

Theoretical backgrounds for light application in diabetes

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Abstract

Glucose can act as an oxidizing agent during this breakdown depending on the composition of surrounding molecules. Glucose reacts nonenzymatically with protein amino groups to initiate glycation, the early stage of the Maillard reaction, leading to crosslinking and browning of the proteins via the formation of advanced glycation end products (AGEs). The AGEs are responsible for various biochemical in tissues which can lead to the development of several complications in diabetes: neuropathy, angiopathy.

The monocyte macrophage plays an important role in this process both by removing the senescent molecules that have accumulated AGEs over time and by initiating the steps that lead to new protein synthesis and tissue remodelling. One of the most important features of the macrophage is its ability to produce and release NO and SOD. The irradiation of macrophages by red light result in a dose-dependent increase in NO production and SOD activity and , laser irradiation of cells in the red range activates the synthesis of SOD and inducible NO-synthase de novo due to photosensitized initiation of free radical reactions.

NO synthase is primarily a cytosolic enzyme which has similarities with cytochrome P450 enzymes. These all have absorption maxima between 446 nm and 452 nm. Several isoforms of the enzyme occur in endothelial cells, as well as in platelets, macrophages, vascular smooth muscle cells, and the brain.

The start of pathogenic levels of Islet cell antibodies (ICA-IgG) may precede the clinical onset of diabetes by several years, even in children. Several attempts have been made to influence the course of type I diabetes by immunotherapy. Plasmapheresis, prednisone, and interferon have proven unsuccessful or only partially successful. Successful methods of preventing diabetes in Worchester rats have been neonatal thymectomy, antiserum to lymphocytes, bone marrow transplantations, and cyclosporine. This demonstrates the strong immunological background of the disease process. Type I diabetic patients have been shown to have inhibition of migration of leucocytes specific for antigens of the endocrine pancreas. Phagocytic white blood cells employ the myeloperoxidase H₂O₂ system to generate reactive oxygen intermediates that kill invading bacteria, viruses, and tumour cells. Partially reduced oxygen species are also potentially damaging to cellular lipids, nucleic acids, and proteins; the production of such species by activated phagocytes has been implicated in the damage of normal tissues under pathological conditions. The initial pathway for oxidant generation involves a membrane associated NADPH oxidase that reduces oxygen to superoxide anion, which then dismutates to form H₂O₂. Myeloperoxidase, a secreted heme protein, amplifies the toxic potential of H₂O₂ by producing reactive intermediates. Production of myeloperoxidase is inhibited by irradiation at 633m 660nm, 820nm, 880nm and 950nm, of which 660nm appears to have the strongest effect. By regulating the amounts of active macrophages, NO, SOD, Myeloproxidase, and the activity of cytochrome P450 and many other substrates by light, it is possible to regulate glucose and AGE breakdown and prevent development of complications of diabetes.

Introduction

Diabetes is not a single disease but the pathological and metabolic state caused by inadequate glucose transport and inappropriate glucose breakdown: a feature common to all types of glucose intolerance.

Diabetes resembles fasting, especially in the responses of liver, muscle cells, and adipose tissues. With low serum ratios of insulin to glucagon and high levels of fatty acids, liver produces glucose while other tissues use fatty acids and ketones instead of glucose. Muscle cells and adipose tissue respond by using ketones and fatty acids.

Although the resemblance between fasting is diabetes are striking, pathologically low serum insulin levels disrupt the efficiency seen during fasting. With low insulin, key glycolytic enzyme activities decrease. Glucose use falls far below levels seen during fasting. Concurrently, hepatic gluconeogenic enzyme activities increase and gluconeogenic rates rise.

Bombarded with free fatty acids, the liver increases gluconeogenesis, secreting large amounts of VLDLs, and accumulates fatty acids in droplet form. A long-term toxic effect of diabetes is accumulation of 25% more lipid than normal. In the diabetic state, the liver oxidizes these fatty acids and produces acetone, acetoacetate, and β -hydroxybutyrate.

Muscle cells and adipose tissue also show major metabolic changes in diabetes.

Muscle glycogen almost disappears, and muscle protein is broken down to support gluconeogenesis. Cardiac and skeletal muscles meet their energy needs from ketones and fatty acids. Fat cells actively release fatty acids under the lipolytic stimuli of glucagon, catecholamines, and insulin deficiency.

Non-insulin-dependent tissues respond to diabetes totally differently. Hexokinase, the key stimulus of glucose use, is increased in jejunal mucosa, renal cortex, and peripheral nerves of diabetic animals.

