Understanding the Pathophysiology of Diabetic Skin May lead to Early Topical Intervention Resulting in Reduced Wounding and Loss of Limb

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Abstract

Purpose: Elucidate early and late stage diabetic skin pathophysiologies that lead to skin breakdown, wounding and potential loss of limb. Glycation, oxidation, inflammation, loss of microcirculation commence the cascade that leads to ischemia, neuropathy, collagen cross-linking and pruritus. The etiologies of this disease on the skin's immune system may best be directed to the field of biochemistry where micronutrients that have demonstrated efficacy against these disorders are now being incorporated into topical corneotherapeutic products. **Materials and Methods:** A review of scientific and medical databases was used and the data was cross referenced. Micronutrients with a strong scientific basis for use against each skin disorder were cataloged. For example, antioxidants were evaluated to determine their effectiveness against oxidative stress known to participate in premature skin cell death, aging, chronic disease and diabetes. Histamine blocking topical micronutrients were evaluated as part of the inflammatory and pruritic process. **Results:** Corneotherapeutic products provide for the administration of micronutrients and medicine by absorption through the skin. Micronutrients with a molecular weight of less that 500 Daltons can enter or exit the skin (500 Dalton Rule). These micronutrients include amino acids, vitamins, antioxidants and polyunsaturated fatty acids (PUFAs) comprised as a balance of n-3/n-6. Research demonstrated that these micronutrients, when applied topically, act at a biochemical level within the skin and reduce the impact

of oxidative damage, inflammation, and glycation. Corneotherapeutic products used at the earliest stages of diabetes may dramatically reduce the risks associated with skin breakdown, wounding and the eventual loss of limb.

Abbreviations

PUFAs - polyunsaturated fatty acids
AGE - advanced glycoslation end-products
FL - fructoselysine
CML - carboxymethyl lysine
ROS - reactive oxygen species
CGRP - calcitonin gene-related peptide
ORAC - oxygen radical absorbance capacity
MDA - malondialdehyde
PMNs - polymorphonuclear leukocytes
MMP - matrix metalloproteinase
RA - retinoic acid
IGF-I – Insulin-like growth factor
RCS - reactive carbonyl species

Background

Diabetes is primarily a metabolic disease and its complication is sequelae of broad-based derangements in fuel metabolism. There is discussion on the sequence of cellular and metabolic events related to diabetes and this speaks to the complexity of the disease. One hypothesis is that carbonyl stress precedes oxidative stress.[1] Both are involved in the pathogenesis of diabetic skin complication. The body of evidence may favor oxidative stress as the metabolic pathway to diabetic skin complications.[1]

The skin and its healing processes are altered in patients with diabetes. The physiological and biochemical implication of the effects of glucose have not been fully elucidated. The skin's altered state leads to chronic complications that

include loss of limbs and mortality. Hyperglycemia, insulin and IGF-I are abnormal in diabetic skin keratinocytes. Exposure to high glucose is associated with changes in cellular morphology, as well as with decreased proliferation and enhancement of Ca^{2+}. Hyperglycemia and impaired insulin signaling might be directly involved in the development of chronic complications of diabetes, such as impaired wound healing. By impeding glucose utilization of skin, keratinocytes as well as skin proliferation and differentiation are diminished. [2] Oxidative stress and oxidative damage that lead to premature skin cell death are presupposed in aging, chronic disease and diabetes. Oxidative stress has a primary role in the pathogenesis of diabetic complication and end-stage tissue damage in the diabetic. This oxidative process may account for increased glycoxidation and lipoxidation products in tissue proteins. These findings have lead Baynes, *et al* to hypothesize that increased chemical modification of proteins by carbohydrates and lipids in diabetes is the result of overload on metabolic pathways involved in detoxification of reactive carbonyl species (RCS). The overload is apparent in a general build up in steady-state levels of reactive carbonyl species. The increase in oxidation in glucose and lipids of tissue proteins in diabetes may therefore be viewed as the result of increased carbonyl stress. The use of lipoic acids for treatment of neuropathy associated with carbonyl stress may provide one pathway to improved tissue physiology.[1]

