Tripeptide reversing the glycosylation (glucose-derived intermolecular) crosslinks in proteins (Advanced Glycation End Products (AGEs)) and the Schiff bases.

Worldwide Patented Combined Composition for the Next-Generation Treatment of Ophthalmic Complications of Diabetes Mellitus (DM) from Innovative Vision Products, Inc.
Summary:

A major consequence of hyperglycemia is excessive non-enzymatic glycosylation of proteins resulting in various protein-protein cross-links and non-crosslinked structures. Advanced Glycation End (AGE) products contribute to long term complications of Insulin Dependent Diabetes Mellitus (IDDM) patients. With the increasing rate of occurrence of IDDM, it is important to increase knowledge about AGEs and AGE-inhibitors. Through research provided at Innovative Vision Products, Inc. it may be possible and beneficial to find substances that can be used to decrease or predict the occurrence of long term complications of AGE formation to improve the quality and length of life for IDDM patients.

Dear Sirs/Madame,

The pathogenesis of diabetic complications continues to be a central issue in current diabetes research. One of the most prevalent metabolic syndromes world-wide, diabetes mellitus (DM), is characterized by hyperglycemia resulting in short-term metabolic changes in lipid and protein metabolism and long-term irreversible vascular and connective-tissue changes.

The microvascular complications accompanying diabetes have a biochemical basis that involves four metabolic pathways: polyol, hexosamine, protein kinase C, and glycation end-products. Understanding these pathways’ role in
the pathological changes that accompany diabetes may assist in diabetes prevention and treatment.

Experimentally induced high molecular crosslinks of vitreous collagen

**Many abnormalities in collagen have been reported in insulin-dependent diabetes mellitus (IDDM), some or all of which have been attributed to increased cross-linking. Recent works have focused on the role of glucose-derived collagen cross-links in the pathogenesis of diabetic complications.**

Structure of fluorophore P (Pentosidine).
These changes include diabetes-specific complications such as retinopathy, nephropathy and neuropathy and complications of the macro-vasculature such as atherosclerosis; potentially resulting in heart disease, stroke and peripheral vascular disease. Links between chronic hyperglycemia and the development of long-term diabetic-specific complications have been discovered and are yet not completely understood.
Glycation (nonenzymatic glycosylation) processes, also known as the Maillard reactions, are a series of reactions between carbohydrates and free amino groups of proteins. The preliminary intermediates, (Amadori products; 1-amino, 1-deoxy, 2-ketoses), ultimately result in the formation of AGES. AGES in humans have been predominantly chemically characterized by the detection of pentosidine and N-carboxy-methyl lysine (CML). Both pentosidine and CML have been found to accumulate in skin and lens collagen matrix at accelerated rates in diabetic patients. Indications are that collagen in IDDM patients undergoes widespread chemical alterations that result in decreased solubility, alter binding affinities to enzymes, increased stability, accelerated cross-linking and increased browning. Accumulation of AGES with structural alterations result in altered tissue properties that contribute to the reduced susceptibility to catabolism and to the aging of tissues. Also, when accelerated by hyperglycemia, AGE accumulation is believed to contribute to the gradual development of diabetic complications. Pentosidine concentrations in the skin of IDDM patients are often elevated and correlate to the severity of complications. It has also been suggested that pentosidine is not just a subset of diabetic complications but rather a general diagnostic feature of the disease process.
Ocular Complications of Diabetes Mellitus

This is the normal appearance of the retina on IVP funduscopic examination.

The eyes can be affected in several ways by diabetes mellitus. Diabetic retinopathy is one of the leading causes for irreversible blindness in the United States.

Diabetic retinopathy is shown here on funduscopic examination at IVP.
Proliferative diabetic retinopathy on funduscopic examination is shown here. This is a particularly serious complication in diabetics that can lead to blindness.

This retinopathy can occur with either type I or type II diabetes mellitus, usually a decade or so after the onset of diabetes. Most persons with type I diabetes and many of those with type II diabetes develop some background (non-proliferative) retinopathy. Proliferative retinopathy is more ominous and is more likely to occur when diabetes mellitus is poorly controlled.

In severe retinopathy, neovascularization may lead to adhesions (synechiae) between iris and cornea or iris and lens.

Neovascularization of the iris leads to secondary glaucoma with blindness.
Glaucoma with marked cupping of the optic disk is seen on funduscopic examination above. The incidence of glaucoma is higher in the diabetic population.

Glaucoma with excavation of the optic cup is shown here microscopically. Diabetics are more prone to develop this complication.

Cataracts are more common in diabetics. This predilection for development of cataracts is felt to result from hyperglycemia leading to accumulation of sorbitol that results in osmotic damage to the crystalline lens.
Cataracts of the crystalline lens with opacification, as shown here, are more frequent in persons with diabetes mellitus.