In hyperglycemia, glucose use increases, and sugars accumulate. Excess glucose accumulation leads to tissue damage. Diabetic rats have 30% more total body glycogen than nondiabetic rats. Glycogen accumulates in renal tubules to values 50 times those in nondiabetic rats. Glycogen accumulation may contribute to tubular dysfunction and susceptibility to damage from x-ray dyes. Unimpeded entry of glucose into many tissues increases cellular glucose, producing linkage of glucose to tissue proteins (glycosylation).

The diabetic state damages non-insulin-dependent tissues, including glomeruli, retinal vessels, nerves, and circulating blood cells.

During periods of stress, epinephrine (adrenaline) plays a key role in inducing an increase in blood glucose. During normal daily living, the blood glucose level is under the dynamic control of insulin and glucagon. Both of these peptide hormones are produced by cells within the islets of Langerhans, cell clusters scattered throughout the pancreas. Insulin, which contains two polypeptide chains linked by disulfide bonds, is synthesized by the β cells in the islets; glucagon, a monomeric peptide containing 29 amino acids is produced by the α cells in the islets.

Insulin acts to *reduce* the level of blood glucose, whereas glucagon acts to *increase* blood glucose. Each islet functions as an integrated unit, delivering the appropriate amounts of both hormones to the blood to meet the metabolic needs of the animal.

Hormone secretion is regulated by a combination of neural and hormonal signals. Glucagon is a powerful stimulator of insulin and somatostatin secretions. Its release is stimulated by hypoglycaemia and amino acids; epinephrine, cholecystokinin (CCK), gastrin, growth hormone and opioids; and both cholinergic and adrenergic innervations. Glucagon secretion is inhibited by hyperglycemia and free fatty acids, and by insulin, somatostatin, glucagon-like peptide (GLP-1), and gastric inhibitory polypeptide (GIP). Glucagon operates by activating adenylate cyclase and generating cAMP within the beta cell. Glucagon receptors are linked to the activation of adenylate cyclase through a stimulatory G protein (Gs). cAMP in turn activates protein kinase A (PKA), which sensitizes the beta-cell secretory machinery to Ca^{2+} ions through the phosphorylation of intracellular proteins.

Glucagon is critical in regulating hepatic glucose production, and is responsible for about two-thirds of the glucose released in the postabsorptive and fasted states. The inappropriately raised plasma glucagon levels found in patients with poorly controlled diabetes play a role in the enhanced neogenesis and excessive glucose release from the liver causing fasting hyperglycemia.

Glucagon brings about the inhibition of pyruvate kinase (PK) in the liver, causing phosphoenolpyruvate (PEP) to accumulate. The level of pyruvate decreases, both because its synthesis from PEP is blocked and because it continues to be converted to PEP, via the pyruvate carboxylase and phosphoenolpyruvate carboxykinase reactions. Accumulation of PEP promotes gluconeogenesis, while the inhibition of pyruvate kinase diminishes the glycolytic flux rate. Glucagon also raises cAMP levels in adipose tissue. There the chief effect of cAMP is to promote triacylglycerol mobilization via phosphorylation of hormone-sensitive lipase, yielding glycerol and fatty acids.

Free radicals in Diabetes

At high concentrations, free radicals and radical-derived, nonradical reactive species are hazardous for living organisms and damage all major cellular constituents. At moderate concentrations, however, nitric oxide (NO), superoxide anion, and related reactive oxygen

species (ROS) play an important role as regulatory mediators in signaling processes. Many of the ROS-mediated responses actually protect the cells against oxidative stress and re-establish "redox homeostasis."

H₂O₂ acts as a second messenger (Reth, M. 2002). He described "a cloud of H₂O₂" emanating from receptors whose activation by other agonists is coupled both to phosphorylation and to ROI production. H₂O₂ transiently inactivates tyrosine phosphatases via reversible oxidation of their active-site cysteinyl residues and thereby augments phosphokinase-mediated signal transmission. Because the specificity is imparted by the receptor and is not preserved by the H₂O₂, H₂O₂ in this situation is not a second messenger in Sutherland's sense. Rather, H₂O₂ is acting in this case as a secondary messenger, modifying the extent or duration of a reaction initiated by another signal. This regulatory role, executed through a reversible covalent interaction, typifies type III specificity. (Rhee, S.G., 2000)

Light and Free radicals

Light has been shown to increase H₂O₂ production at red visible wavelengths. Recent studies have showed that blue and red light irradiation in vitro and in vivo can increase production of nitric oxide (NO), superoxide anion, and related reactive oxygen species (ROS) (Klebanov G.E. *et al.*, 2000). These can modulate the production and secretion of several cytokines and other mediators and play an important role as regulatory mediators in signaling processes which can then modulate the production, mobilization and homing of stem cells (Jozkowicz A. *et al.*, 2004, Gasparyan L. *et al.*, 2004).