Glycation (cross-linking of proteins and sugars to form non-functioning structures in the body) modifies tissue proteins, resulting in chemical reactions

between glucose and primary amino groups. The Maillard reaction (also known as advanced glycoslation end-products [AGE products]) is used to measure modification of insoluble collagen associated with oxidative stress and glycation. End product chemicals present according to the stage of glycation. Early stage glycation is identified with fructoselysine (FL). The glycoxidation products are N-epsilon (carboxymethyl) lysine (CML) and pentosidine which form later in the process.[3] Studies support the description of diabetes as a disease characterized by accelerated chemical aging of long-lived tissue proteins.[4]

Dyer DG, *et a*l studied Maillard reaction products in skin collagen from 39 type 1 diabetic patients and 52 non-diabetic control subjects. The Maillard reaction is created by damaged proteins and is associated with aging of the extracellular matrix and diabetes. Proteins undergo increased stiffening and loss of elasticity during the process. In non-diabetic patients glycation of collagen FL content increased 33% between age group 20-85 years of age. In contrast CML, pentosidine and fluorescence increased five fold, correlating directly with age. In diabetic patients, collagen FL was increased threefold compared to non-diabetic subjects, correlating strongly with glycated hemoglobin but not with age. Collagen CML, pentosidine and fluorescence were increased up to two fold in diabetic subjects compared with control subjects. This can be explained by the increase in glycation alone. Increased glycation can be invoked without increased oxidative stress. There is a strong correlation between age-dependent CML, pentosidine and fluorescence modification of collagen via the Maillard

reaction and acceleration in the process chemical tissue aging in diabetes. Diabetic patients who are also under high oxidative stress, and are therefore rapid accumulators of glycoxidation products (AGE), may be particularly vulnerable to the development of complications associated with skin collagen and poor wound healing.

Oxidative reactions are a normal attribute of aerobic life that causes structural damage to DNA, proteins, carbohydrates and lipids. When this oxidative damage is inflicted by reactive oxygen species (ROS) it is known as "oxidative stress." Oxidative stress occurs when the balance of prooxidant/ antioxidant forces favor the prooxidant process.[5] Oxidative stress is apparent in the inflammatory process, aging and diabetes and is implicated in increased glycoxidation and lipoxidation leading to cell death.

Diabetic vascular complications are also resultant of oxidative stress. Oxidative stress may play an important role in the pathogenesis of vascular diseases characterized by an increased formation of free radicals and a corresponding depletion of antioxidant reserves.[6] Events that take place in macrovascular disease cascade leading to microvascular disease events. Complications related to microvascular blood flow create their own sequelae of endothelial dysfunction, neuropathy, foot ulceration and amputations.

Diabetic microangiopathy progresses slowly due to a combination of sequential circulatory changes including; (1) altered basement membrane, (2) altered cellular function, (3) cell metabolic changes, (4) altered blood flow properties, (5) disturbed hemostasis, (6) altered oxygen transport, and (7) altered hormone production.[7]

Skin blood flow is altered in diabetes. Some of the functional disturbances are improved through metabolic control. These microvascular alterations reduce blood flow to the skin and result in a reduction of micronutrient support.[8] It appears that disturbances in the microvascular system may play a role in the pathogenesis of diabetic neuropathy.[6]

Dermal neurovascular dysfunction stems in part from decreased microvascular blood flow and increased vascular resistance. These alterations involve impaired dilator response to substance P (a neuropeptide-neurotransmitter), calcitonin gene-related peptide (CGRP) and reactivity to nociceptive stimulation. Unmyelinated C-fibers, which constitute the central reflex pathway, are assumed to be damaged in diabetic neuropathy.[9] Insulin/C-peptide deficiency in diabetic neuropathy is linked to the metabolic abnormality of oxidative stress.[10] Neuropathies affect more than half of all diabetics. As the disease progresses, the deterioration results in peripheral and autonomic nerve dysfunction. Diabetic neuropathy is the most common cause of non-traumatic amputations affecting 15% of all diabetic patients with neuropathic disease.[11]

The pathophysiologies of diabetes commence with alteration in the metabolic processes. These unseen changes lead progressively to a disease state that involves the loss of limb and life. Early intervention with corneotherapeutic products may spare diabetics this fate.