Glycation, a chemical modification of proteins with reducing sugars, indicates a possible explanation for the association between hyperglycemia and the wide variety of tissue pathologies. Research suggests that reducing sugars can react with the amino groups of long-lived proteins to produce non-enzymatic cross-links. Formations of these cross-links occur as end-stage products of the Maillard reaction; they are known as advanced glycation end-products (AGEs).
The chemical nature of AGEs \textit{in vivo} is largely unknown, but there is a growing population of structurally-defined AGE adducts such as pyrraline, pentosidine, N-carboxy-methyl lysine (CML) and crossline that are found to be elevated in diabetic tissues. The best found chemically characterized AGEs in humans are pentosidine and CML (see Figures). Some of the highest levels of pentosidine have been detected in individuals afflicted with DM. Evidence has shown that elevated skin pentosidine levels in individuals with DM correlate with the severity of the complications. Initial investigations have shown that pentosidine can be detected in smaller levels in various tissues of noncollagenous origin, including the blood and the human lens.

\textit{Fig 3}

Advanced glycation products in vascular pathology. This diagram depicts some of the key points discussed in the text on the role of AGE products in microangiopathy as well as in macroangiopathy.
Advanced glycation products: Role of receptors. Many cells bear receptors that recognize AGE. This diagram delineates some of the effects of this interaction with regards to microangiopathy.

The Maillard reaction and diabetes mellitus (Contribution from Dr Alejandro Gugliucci MD, PhD)

AGEs are a class of complex, often unstable, reactive compounds formed in excess during aging and diabetes mellitus. According to the “glycation hypothesis,” accumulation of AGEs alters the structural properties of tissue proteins and reduces their susceptibility to catabolism. It has been shown that the process of AGE formation is accelerated by hyperglycemia. Some of the protein alterations observed in diabetic patients resemble those in much
older, non-diabetic patients, suggesting ‘diabetes induced early aging’.

Structure of carboxyl methyl lysine (CML).

Protein glycation and AGE formation are accompanied by
increased free radical activity that contributes to the bimolecular damage in diabetes. AGEs act as mediators and can initiate a wide range of abnormal responses in cells and tissues such as the inappropriate expression of growth factors, alterations in growth dynamics, accumulation of extra-cellular matrix and initiation of cell death, through decreased solubility, elasticity and enzymatic affinities in long-living proteins such as collagen.

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Effects of advanced glycation end products on hyaluronan photolysis: a new mechanism of diabetic vitreopathy.


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**Purpose:** To test the effects of advanced glycation end products (AGEs), which are increased in vitreous of diabetic patients, on photolysis of hyaluronan. **Methods:** Pullulan standards were used as molecular weight (MW) markers to obtain a calibration curve. 0.02% hyaluronan solutions were divided into AGE-added and AGE-free samples; each sample was irradiated using a xenon lamp or kept in the dark. Retention time (RT) was measured for each sample using high-performance liquid chromatography. **Results:** RTs and logarithm of MW of pullulan standards were negatively correlated. In hyaluronan samples exposed to light, RT increased significantly for both AGE-added and AGE-free samples compared with samples kept in the dark. RT in AGE-added samples was greater by 3% than that in AGE-free samples (p = 0.02). **Conclusions:** Exposure to light decreases MW of hyaluronan; addition of AGEs promotes this change. The photosensitizer activity of AGEs may be associated with accelerated depolymerization of hyaluronan in diabetic patients.
Non-enzymatic glycosylation occurs with many proteins including hemoglobin, lens crystallins, collagen in the vitreous body, skin and other tissues an attachment that results in changes in the physical and chemical properties.

Test to determine the degree of glycation of hemoglobin in blood

The role of advanced glycation end-products (AGEs) in the development of complications in individuals with insulin-dependent diabetes mellitus (IDDM) has been explored by previous studies.

Glucose in solution exists as a stable pyranose ring in equilibrium with the open chain aldehyde form. The reaction of monosaccharides or aldehydes derived from lipid peroxidation with proteins consists of the covalent linkage of the double-bonded oxygen of the aldehyde function with an NH2 group, either on the alpha-amino group of the N-terminal amino acid or on the epsilon-amino group of lysine. This condensation results in the formation of a Schiff base or aldimine, and is a reversible reaction. However, following the formation of the Schiff base in the tissues, there is an internal reconfiguration of the molecule, the so called Amadori rearrangement, resulting in formation of a ketoamine which tends to not revert back to the Schiff base. The rate of reaction of various carbohydrates with protein correlates with the extent to which the sugar exists in the open ring (aldehyde) form.
Following the condensation and reconfiguration, the Amadori products undergo a series of further reactions with amino groups on other proteins to form glucose-derived intermolecular crosslinks in the tissue proteins.

These collagen modifications result in a color change which has been demonstrated by spectrophotometric measurement to correlate with diabetic complications.

One of these advanced glycosylation products, a yellow compound, 2-(2-furoyl)-4(5)-(2-furanyl)-1H-imidazole, has been identified. Quantitation of another advanced glycosylation end product in the skin, the amino acid pentosidine, has also been demonstrated to correlate with a cumulative score of diabetic complications.

The process of non-enzymatic glycosylation occurs to a minor extent at normal blood sugar concentrations.