It has been demonstrated that an enzyme, superoxide dismutase (SOD), specifically catalyses the dismutation of superoxide. The function of SOD can be regulated by activating the redox sites of copper and zinc by their absorption maxima at 404 nm, 450 nm, 584 nm, 620nm, 632nm, 680nm, 685nm, 760nm, 780nm or 820 nm. There are two main types of SOD

1. Cyanide-sensitive, copper and zinc containing enzymes; with absorption maxima at 404 nm, 450 nm, 584 nm, 620nm, 632nm, 680nm, 685nm, 760nm, 780nm or 820 nm.
2. Cyanide-sensitive enzymes containing iron and manganese. SOD from liver mitochondria contain manganese. with absorption maxima at, 450 nm, 584 nm, 620nm, 632nm, 680nm, , 760nm.

Several hematopoietic growth factors , including granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin-3 (IL-3), steel factor (SF), and thrombopoietin (TPO) induce a rapid increase in reactive oxygen species (ROS) in quiescent cells , and some of the biochemical and biologic effects of these cytokines are mimicked by H₂O₂ and superoxide. (Nathan, C, 2003)

These effects include tyrosine phosphorylation of cellular proteins, increased migration, c-FOS gene expression, and G1 to S-phase cell cycle progression. (Dalton, T.P.,1999)

Glucose and oxidation

The breakdown of carbohydrates, particularly glucose, is a major source of cellular energy. Glycolysis, the initial stage in the breakdown of glucose, is common to virtually all cells. However glucose itself can also act as an oxidizing agent during this breakdown depending on the composition of surrounding molecules.

The molecular mechanism of biological oxidation by glucose was first identified in 1912 by Louis Maillard. This French chemist described a brown colour that formed from heating solutions of carbohydrates and amines and termed this process the "reaction du Maillard." Glucose reacts nonenzymatically with protein amino groups to initiate glycation, the early stage of the Maillard reaction. This process begins with the conversion of reversible Schiff base adducts to stable, covalently bound Amadori rearrangement products. The levels of the Amadori products on numerous proteins are elevated in proportion to the degree of hyperglycemia in diabetes mellitus. In the intermediate stage of the Maillard reaction, the Amadori products can then undergo multiple dehydration and rearrangements to produce highly reactive carbonyl compounds such as

3-deoxyglucosane (3-deoxy- D-erythro- hexos-2- u lose, 3-DG, which reacts again with free amino groups, leading to crosslinking and browning of the proteins via the formation of advanced glycation end products (AGEs) in the late stage of the Maillard reaction.

The modification of long-lived proteins with AGEs has been hypothesized to contribute to the development of pathologies associated with diabetes mellitus, aging], dialysis-related amyloidosis, and Alzheimer disease]. The in vivo presence of AGEs has been demonstrated in haemoglobin lens crystalline, beta 2-microglobulin (beta 2m)], beta-amyloid peptide and tau protein. It has also been shown that AGE peptides accumulate in the circulation of diabetic and nondiabetic patients with uremia. Immunohistochemistry using antipyrraline antibody and anti-AGE antibody showed that pyrraline and AGE have been reported to be localized in the extracellular matrix of sclerosed glomeruli from diabetic patients with advanced nephropathy.

Recent studies have implicated the Maillard reaction in ageing and in the normal turnover of proteins. Studies of the nonenzymatic glycosylation or advanced glycosylation endproducts (AGEs) in vivo began with the discovery of elevated amounts of the minor haemoglobin, HbA1 C, in the blood of patients and animals with diabetes. The Maillard reaction accounts for the glucose-dependent, nonenzymatic covalent modification of proteins that accompanies hyperglycemic states. Initially, the Maillard reaction involves the combination of the aldehyde group of glucose in the open-chain form with amine groups on proteins to form a Schiff base followed by Amadori rearrangement to form fructose lysine. This reversible glycosylation of amino groups, or glycation, underlies the formation of HbA1 C, the well-recognized marker of chronic glycemic control in diabetes mellitus. The final stage of the Maillard reaction involves the irreversible oxidation, or glycooxidation, of fructose lysine to yield a host of advanced glycation end products (AGEs) such as Nepsilon-(carboxymethyl)lysine, pentosidine, and pyrroline, the formation of which correlate directly with the vascular and renal complications of diabetes mellitus. These carbohydrate-derived protein oxidation products are readily quantified with gas chromatography/mass spectroscopy and are more abundant in diabetics than in age-matched control subjects. The pentosidine content of collagen-rich tissues has been demonstrated to increase with age. Both skin and plasma content is further increased in the presence of diabetes, where levels are found to correlate with severity of complications. The highest levels of pentosidine are found on tissue and circulating proteins from patients with renal failure.