Discussion

Considerable evidence exists demonstrating the role hyperglycemia plays in the production of ROS. ROS leads to increased oxidative stress in diabetic tissue. When the endogenous antioxidant network becomes overwhelmed (redox imbalance) intercellular signaling pathways are activated. Redox reactions involve the transfer of electrons. With redox, the compound that loses an electron is said to be "oxidized" while the compound that gains an electron is said to be "reduced." A major consequence is the production of gene products that cause cellular damage that lead to late-stage diabetic complications. Studies suggest that treatment with exogenous antioxidants may be an effective treatment against redox imbalance providing a balance between prooxidant/antioxidant forces.[12]

ROS (including free radical damage) tissue damage first presents in the sub-epidermal tissues with damage of proteins, lipids, and DNA. The endogenous antioxidant system has become overwhelmed causing cell apoptosis slowly progressing to present as skin lesions and more serious wounds. The

microvascular system responsible for nourishing the epidermis is in a state of oxidative stress due to redox imbalance.

The issue becomes how can intervention reverse the process and create a more homeostatic environment for the dermis, epidermis and stratum corneum? The epidermis is metabolically regulated by the integrity of the stratum corneum. When the barrier permeability is altered (lesions) the epidermis becomes compromised.

Corneotherapy

The term corneotherapy was coined by Albert M. Kligman, MD, in the mid-1990's.[13] Today, it is a field of research that is leading to an improved understanding of the skin's role as gatekeeper. In the past decade, researchers have discovered which topically applied substances will pass over its threshold. According to Kligman, "The ultimate development in the quest for understanding the stratum corneum was corneotherapy, focusing therapy toward correcting the defective horny layers associated with chronic diseases." [14] Among the complement of corneotherapeutic substances that can be delivered via the stratum corneum as "active agents" are amino acids, lipids, vitamins and extracts.[15]

500 Dalton Rule

Corneotherapy is made possible by what is known as the 500 Dalton Rule. The 500 Dalton Rule is used in the development of topical drugs and transdermal delivery systems. The skin's barrier is effective in blocking molecules with an atomic weight greater than 500 Daltons, but molecules with less weight pass through the skin's barrier. Topical drugs like cyclosporine, tacrolimus and ascomycins can be effectively delivered through the skin because the molecules of the drugs are all under 500 Daltons.

While the stratum corneum's physicochemical barrier resists the penetrations of large molecules, smaller molecules with a molecular weight of less than 500 Daltons surpass transcutaneously.[16] Molecular size is an important factor governing passage of substances through the skin, giving substances with higher molecular weights self-limiting properties.[17] Passive delivery of substances, due to their low molecular weight, provides novel delivery opportunities.[18] Included in these low molecular weight substances are vitamins, amino acids, essential fatty acids n3 and n6, and antioxidants like hydroxytyrosol.

Essential Fatty Acids

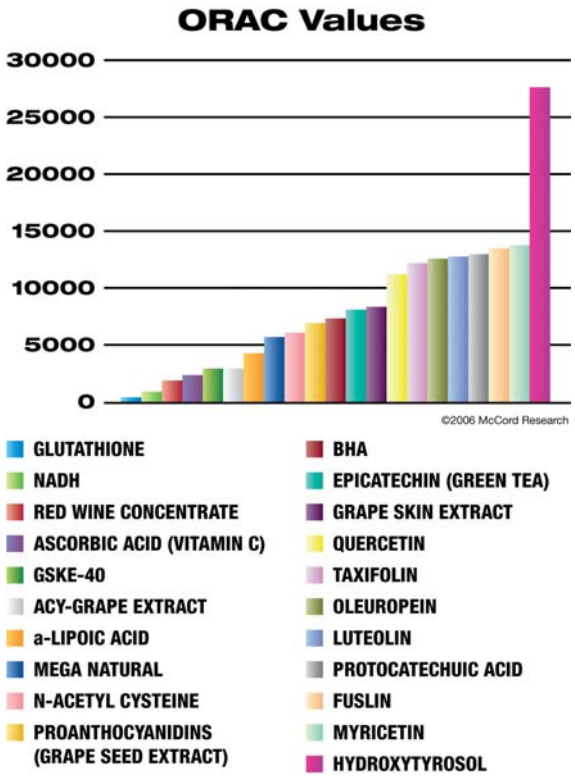
The inflammatory response is among the physiological events at the site of injury. Fatty acids are involved in the modulation of wound (injury) closure either in the form of phospholipids anchored in the cell membrane or as soluble lipoic mediators. This favorable modulation demonstrates a role for fatty acids as therapeutic agents at the wound site.[19]

