Hyperglycemia-Related Pathophysiologic Mechanisms and Potential Beneficial Actions of Melatonin

Ahmet Korkmaz[1,2], Turgut Topal[1], Sukru Oter[1,*], Dun-Xian Tan[2] and Russel J. Reiter[2]

[1]*Department of Physiology, School of Medicine, Gulhane Military Medical Academy, Ankara, Turkey;* [2]*Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX USA*

Abstract: Chronically-elevated blood glucose initiates a harmful series of processes in which toxic reactive species play crucial roles. Oxidative as well as nitro-oxidative stress is harmful for virtually all biomolecules including lipids, proteins and DNA. Such pathophysiologic mechanisms eventually results in cellular dysfunction, apoptosis or necrosis. Melatonin is a multifunctional indolamine which counteracts several pathophysiologic steps and displays significant beneficial effects against hyperglycemia-induced cellular toxicity. These are related to melatonin's antioxidative, anti-inflammatory and possibly epigenetic regulatory properties. Current knowledge encourages using this non-toxic indolamine either as a sole treatment or in conjunction with other treatments for inhibition of the biohazards of hyperglycemia.

Key Words: Hyperglycemia, free radicals, peroxynitrite, DNA damage, melatonin.

INTRODUCTION

Diabetes has long been viewed as a disorder of carbohydrate metabolism due to its hallmark feature of hyperglycemia. Indeed, hyperglycemia is not only the cause of the acute symptoms such as polydypsia, polyuria, and polyphagia [1], but also the long-term complications including retinopathy, nephropathy, and neuropathy. In addition, hyperglycemia may contribute to the development of macro-vascular disease, which is associated with the development of coronary artery disease, the leading cause of death in individuals with diabetes [2]. Thus, a primary goal in the prevention and the management of diabetes is the regulation of blood glucose to achieve near-normal levels. Hyperglycemia affects many tissues including vascular endothelium and pancreatic β-cells which leads to their dysfunction [3, 4]. In this progression, the initial stage is a relatively long period of chronically-elevated blood glucose. Before diseases such as diabetes, hypertension, renal and cardiovascular diseases, hyperglycemia persists and compromises metabolic activity leading to endothelial dysfunction (ED), β-cell dysfunction and disrupted vascular smooth muscle relaxation [5, 6]. The mechanism by which hyperglycemia is harmful remains a challenging question to be answered.

Several works suggest that the levels of all biomarkers of oxidative stress are elevated in diabetic patients; this indicates an over-production of free radicals. The main source of oxidative stress in diabetes appears to be hyperglycemia and excessive generation of toxic species which play a key role in the pathogenesis of diabetic complications [7]. In particular, recent studies show that a hyperglycemia-mediated process of superoxide ($^{\cdot}O_2^-$) formation, due to the leakage of electrons from the mitochondrial electron transport chain, is an initial and essential event in the activation of pathways involved in the pathogenesis of secondary negative events of diabetes [8, 9].

VASCULAR ENDOTHELIUM, PANCREATIC β-CELLS AND HYPERGLYCEMIA

The endothelium, one of the largest cell populations in the body, is strategically located between the wall of blood vessels and the streaming blood. In adults, approximately ten trillion (10^{13}) cells form a layer of cells: the vessel endothelium. It senses mechanical stimuli such as pressure and shear stress and responses to hormonal stimuli including vasoactive substances [10]. Endothelial cells have finally emerged as key immunoreactive cells involved in host defense and inflammation. These cells both produce and react to a wide variety of mediators including cytokines, growth factors, adhesion molecules, vasoactive substances and chemokines, with effects on many different cells. Endothelial cells are also intimately involved in the manifestation of infection, atherogenesis, hypertension, diabetes and cancer [11]. These cells are known to be influenced by hyperglycemia which leads to their dysfunction earlier than other tissues in the organism. ED was first described in human hypertension in the forearm vasculature in early 1990's [12]. Impaired vasodilation in hypertensive subjects has been confirmed by many studies in different vascular beds including small resistance vessels [13]. Impaired vasodilation has also been described in type 1 [14] and type 2 diabetes [15], coronary artery disease [16], chronic heart failure [17, 18] and chronic renal failure [18, 19]. It is now known that ED is the key element involved in pathogenesis of chronic diseases prior to their clinical manifestation.