High circulating levels of AGEs which correlate positively with elevated creatinine levels have been described in renal failure patients. Circulating low molecular weight AGE fractions and, by implication, free pentosidine derives from breakdown of AGEs on tissue or on high molecular weight proteins. Direct reactivity of these break-down products has been demonstrated in vitro and in vivo. Therefore the accumulation of AGE fragments in patients with renal failure may mediate many uremic toxic effects.

During the course of their studies of AGE formation, researchers were struck by the fact that tissues isolated from live subjects have less AGEs than predicted from the incubation in vitro of proteins and glucose. This prompted the hypothesis that a removal system must exist in vivo. Macrophages, well known scavenger cells of the body, were found to possess specific AGE-receptors that could mediate the uptake and eventual breakdown of AGE-proteins. AGE-receptors have been since noted to occur on a number of other cells as well, including endothelial cells, smooth muscle cells, mesangial cells, monocytes and fibroblasts.

Macrophage scavenger system

The identification of a macrophage scavenger system for AGE-modified proteins has opened up a new area of investigation into the role of AGE modification in connective tissue homeostasis and the development of metabolic diseases. Although connective tissue matrix proteins, including collagen, have extended life span in vivo, they are slowly removed and replaced throughout adult life.

The monocyte macrophage plays an important role in this process both by removing the senescent molecules that have accumulated AGEs over time and by initiating the steps that lead to new protein

synthesis and tissue remodelling. The net accumulation of AGE proteins in tissues is a reflection of the balance of the reaction of glucose with the matrix proteins and their removal by the macrophage. Monocyte and macrophage production has been found to increase after manipulation of points 'Shangjuxu' St 37 and Tianshu UB10 and Dazhui Dul 4. T-helper lymphocyte production is increased by treating the points Jingmen GB 25, Zhaohai K6 and Dashu UB1 1 and suppressed by Ququan Liv 8.

Phagocytic white blood cells employ the myeloperoxidase- H_2O_2 system to generate reactive oxygen intermediates that kill invading bacteria, viruses, and tumour cells. Partially reduced oxygen species are also potentially damaging to cellular lipids, nucleic acids, and proteins; the production of such species by activated phagocytes has been implicated in the damage of normal tissues under pathological conditions. The initial pathway for oxidant generation involves a membrane associated NADPH oxidase that reduces oxygen to superoxide anion, which then dismutates to form H_2O_2 . Myeloperoxidase, a secreted heme protein, amplifies the toxic potential of H_2O_2 by producing reactive intermediates. Production of myeloperoxidase is inhibited by irradiation at 633nm, 660nm, 820nm, 880nm and 950nm, of which 660nm appears to have the strongest effect. Production of myeloperoxidase appears to be inhibited by low frequency electro-acupuncture at Mingmen Du 4 and also points Pishu UB20 and Jinayu L15.

The major product of myeloperoxidase at plasma concentrations of chloride ion is hypochlorous acid (HOCl). This potent cytotoxin chlorinates electron rich substrates and oxidatively bleaches heme proteins, nucleotides and carotenoids. Indirect evidence suggests that reactive carbonyls form in proteins and amino acids exposed to myeloperoxidase-generated HOCl, and trace quantities of aldehydes have been detected in amino acids exposed to high concentrations of reagent HOCl under strongly acidic conditions. Neither the reaction pathways nor the physiological relevance of the products generated in these reactions have been clearly established.

L-Serine and L-threonine are both present in concentrations up to 200 micro M in plasma, suggesting that hydroxy-amino acids may be substrates for oxidation by myeloperoxidase in vivo. Myeloperoxidase is a component of human atherosclerotic lesions and the patterns of immunostaining for the enzyme and for HOCl modified proteins are remarkably similar to those reported for oxidation-specific epitopes in rabbit atherosclerotic lesions. A wealth of evidence indicates that LDL, the major carrier of cholesterol in humans, must be oxidized to trigger the pathological events of atherosclerosis. LDL isolated from human aortic tissue exhibits immunoreactivity with polyclonal antibodies specific for acrolein modified proteins. These observations suggest that myeloperoxidase may be a catalyst for LDL oxidation in vivo, and that acrolein generated by myeloperoxidase may play a role in converting LDL into an atherogenic particle. Points used for hyperlipidaemia are Neiguan P6, Taichi Liv 3 and Fenglong St 40.

