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Possible Clinical Applications of Electron Discharge at Extremely Low Energy Level for Suppression of Oxidative Stress: Introduction of "e-Vitamin" therapy

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ABSTRACT: Antioxidative treatment delivered using an electron discharge at extremely low energy, also known as "e-Vitamin" therapy, has been demonstrated *in vitro* by the exposure of a physiological saline solution to the electron discharge, which results in an increase of pH and negative value of oxidation and reduction potential indicating an increased electron density in the solution and the reduction of Fe^{3+} and glutathione disulphate. More importantly, a significant reduction of liver damage was observed on administration of the treated saline solution to rats in the hepatic ischemic reperfusion model. In addition, e-Vitamin therapy delivered using a direct current-type dielectric barrier discharge system significantly decreased both alanine aminotransferase levels (an indicator of liver inflammation) and hepatic malondialdehyde levels (an indicator of oxidative stress), as well as reducing blood glucose levels in rats with type 2 diabetes and hepatic steatosis. These results suggest that e-Vitamin therapy merits investigation as a clinical intervention in patients with type 2 diabetes and fatty liver disease.

KEY WORDS: oxidative stress; type 2 diabetes mellitus; NASH; electron therapy; dielectric barrier discharge

I. INTRODUCTION

Oxidative stress plays an important role in the progression of type 2 diabetes mellitus,[1] and nonalcoholic steatohepatitis (NASH).[2,3] Systemically elevated oxidative stress in these pathologies is reflected in the increased levels of oxidative markers such as malondialdehyde (MDA), lipid peroxides, and 8-hydroxy-deoxyguanosine in the blood and tissues of patients. It is known that these excess peroxides and metabolites, which damage both cell membranes and DNA, are produced by an increase of intracellular free radicals, reactive oxygen species (ROSs), and hydroxyl radicals, as well as the leakage of free electrons from mitochondria resulting from mitochondrial dysfunction.[4]

Current antioxidative therapeutic approaches are focused on the use of contemporary antioxidant compounds such as α-tocopherol and ascorbic acid. However, these

vitamins have failed to exhibit sufficient effectiveness in the treatment of either type 2 diabetes or NASH.[5,6]

We report here that electron discharge at very low energy level, called "e-Vitamin" therapy, can mediate antioxidative effects in biological systems. The possible clinical applications in type 2 diabetes and NASH are discussed.

II. INDUCTION OF REDUCING POTENCY BY ELECTRON DISCHARGE AT EXTREMELY LOW ENERGY LEVEL

The reduction reaction in the basic chemical functions of life is theoretically defined as the gaining of an [e^-]. We thus attempted to induce reductive potency by the delivery of an electron to physiological saline solution using a system that exposes the electron discharge onto the surface of the saline solution in air as described elsewhere.[7,8] The cathode electrode was placed in a position where metal electrodes were kept a distance from the surface of the solution. The anode metal electrode was also separated from the solution by placing hydrophobic materials between the solution and the electrode, a process distinct from saline electrophoresis.

This treatment resulted in a time-dependent increase of pH and negative value of oxidation and reduction potential (ORP), indicating an increased electron density in the solution.[7] Furthermore, the reductions of Fe^{3+} and glutathione disulphate (GSSG) were also observed in the same system.[8] These observations indicate that reductive potency can be induced by the electron treatment and is capable of the detoxification of ROSs and the hydrogenation of oxidized antioxidants such as GSSG, thus mimicking the biochemical role of NADPH. Most importantly the antioxidative activity induced in the physiological saline solution was transferable to biological systems *in vivo*, as the administration of the electron-treated saline solution markedly inhibited the severe liver injury caused by excessive oxidative stress in the rat hepatic ischemic/reperfusion (I/R) model.[7]

Since the reduction reaction requires a transfer of proton-coupled electrons to oxidized molecules in the biochemical reaction,[9] we speculate that the electron treatment of the saline solution presumably induced a proton-coupled electron transiently, and absorbed into cluster molecules formed with water molecule, NaCl, and dissolved oxygen and carbon dioxide gases in the solution. Then, a variety of the cluster molecules associated with proton-coupled electron are capable to donate the proton-coupled electron to oxidized molecules as a non-enzymatic hydrogenation activity. Further studies on identification of the reducing anions or cluster molecules are needed to understand the exact mechanism for the induction of the reductive potential by the treatment of electron discharge at a very low energy level in physiological saline solution.

