreciable quantities. High speed responses of these enzymes (superoxide dismutase, catalase) determine their efficiency and ability to act regardless of availability of substrates or coenzymes [3].

Superoxide dismutase (SOD) contains in the active center atoms of transition metals, Cu, Zn, Mn. There are two forms of intracellular localization: one consisting of two identical subunits, non-covalent containing 2 atoms of Cu and 2 atoms of Zn for a molecular weight of 33,000 and the other containing 2-4 atoms of Mn-molecule, a molecular weight of 88,000, composed of four subunits. New enzyme forms containing Fe, Cu and Zn are the effect of diversification of forms of life and increase the concentration of O_2 [4]:

$$M^{(n+1)+}\text{-SOD} + O_2^- \rightarrow M^{n+}\text{-SOD} + O_2$$
$$M^{n+}\text{-SOD} + O_2^- + 2H^+ \rightarrow M^{(n+1)+}\text{-SOD} + H_2O_2.$$

where M = Cu (n=1)° ; Mn (n=2)° ; Zn (n=2)°

The form containing Cu-Zn is the form of intracytoplasm superior organisms, and the form containing Mn is intramitochondrial. Marklund *et al.*[7] found extracellular dismutase in the interstitial space, especially in the lung. It has a molecular weight of 135,000 for the four subunits and contains 4Cu and 4Zn. In plasma, normal values are 5-20 U / ml.

SOD exerts a protective effect, catalyzing the dismuting of superoxide anion and the formation of H_2O_2, which will then be degraded by catalase. It may inhibit the formation of singlet oxygen and indirectly polyunsaturated fatty acids peroxidation.

Nonenzymatic antioxidants: Glutathione (GSH) is the most important nonenzymatic antioxidant, being the form of transport for groups of SH type. These groups show a high reactivity, due to ability to deliver a pair of electrons, forming multiple covalent bonds or electrovalent bonds, reversible or irreversible. Unlike the composition of the SH groups of enzymes, which are protected by the polypeptide chain, the structure of GSH groups is the first target of free radical (FR) action. Acting nonenzymatic or as a coenzyme of GSH-peroxidase and transferase, GSH neutralizes a number of endogenous and exogenous toxic compounds [5].

In physiological conditions, there is a balance between the production of FR and antioxidant defense capacity. The emergence of an imbalance in favor of production of FR and/or reduced antioxidant (enzymatic and/or nonezymatic) causes oxidative stress (OS), the activation of O_2 becomes an aggressive factor,

with repercussions in one organ or whole organism [6].

The pharmacological action of alpha-lipoic acid is characterized by enhancing antioxidant protection mechanisms, decreased production of free radicals, stimulation of glucose using increased blood flow endoneural and growth of nerve conduction velocity.

This study was to assess the endogenous antioxidant defense capacity, the metabolic balance, respectively, nerve conduction speed in a group of patients with type II diabetes after two months of treatment with lipoic acid.

Experimental

The objective was to evaluate the antioxidant defense capacity reduction expressed by superoxide dismutase (SOD) and reduced glutathione (GSH) in 50 patients with diabetes mellitus type II non-insulin-dependent (NIDDM) before and after treatment for 2 months with alpha-lipoic acid orally administered in two doses of 600 mg. Of this group 23.67% patients were male and 76.33% females. Also, the group of patients was classified according to age group (Table 1) and disease duration (Table 2).

Table 1. Distribution of cases by age.

Age groups	No.of cases	Subjects (%)
41-50	10	20
51-60	8	16
61-70	25	50
71-80	7	14

Table 2. Group structure according to the disease duration.

Time (Ages)	Number of cases	Subjects (%)
1-4	24	48
5-10	12	24
11-15	7	14
16-20	2	4
21-25	5	10

Evaluation of metabolic balance, neuropathy and oxidative stress was performed on study group before and after treatment for 2 months with alpha-lipoic acid administered 2 oral doses of 600 mg.