The high yield of the L-serine and L-threonine oxidation products suggests that other free amino acids may be substrates for oxidation by myeloperoxidase. The total concentration of free amino acids in plasma is [approximately] 4 mM, suggesting that reactive aldehydes derived from amino acids may be major products of phagocyte activation in vivo. Zhou et al noted a significant increase in the phagocytic activity of the neutrophils of patients who were given acupuncture at Zusani St 36, Neiguan P6 and Sanyinjiao Sp6 in comparison with no-treatment controls.

Other likely targets for oxidation by myeloperoxidase-generated HOCl include plasma antioxidants and protein amino groups. The mass spectrometric quantification of the levels of glycolaldehyde, 2-hydroxypropanal, and acrolein in inflammatory tissue should provide a powerful tool for investigating the role of hydroxy-amino acids as substrates for oxidation in vivo by myeloperoxidase.

Alpha-Hydroxy and alpha, beta-unsaturated aldehydes are highly reactive species that have been implicated in disorders ranging from ischemia-reperfusion injury to DNA damage and aging. Because the generation of reactive aldehydes by myeloperoxidase is nearly quantitative at plasma

concentrations of L-serine, L -threonine, and chloride, phagocyte-mediated formation of these products may be of central importance in promoting tissue injury at sites of inflammation.

Calmodulin

Calmodulin is a small protein (Mr ~17,000) which contains four calcium ion binding sites. Each site binds Ca⁺⁺ with a KD of about 10⁻⁶ M, consistent with observations that calcium can effect intracellular metabolic changes in concentrations as low as 1 mM.

Binding of calcium stimulates a major conformational change in the protein, leading to a more compact and more highly helical structure, which augments the affinity of calmodulin for a number of regulatory target proteins. When bound to calcium, calmodulin plays a special role as an integral subunit of the glycogen metabolism enzyme, phosphorylase b kinase. Hence, the glycogenolysis cascade depends on intracellular calcium concentration as well as on cyclic AMP levels. This dependence is particularly important in muscle, where contraction is stimulated by calcium release. Thus, Ca⁺⁺ plays a dual role, in provision of the energy substrates needed to support muscle contraction and in contraction itself.

In addition to phosphorylase b kinase, the calmodulin-calcium complex binds to other proteins, including the myosin light chain kinase in muscle, which helps to stimulate muscular contraction.

Calmodulin is involved in a wide range of diverse cellular processes such as cell cycle control, cell motility, smooth muscle contraction, and intercellular signaling. It activates various kinases (CaM kinase I and II and myosin light chain kinase), phosphatases (calcineurin), ion channels, and other cytosolic enzymes .

Calmodulin specifically decreases Smad2-dependent effects and increases Smad1 actions. Both Smad1 and Smad2 contain two distinct calmodulin-binding sites. RTK signaling also modifies Smad function. It has been found that calmodulin binding inhibits Erk2 phosphorylation and that Erk2 phosphorylation inhibits calmodulin binding *in vitro*. These data suggest cross-talk between Ca²⁺/CaM, TGF- β , and , receptor tyrosine kinase (RTK) signaling. Cytochrome P450 enzymes, NO synthase and calmodulin all have absorption maxima between 446 nm and 452 nm.

Diabetes and inflammation

The Barts-Windsor study in the early 1980's showed that there can be a long latency period in type I diabetes development. The start of pathogenic levels of Islet cell antibodies (ICA-IgG) may precede the clinical onset of diabetes by several years, even in children. Other classes of auto antibodies have been described in recent-onset type I diabetics- anti-insulin antibodies prior to initiation of insulin therapy, antibodies to the insulin receptor, and cold-reacting lymphocytotoxic antibodies.