There are many plant oils that provide n3 and n6 fatty acids to the skin for therapeutic applications. Among them are olive oil and canola oil which were the focus of our research. Olive oil is unique in that it delivers an additional benefit as a free radical scavenger. 3,4-dihydroxyphenyl ethanol or hydroxytyrosol, is a potent anti-inflammatory and antioxidant found in olive oil as well as the pulp of green olives.[20]

In a study to evaluate olive oil, with its hydroxytyrosol component, it was found to block activation of nuclear factor-kappaB, signal transducer an activator of transcription-1 alpha and interferon regulatory factor-1. In addition, it down-regulated inducible nitric oxide and COX-2 gene expression by preventing nuclear factor-kappaB. These findings suggest that olive oil/hydroxytyrosol may represent a non-toxic agent for the control of pro-inflammatory genes.[21]

Olive phenolics possess the highest antioxidant activities of all known antioxidants. They scavenge superoxide and other reactive oxygen species, inhibit neutrophil respiratory burst, and increase plasma antioxidant capacity.[22] Hydroxytyrosol has an oxygen radical absorbance capacity (ORAC) value nearly six times that of alpha lipoic acid (antioxidant).

Table I



Hydroxytyrosol, from olives, is currently the most potent antioxidant available and provides protection nearly three times that of grapeseed extract.

Canola oil is a good source of alpha-linolenic acid (11%) and it has an optimal n6:n3 ratio.[23] Canola oil's balance of the essential polyunsaturated fatty acids, linoleic and alpha-linolenic acid (two fatty acids that the body cannot produce) provide lipids necessary for cell membrane repair and cellular respiration.

In a study to see if topically applied lipids interfere with the structure and function of the permeability barrier, several oils and butters were evaluated. The skin of 21 healthy subjects was irritated by sodium lauryl sulphate. Canola oil assisted in the barrier repair and did not interfere with the repair process. The observed effects of canol oil on sodium lauryl sulphate-induced irritation was that canola oil assisted the damage barrier by providing adequate lipids. In this

study canola oil out performed borage oil, sunflower seed oil, shea butter and petrolatum.[24]

Amino Acids

Less than two dozen amino acids join together to orchestrate the body's tissue and skeletal structures. These small molecules join forces to create macromolecules like collagen, elastin and fibronectin. Amino acids are also involved in thousands of chemical reactions each second that have nothing to do with tissue or skeletal formation. Two of the body's most vital amino acids are L-taurine and L-cysteine.

Taurine (2-aminoethane sulfonic acid) is of importance in the management of diabetic skin. Taurine is a sulfur-containing amino acid found in almost all tissues.[25] It is responsible for a myriad of important physiological roles in each organ. Taurine plays a pivotal role as; a) an antioxidant and detoxifying agent against ROS, b) enhancer of cell proliferation, c) reducer of inflammation, d) key as an osmosregulator and, e) a stimulatory of glycolysis and glycogenesis. The role of taurine is determined by the cell type.[26] At the wound site, taurine increases tensile strength by reducing lipid peroxide formation-malondialdehyde (MDA). Further, taurine is vital to neurotransmission and serves as a neuroprotectant.