**Dehydroascorbate can act as the glycosylating agent in proteins. This is a most plausible mechanism of age-related cataract progression.**

Grade 4 Nuclear Sclerosis (NS) Cataract Formation
Grade 1 Nuclear Sclerosis (NS) Cataract Formation

The gradual glycosylation of proteins may be responsible for some of the sensitive tissue changes associated with aging, and this process is apparently accelerated in persons with elevated blood sugars. Most proteins evaluated seem to be involved by this reaction which results in changes in the physical and chemical properties.

Glucosylation of the red cell membrane is apparently responsible for the stiffness of diabetic erythrocytes.
Glucosylation of collagen results in increased stiffness and resistance to enzymatic degradation, mechanical changes of collagen which are also characteristic for aging.
Protein glycosylation with changes in tertiary structure and solubility of proteins could conceivably be responsible for many of the ophthalmic complications.

Advanced glycation end products (AGEs) from the Maillard reaction contribute to the pathogenesis of diabetes-associated complications.

Non-Proliferative Diabetic Retinopathy

In therapeutic interventions for reducing AGEs, few compounds have been reported as AGE inhibitors.
AGE inhibitors

Due to detrimental effects of AGEs, researchers attempt to find inhibitors of the advanced glycation process. Brownlee et al. suggest that optimal future therapies to minimize tissue damage may require pharmacologic agents that directly interfere with the self-perpetuating component of hyperglycemia-initiated tissue damage. Aminoguanidine (AG), an inhibitor of advanced glycation reactions in vitro, has been found to inhibit the development of diabetic complications in animal models of diabetes. Booth et al. suggest that these inhibitors can potentially react as a hydrazine with carbonyls of Amadori intermediates or can hunt for reactive dicarbonyls through its guanidinium moiety. However, the mechanism of AGE formation is only partially understood, making it difficult to identify the precise chemical products responsible for in vivo damage and thus impede the development of specific inhibitors.

Problems of toxicity have been encountered in a phase III clinical trial with aminoguanidine, so this drug should be considered a prototype. Various classes of drugs are able to interfere with the formation of AGEs or the cross-linking of proteins by AGEs. Other strategies include the use of:

- Pyridoxamine
- Benfotiamine
- Cross link breakers

Aminoguanidine, pyridoxamine, 2,3-diamino-phenazine, OPB-9195, and
tenilsetam inhibit AGE formation by scavenging reactive carbonyl intermediates, $N$-phenacylthiazolium bromide (PTB) and ALT-711 are AGE-cross-link breakers. Furthermore, signal transduction through RAGE can be inhibited by antisense oligodeoxynucleotides (AS-ODNs), RAGE antibodies, or soluble RAGE. Drugs targeting other systems have also shown some effects on AGE-related pathways.
We now propose for your attention the recently developed and worldwide patented by Dr. Babizhayev/Innovative Vision Products inc. tri-peptide derivatives which are most effectively scavenging toxic aldehydes, glycotoxins, have the unique ability to reverse the glycation process and what is most intriguing, to reverse effectively the already formed glycosylation (glucose-derived intermolecular) crosslinks in the tissue proteins via the transglycosylation mechanism.

GMP production and development of pharmaceutical peptides from mg to kilograms

- Development
- Upscaling
- Routine production
- Purification and analysis

This transglycosylation process indicates on the benefits of the combined use of a tripeptide formulations in the patented by Dr. Babizhayev/Innovative Vision Products, Inc. novel therapeutic compositions (including ophthalmic compositions) for the treatment of Diabetes complications.

The described tripeptide compounds and ophthalmic formulations thereof are useful as therapeutic agents for the treatment of complications arising from diabetes. Pharmaceutical compositions containing the tripeptide compounds and a method of treating diabetic complications are also disclosed.
For instance, the proposed tripeptide is endowed with the unique transglycating activity suitable for the therapy of Diabetic Retinopathy (DR).

The accumulating evidence suggests that formation of advanced glycation end products (AGEs) within the diabetic milieu is one of the contributing factors to neural and microvascular abnormalities as retinopathy progresses.

The data highlight the pathogenesis of diabetic retinopathy, with special emphasis on AGEs, their receptors and the potential role of AGE-inhibiting, mainly AGE reversing agents (tripeptide) ophthalmically acting through its transglycosylating activity. Retinopathy may be associated with an upregulation of the receptor for AGEs (RAGE) in a proinflammatory axis, concomitant with increases in AGEs.

Our studies detail the role of RAGE in AGE trafficking, its various ligands and the possibilities for targeting RAGE and AGE reversing through the transglycosylation process for therapeutic exploitation of the tripeptide as the ophthalmic Rx drug in the therapy of Diabetic Retinopathy.

The tripeptide Product is also very promising in the treatment of cataracts (including age-related and diabetic cataracts) associated with the glycosylation problems.
The product also addresses Neuro-ophthalmic associations and complications of diabetes mellitus.

These patented applications are considered as the potent new therapies of the Diabetes and Diabetes ophthalmic complications with the ultimate goal in prevention of clinical disease.
We propose to discuss this Particular business opportunity and possible issues for cooperation/Partnering between our Companies and the Groups in the Project.

Please, let IVP know your consideration comments.

Respectfully submitted,

Cordially, Dr. Mark A. Babizhayev, Ph.D., Executive Director

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