In contrast to the low energy discharge of e-Vitamin treatment, when electron discharge was employed at high energy levels, intracellular oxidative stress was increased with the production of hydroxyl radicals and the destruction of skin cancer cells.[10,11] It

is thus evident that the induction of reductive potentials, and the detoxification of cytotoxic free radicals and lipid peroxides by the electron discharge are critically dependent upon the very low energy level of the discharge used.

III. DIRECT CURRENT-TYPE DIELECTRIC BARRIER DISCHARGE (DC-DBD) APPARATUS

We have designed a DC-DBD apparatus to administer an electron discharge at very low energy level to the body for the induction of antioxidative activity (Fig. 1A). Utilizing a semiconductive planate dielectric rubber sheet insulated with a pulp fiber cloth, the electron discharge is delivered as a unidirectional and plenary current at 5 μA, 25 V DC. This device enables the electron discharge to be safely applied onto the skin surface without sensing electric current.[9] The possible electric shock resulting from an accumulation of electrons on the surface of the cathode electrode was avoided by placing the dielectric rubber sheet and the insulating pulp fiber cloth materials on the anode electrode. Also a similar reductive potency obtained in the electron-treated saline solution can be observed by placing the cylinder containing the saline solution between the two pulp cloths in Fig 1A.

As shown in Fig. 1B, an electron discharge resembling an electron shower was observed in the air gap between the electrodes when DC voltage was applied onto the cathode electrode.[12]

IV. THE EFFICACY ON TYPE 2 DIABETES INDUCED NASH MODEL RATS

We examined the efficacy of electron discharge at very low energy, "e-Vitamin" therapy, on the type 2 diabetic NASH rat model using the DC-DBD apparatus.[8] In this model, pathology is induced by neonatal administration of streptozotocin and a high fat diet.[8] Adult male rats exhibiting pathologically high levels of oxidative stress, liver inflammation, and blood glucose were treated noninvasively for 4 weeks with electron discharge at 5 μA using the DC-DBD apparatus. The treatment resulted in a significant decrease of serum alanine aminotransferase (ALT) and hepatic MDA levels as compared to animals in the untreated group. These decreases were also associated with gradual but steady reduction in high glucose levels, as compared to the untreated group, though these values did not reach statistical significance. It is nonetheless possible that the beneficial antioxidative efficacy of electron treatment could also be due to modulation of abnormal glucose uptake in the NASH model rats; further studies are needed to draw any clinically significant conclusions regarding the mechanism and impact of electron treatment on these signs of diabetes.

Interestingly, since the serum values of MDA were significantly correlated with both the values of ALT and glucose levels, it is likely that systemic oxidative stress is

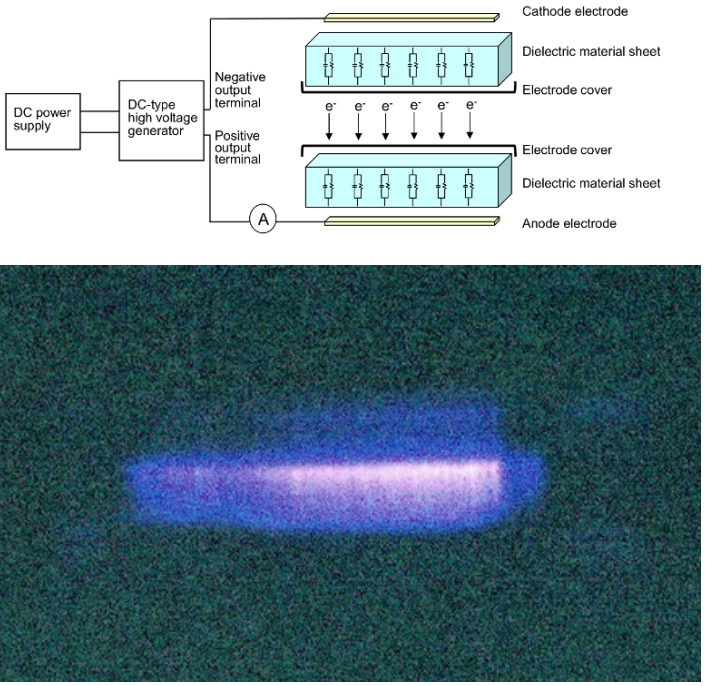

FIG. 1: A schematic diagram of DC-type dielectric barrier discharge system. **(a)** DC-DBD apparatus is designed to decrease oxidative stress for the purpose of studies *in vivo*. **(b)** A unidirectional electron discharge was observed in the air gap between the cathode and anode dielectric electrodes covered with an insulation material made of pulp sheets