Taurine prevents high-glucose-induced vascular endothelial cell apoptosis or programmed cell death. In vitro and in vivo studies have demonstrated that high glucose selectively triggers apoptosis. Taurine has cytoprotective properties through its actions as an antioxidant, osmoregulator and intracellular Ca^{2+} flux regulator. Taurine has been shown to reduce the cell damage associated with the ischemia-reperfusion phenomena (ROS and no-flow of fluid after injury). Subjects with insulin-dependent diabetes have been found to be deplete in taurine, giving this amino acid pharmacological value in the treatment of diabetic patients.[27]

AGEs accumulate earlier and faster in long-term diabetes than in aging. The accumulation of AGE and oxidative stress enhance the synthesis of extracellular matrix and the release of toxic cytokines. Cysteine, in combination with taurine, was effective in preventing AGE in the treatment of advanced diabetes.[28]

Peripheral nerve conduction velocity deficits are dependent on decreased nerve perfusion which is related to free radical activity that is not impaired via endogenous protection in the redox cycle. In a controlled study using diabetic rats that had been either treated with cysteine or left untreated, followed by the application of a liquid nitrogen-cooled probe to form a lesion on the myelinated nerve fiber, it was found that the cysteine treatment prevented damage.[29] This study has implications in the treatment of diabetic neuropathy.

Vitamins

Vitamins are organic compounds that are required by the body. Our bodies are incapable of synthesizing vitamins and vitamin deficiency is associated with many diseases. The body is better able to transform the vitamins provided through the food we eat when we are young. With age, vitamin synthesis decreases and vitamins like A, B, C and D are not available for many of the metabolic processes associated with cell viability.

According to Vernon R. Young, Ph.D. of the Massachusetts Institute of Technology (MIT), one area of exciting research deals with vitamin D. According to Young, vitamin D requirements of our cells are met in part by the conversion of a precursor of cholesterol through the imposition of ultraviolet light. This precursor of cholesterol is converted to a product which subsequently undergoes a metabolic transformation in the liver. Then it leaves the liver and goes to the kidney, and finally the active form of vitamin D, 1,25-dihydroxyvitamin D (1,25[OH]2D) is formed. Vitamin D is carried to the tissues where it is required by a protein in the blood stream. Studies suggest that with age the precursor of vitamin D declines reducing the conversion of 7-dehydrocholesterol to the active form of vitamin D. Diabetes is a disease that, due to the creation of ROS, accelerates the aging process on a cellular level.

1,25 (OH) 2D down regulates inflammatory markers and is important for the paracrine regulation of cell differentiation and function. For this reason vitamin

D deficiency can play a role in the pathogenesis of auto-immune diseases like diabetes.[30] Vitamin D deficiency predisposes individuals to type 1 and 2 diabetes and has been shown to impair insulin synthesis and secretion in humans suggesting that it has a role in type 2diabetes. Vitamin D deficiency may, therefore, be involved in the pathogenesis of both forms of diabetes.[31]

Studies to ascertain the capacity of the epidermis to produce Vitamin D3 in young and aged skin was studied. The skin of both groups was exposed to ultraviolet light and then evaluated for Vitamin D_3 levels. When comparing Vitamin D_3 levels in the skin of subjects between the ages of 8-18 years of age with those of subjects between 77-88 years of age, it was shown that Vitamin D_3 epidermal levels were reduced two-fold in the aged subjects.[32] Such research may reinforce the need for corneotherapeutic Vitamin D_3 in patients with diabetes and/or aged skin.

Retinyl palmitate (Vitamin A) is essential for normal skin development and is known to have physiological and biochemical effects. It serves as an important regulator of keratinocytes terminal differentiation. It has the potential to alter the expression of protein molecules in both the epidermis and dermis. It is directly involved in collagen synthesis and the type of collagen synthesized. A study using topical administration of retinyl palmitate (0.1%-5% w/w) for 14 days on hairless mice demonstrated an up to 128% increase of collagen per unit of skin surface area in response to retinyl palmitate administration when compared to

control. There was a significant thickening of the epidermis and in increase in DNA content.[33]

It is well known that retinoids are effective in the treatment of dermatological disorders. It has been reported that retinoids have inhibitory effects on the generation of superoxide O_2 (ROS generated during inflammation) by stimulated polymorphonuclear leukocytes (PMNs). In a study to evaluate the effect of retinoids on PMNs and ROS, it was found that retinoids served as effective antioxidants and reduced PMN inflammation associated with inflammatory skin disorders.[34]