Several attempts have been made to influence the course of type I diabetes by immunotherapy. Plasmapheresis, prednisone, and interferon have proven unsuccessful or only partially successful. Successful methods of preventing diabetes in Worchester rats have been neonatal thymectomy, antiserum to lymphocytes, bone marrow transplantations, and cyclosporine (the immunosuppressive drug highly effective against T cell responses). Treatment with cyclosporine initiated within 6 weeks after diagnosis has resulted in improved insulin secretion and cessation of then need for exogenous insulin. However, interrupting cyclosporine treatment was followed by relapse, and there was significant drug-related toxicity, especially nephrotoxicity. This however demonstrates the strong immunological background of the disease process. Administration of silica has also been reported to prevent diabetes in rat models, suggesting a critical role for macrophages in the pathogenesis of the process.

Type I diabetic patients have been shown to have inhibition of migration of leucocytes specific for antigens of the endocrine pancreas. Such inhibition, confirmed using purified T lymphocytes, can be reversed by addition to the test system of normal lymphocytes, suggesting that the diabetic patients have deficient suppressor T cell function. Suppressor T cell activity, demonstrated at the clinical onset of diabetes, has been shown to return to normal within 6 months after diagnosis. Differentiation of T cells, induction of granulocytic differentiation by granulocyte and macrophage colony-stimulating factor (GM-CSF), and differentiation of hematopoietic progenitors by

erythropoietin (EPO) are associated with local production of Insulin-like growth factor I (IGF-I), which acts through the IGF-I receptors that are widely expressed on all myeloid and lymphoid cells. In addition to stimulating proliferation and differentiation of these cells, IGF-I exerts chemoattractant effects on T-cell progenitors migrating from hematopoietic tissues to the thymus, where they are further differentiated by cytokines. At sites of inflammation, macrophage-derived IGF-I, together with interleukin-1 (IL-1), enhances migration of T lymphocytes to the site. These cytokine-activated T cells undergo clonal expansion in response to IGF-I.

The therapeutic effects of light are based on its versatile influence on all levels of the living matter. At the subcellular level the following changes take place: excitation of molecules, their stereochemical restructuring, increase of the protein as well as ATP, RNA, DNA synthesis rate and, consequently, activation of the nuclear apparatus of the DNA-RNA-protein system and biosynthetic processes. Some other processes are observed as well. For instance, elastic vibrations of protein structures, acceleration of the collagen and its precursors synthesis, changes of the oxygen balance and redox activity, inhibition of lipid peroxide oxidation and free radicals formation, potentiation of the antioxidants action. At the same time there is an increase of catalase and peroxidase activity as well as activation of other enzyme systems of the cell, namely, succinate-dehydrogenase, cytochromeoxidase, aldolase, acetyl-cholinesterase, ATP phase, acid and alkaline phosphatases. Some effects occur due to the transformation of the light photon energy into the energy of chemical bonds

Low energy laser treatment

Low energy laser treatment of blood results in the stimulation of phagocytosis and leukocyte migrational activity, stabilization of erythrocytes, stimulation of the erythropoietic function of the bone marrow, increase of the oxygen blood capacity, development of the desensitizing effect, reduction of the blood viscosity and coagulation, improvement of the rheological qualities, reduction of glucose, cholesterol, low-density and very low-density lipoproteins concentrations. Restoration (normalization) of functions and their stimulation occur at the organ level. Increase of adaptational ability, stabilization of the neuropsychic status, occurrence of the immunomodulating effect, stabilization of the hormonal status, normalization of arterial blood pressure take place at the whole-body level.

According to modern views, the membrane structures of the cell are of great importance in the formation of the response to laser emission. Their high sensitivity is explained by the fact that they represent natural boundaries of phase division. Light causes reorientation of the lipid bilayer polar groups. Since there is a close contact between the lipids and proteins, conformational changes of the lipid bilayer can influence membranes, cell energy production and enzyme reactions. The results of a great number of studies have shown that the membrane may restructure under the influence of light. For example, there are changes in the membrane permeability, restructuring in the composition of lysosomal, neuronal, microsomal, mitochondrial membranes, human cell membranes in the culture as well as artificial phospholipid membranes

The immunological behavior of cells depends not only on the structural and functional state of the membrane, but also on the pre-membrane layer - glycocalix - originating from the calcium ion. Stimulation of the phagocyte activity of leucocytes in blood, that of T- and B-lymphocyte rosette-formation ability, as well as their ability to blast-transformation under the light exposure of both the whole body and isolated cells, described in a number of papers, may be explained by physical and chemical changes in the state of cell membranes and pre-membrane layer, in particular

At the cellular level, changes in electric charge and the membrane potential occur. Stimulation of the nuclear functions raises the mitotic activity of the cell, activates reproduction processes as well as intra- and extra-cellular physiological and reparative regeneration. Low-energy laser emission in this case has an antimutagenic effect, the latter being its great advantage compared to

other forms of medical treatment. At the tissue level light irradiation leads to the increase of tissue oxygenation, pH changes of intercellular fluid, morphofunctional activity, substantial expansion of microcirculatory bed, antiedemic and anti-inflammatory, as well as analgetic and antipruritic effects.