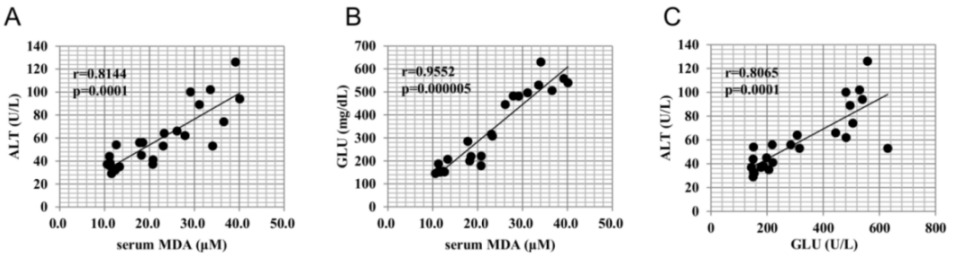

FIG. 2: Relationships between MDA, ALT, and glucose levels. These figures are from Ref. 8." The reprint permission has been obtained from the Publisher. Linear regression analysis was used to examine the relationships between individual levels of serum MDA, ALT, and glucose measured in the electron-treated and untreated NASH and normal rats after 4 weeks of treatment. A significant correlation was observed between the serum MDA levels and those of both ALT (r=0.8144; p<0.001) and glucose (r=0.9522; p<0.01, Figs. 2a–2c).

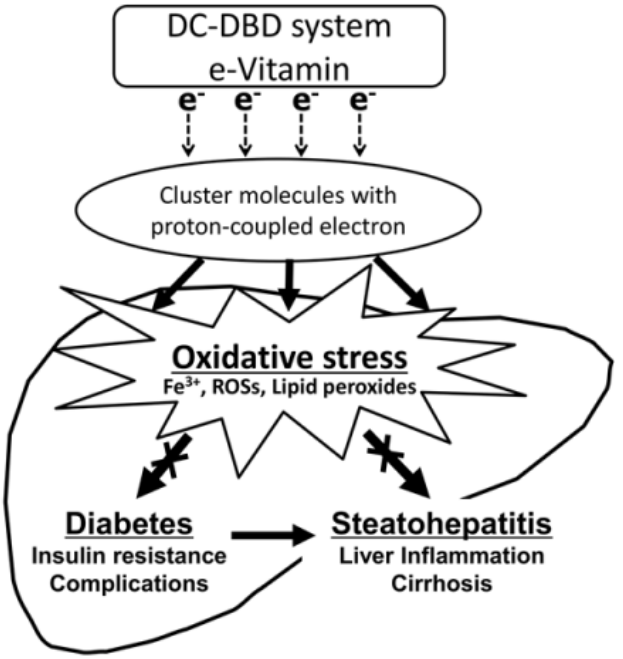

FIG. 3: A hypothesis for the effectiveness of e-Vitamin therapy on type 2 diabetes induced NASH.

linked to the pathogenesis of liver inflammation and the high glucose levels observed in type 2 diabetes and hepatic steatosis.[8]

V. NEXT STEP

Very low energy electron treatment, called "e-Vitamin" therapy, is capable of reducing harmful intracellular free radical producing molecules, such as trivalent iron,[8] ROSs, and lipid peroxides in excess oxidative stress. Also, the proton-coupled electron associated with cluster molecules may contribute to increased antioxidative network activity through elevation of nonenzymatic hydrogenation activity. We have summarized the possible mechanism of the e-Vitamin therapy as described in Fig. 3.

Since e-Vitamin therapy presumably offers antioxidative effectiveness without undesirable events in type 2 diabetes-associated NASH patients, we suggest for the next step that this therapy should be investigated in a clinical intervention, not just in type 2 diabetes and fatty liver disease, but also in aging and other lifestyle-related diseases which are thought to be mediated by pathologically elevated antioxidative stress.

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