Vitamin A can be absorbed through the skin in physiologically significant quantities.[35] Diabetics have an increased susceptibility to chronic skin ulcerations. The etiology of chronic wound formation in diabetic individuals is multifactoral but may be accelerated by changes in the structure and function of the skin secondary to impaired fibroblast proliferation, decreased collagen synthesis and increased matrix metalloproteinase (MMP) expression. In a study to determine the effects of retinoids on diabetic skin, 2mm skin biopsies from the hip or ankle were obtained and then incubated for 9 days in the presents of retinoic acid (RA). The study's data suggest that RA has the capacity to improve the structure and function of diabetic skin, and that a major effect is on reduction of collagen-degrading MMPs.[36]

A feature of aged skin is the flattening of the epidermal-dermal junction, evidenced in histological sections as a loss of rete ridges and the disappearance of papillary projections. Diabetics suffer from premature skin aging are subject to this loss of dermal-epidermal junction integrity. In a study to determine if topically applied Vitamin C could increase the density of dermal papillae in aged human skin, it was determined that Vitamin C had the potential to enhance the density of dermal papillae, perhaps through the mechanism of angiogenesis. Further, it was determined that topically applied Vitamin C may have therapeutical effects for correction of the regressive structural changes associated with the aging process.[37]

Vitamin C is known for its antioxidant potential and activity in collagen biosynthetic pathways. The photo-protective properties of topically applied Vitamin C have also been demonstrated, placing this small molecule as a potential candidate for use in the prevention and treatment of skin aging.[38] In addition, Vitamin C selectively restores the impaired endothelium-dependent vasodilatation in the vessels of patients with insulin-dependent diabetes mellitus through its antioxidant effects against NO degradation.[39]

Niacinamide (Vitamin B_3) prevents or delays insulin-deficient diabetes in several animal models of type 1 diabetes and improves cell function.[40] Vitamin B_3 acts as an antioxidant and can reduce inflammation and may also improve glucose control in type 2 diabetics. In multiple chronic clinical studies, topical Vitamin

B_3 has been observed to be well tolerated by skin and to provide a broad array of improvements for aging skin.[41]

Vitamin B_6 participates as a cofactor in the synthesis of prostaglandin hormones that, in part, dertermine the smoothness and texture of skin. Virtually all B-Vitamins are required at sufficient doses to ensure healthy development of skin cells. Deficiencies in B-Vitamins directly result in various types of skin conditions, skin diseases and alterations in the normal appearance of skin.[42]

Conclusion

Corneotherapy is an effective adjunctive treatment in patients with diabetes for the prevention of skin breakdown and treatment of the peri-wound area. The literature supports the positive effects of topically administered small molecules such as amino acids, fatty acids, vitamins and specific antioxidants such as hydroxytyrosol. Topical products should be reviewed to determine their ability to deliver corneotherapeutic ingredients to diabetic patients for the prevention of skin breakdown and wounding.

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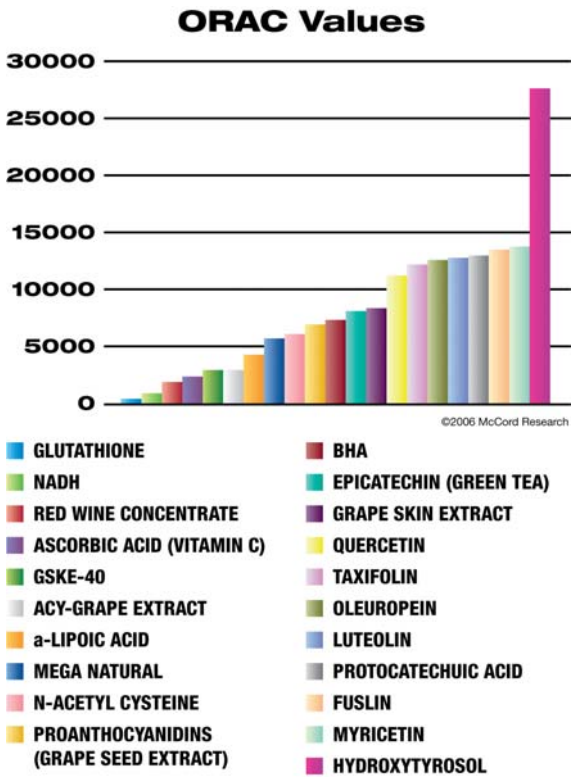
Table I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