Klebanov, 2003 found that lipopolysaccharides increased NO production and SOD activity in macrophages in a concentration-dependent manner. The irradiation of macrophages by red light resulted in a dose-dependent increase in NO production and SOD activity. The incubation of irradiated cells in the presence of 10 microM cycloheximide abolished the increase. The presence of antioxidants (mexidol and ascorbate) also significantly inhibited the laser-induced activity of macrophages. Thus, laser irradiation of cells in the red range activates the synthesis of SOD and inducible NO-synthase de novo due to photosensitized initiation of free radical reactions.

The aim of the treatment was to prevent and decrease complications connected to diabetes as well as balance glucose levels. Insulin dosage was not changed until glucose levels were low enough to necessitate the decrease.

Treatment protocols

Treatment was carried out in two modalities. Clinic treatment with a series of acupuncture points irradiated and blue clusters placed according to following instructions (see below) once a week. Patient was asked to reinforce the treatment using blue clusters at home daily.

Clinical treatment

Acupuncture points were irradiated with red laser (635 nm), 5 mW (ELAPS 04, Emred OY, Finland), 25-30 seconds each, using acupuncture light. The most important points used in treatment of diabetes are ;Liv 14, GB 24, REN 12, REN 10, REN 6, Liv 13, Lu 10, LI 11, Sp 6, St 40, Liv 3, Pijeh (ex 35), GB 21, UB 13, UB 17, UB 20, UB 21, UB23. Results obtained were significantly better when using a combination of laser and low amperage electric stimulation simultaneously. Combined use gave better results than either method used separately.

Variation in the used of points occurred according to type of diabetes, and according to accompanying complications.

The two varying treatment protocols are illustrated in diagrams 5 and 6.

Blue cluster of 1.6 – 2 mW/cm² at 458nm was used on midepigastic, Ren 12-14 area and dorsally both sides 10-12 rib area 4-5 cm laterally from midline. To begin with 5 minutes each area was irradiated and dose was increased up to 10 minutes according to glucose levels.

For peripheral neuropathy, red/infrared or blue cluster(s) were placed according to diagrams (Diag 1, 2, 3, 4)

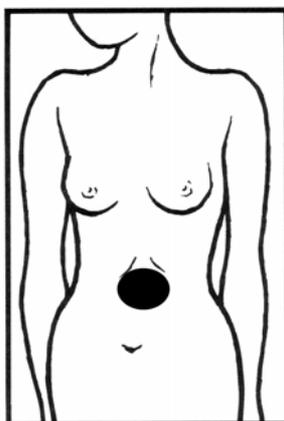


Diagram 1

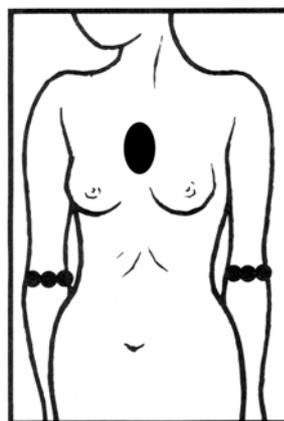


Diagram 2

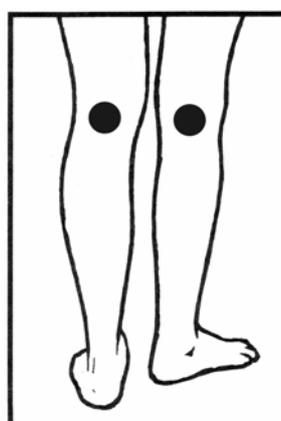


Diagram 3

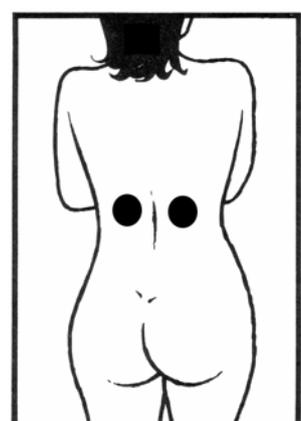


Diagram 4

Treatments were started proximally where numbness/pain begins and moved distally toward feet as pain/numbness resolved.

Treatment frequency was once a week for four weeks, thereafter once every two weeks for two months and then once a month according to need.