Neuropathy was assessed by the presence of clinical signs and nerve conduction velocity value (NCV) in the motor fibers. NCV determination was made using electromyography provided with neurostimulator. We used surface electrodes to stimulate both nerve and also to collect muscle evoked potential of the corresponding muscle. Muscle evoked potential parameters of activity are represented by amplitude and latency of response. Potential amplitude is given by the number of fibers that generate response. Impulse response latency is the time required to travel long distances from the point of stimulation to the collection. The difference between proximal and distal latency (as measured by electromyography screen) is the time required for the impulse to walk between the two points of stimulation. NCV is the distance between two points of stimulation and the latency difference between proximal distal, respectively. NCV normal value in peripheral motor nerves (cubital, median, radial, external popliteal sciatic) is 45-65 m/s.

Determination of antioxidants: Superoxide dismutase is the enzyme that catalyzes the O_2-dismuting to H_2O_2. To determine the enzymatic activity requires a feedback system in which to produce O^{2-} and a substance by which to measure the amount of O^{2-}. The production of O^{2-} redox couple is composed of methylene blue and tetramethylendiamine (TMDA) and the substance is an indicator tetrazolium nitroblue (NBT). In a redox system in the presence of superoxide anion O^{2-}, NBT indicator is reduced to formasan a colored product having maximum absorption at 560 nm. Corresponding absorption is directly proportional to the concentration of O^{2-} from the reaction mixture. In the presence of SOD, the number of superoxide anions is decreased and production of formasan is reduced. All samples should be read in spectrophotometer at $\lambda = 560$ nm, compared with phosphate buffer control. SOD is considered a unit, that quantity of enzyme that reduces by 50% the amount of formasan the blank reaction. Reduced glutathione has a role in detoxification by degradation of reactive oxygen species, produced spontaneously or enzymatically. In these reactions, reduced glutathione acts as a hydrogen-ion donor. Its shape will be reduced again oxidized GSH reductase, using NADPH as substrate. Glutathione blood acid reacts with 5,5-tiobis (2-nitrobenzoic) (DNTB) and produces a colored product having maximum absorption at 420 nm. Normal values are 303-563 mm/l.

Results and Discussion

The amount of SOD was significantly reduced in patients with type II diabetes in the study group 6.54 ± 1.49 IU / ml ($p < 0.001$) mean normal value is 10 IU / ml. Treatment for 2 months with lipoic acid significantly increased the amount of SOD, reaching 8.70 ± 1.01 IU/ml ($p < 0.001$).

The amount of GSH was also reduced: 353 ± 31 μM/L ($p < 0.01$). Normal average value is 433 μM/L). Treatment for 2 months with alpha-lipoic acid significantly increased the amount of GSH, reaching 395 ± 11 μM/L ($p < 0.01$)

Blood sugar value decreased from (before treatment) $187\% \pm 2.91$ mg to $157\% \pm 3.78$ mg. NCV decreased significantly in older patients with diabetes, the form with a history of more than one year. In this category NCV value was inversely proportional to disease duration ranged between 1 and 25 years.

Conclusions

Superoxide dismutase and reduced glutathione express endogenous antioxidant defense capacity which is significantly lower in patients with type II diabetes, in correlation with oxidative stress intensity. The results of this study also revealed a strong correlation between oxidative stress parameters and blood glucose NCV. Clinically, diabetic motor neuropathy is manifested by muscle weakness, fall planting vaults and high pressure points, and motor function by reducing the NCV.

After treatment 78% of patients went from stage 1 to stage 2 neuropathy and 8% of cases showed no obvious signs of improvement, leaving stage 2 neuropathy.

In the group of diabetic patients we observed a significant decrease in antioxidant defense capacity, expressed by two parameters, SOD, GSH, as a result of increasing oxidative stress. NCV value was correlated with disease duration and blood glucose (between these parameters is an inverse relationship). Treatment with alpha-lipoic acid is effective in improving diabetic neuropathy subjective signs but also of those objectives (absence of neuropathy and increased NCV).

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