Home treatment

Patients were asked to follow daily the cluster treatment protocol similar to the clinic treatment protocol.

Results

7 type I and 17 type II diabetic patients have had treatments for a year or longer. All of the patients reported improvement of complications, neuropathy, ulcers, microcirculation. Long term glycolyzed hemoglobin levels lowered significantly within the first two months after which amount of medication used was slowly lowered. Type II diabetics had a faster response than type I. Of these type II diabetics 15 have been able to remain without medication for at least 6 months on average, type I diabetics have reduced amount of insulin used on average from 42 units of insulin to average of 14 units of insulin per day. Some younger patients have been able to cut down to minimal doses of only a few unit of insulin per day.

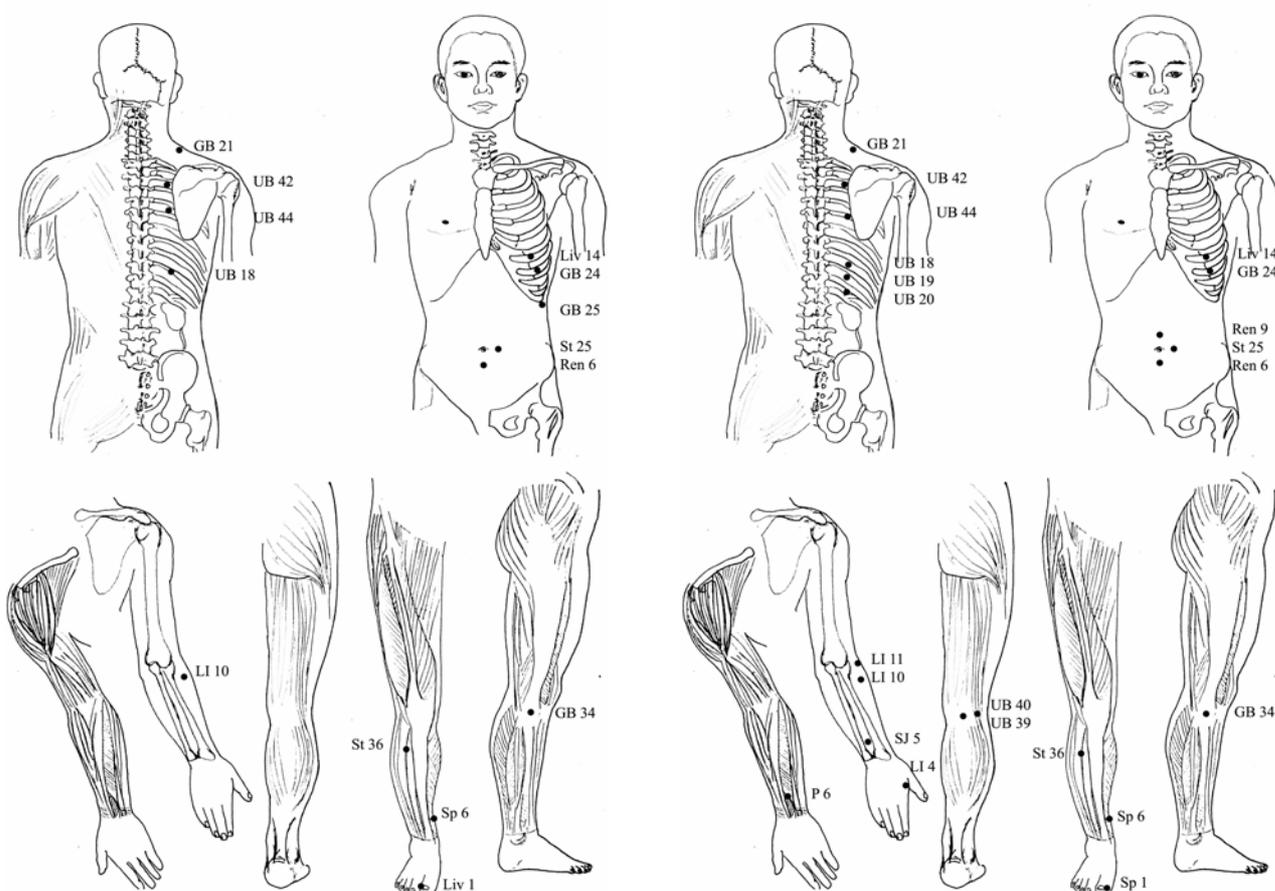


Diagram 5. Acupuncture points for type I diabetes

Diagram 5 Points used for type II diabetes

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