Chronically-elevated glucose is damaging to the structure and function of organs, including the vascular endothelium and pancreatic islets. Multiple biochemical pathways and mechanisms of action for glucose toxicity have been suggested. These include increased glucose autoxidation, augmented polyol pathway flux, increased advanced glycation end-products (AGE) formation, activation of protein kinase

*Address correspondence to this author at the Gulhane Askeri Tip Akademisi, Fizyoloji Anabilim Dali, 06018 Ankara, Turkey; Tel: +90 312 3043602, +90 532 6529178; Fax: +90 312 3043616;
E-mail: oters@gata.edu.tr; fizyoter@gmail.com

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C (PKC) and nuclear factor kappa B (NF-κB), and increased hexosamine pathway flux, sorbitol formation, and oxidative phosphorylation [20]. Hyperglycemia also promotes directly as well as through the activation of NF-κB, increased expression of inducible nitric oxide synthase (iNOS), accompanied by excessive generation of nitric oxide (NO), and an overactivity of NAD(P)H, which, in turn, produces an over abundance of $^{\cdot}O_2^-$ [21].

One potential central mechanism for glucose toxicity is the formation of excess reactive oxygen species (ROS), which are generated *via* multiple mitochondrial and non-mitochondrial pathways [22]. The pancreatic islets are especially vulnerable to ROS because of their low intrinsic level of antioxidant enzymes. It has been long known that pancreatic islets contain relatively small amounts of the antioxidant enzymes Cu,Zn-superoxide dismutase (Cu,Zn-SOD; also known as SOD1), Mn-SOD (SOD2), catalase (CAT) and glutathione peroxidase (GSH-Px), and as a result, they are readily damaged by chronic oxidative stress [23, 24]. Moreover, β-cells of rat islets were shown to be especially sensitive to hydrogen peroxide (H_2O_2) as a result of a deficiency GSH-Px, the enzyme which metabolizes it to innocuous products [25, 26]. The beneficial effects of superoxide dismutase (SOD) treatment in the prevention of alloxan-induced diabetes in mice is also known [24]. These and many other observations have reinforced the notion that the intrinsically low level of antioxidant activity of the pancreatic islets renders them particularly at risk for ROS-induced damage. Moreover, chronically-elevated glucose and ROS levels can cause reduced insulin gene expression [27] and insulin resistance [28]. Apart from hyperglycemia, dyslipidemia is a well known contributor of endothelial and β-cell dysfunction although the exact mechanism remains unknown. However, there is evidence that dyslipidemia, like hyperglycemia, causes excessive ROS production [29]. Thus, endothelial and β-cell dysfunction are the result of (i) chronic exposure to hyperglycemia, (ii) chronic exposure to free fatty acids (FFA) and (iii) a combination of chronic hyperglycemia and FFA leading to an excess ROS production [30].

This pathophysiologic sequence sets the scene for considering antioxidant therapy as an adjunct in the management of insulin resistance and diabetes where oxidative stress is elevated. It seems likely that chronic oxidative damage is a major contributor to hyperglycemia-induced damaging processes. One means of testing this hypothesis would be to determine whether better maintenance of glucose levels in hyperglycemic patients is accompanied by lower levels of ROS and improved insulin secretion and/or insulin resistance. Another would be to add antioxidants to conventional therapy to determine whether this maneuver would prevent continued deterioration in β-cell and endothelial functions, despite continued hyperglycemia.

Contrary to expectations, antioxidant therapy with commonly used antioxidants such as vitamins E and C or others, seem not to function in the preservation of endothelial and pancreatic β-cell physiology [30, 31]. A theoretical explanation for the failure of these antioxidants relates to the fact that with the discovery of NO/NOS family, oxidative stress became more complex then previously realized. Thus, it is believed that hyperglycemia-induced oxygen-based free radicals are only an initial step in the pathophysiologic mechanisms leading to clinical manifestations such as hypertension and diabetes [30, 32].

PHYSIOLOGIC AND PATHOLOGIC ROS PRODUCTION

Under normal circumstances, cells are able to balance the production of oxidants and antioxidants, resulting in redox equilibrium. Oxidative stress occurs when cells are subjected to excess levels of ROS or as a result of depletion of antioxidant defenses [33, 34]. Several environmental and biochemical changes cause elevated ROS production; these include hazardous contaminants (e.g., nitroso compounds, polycyclic hydrocarbons, alcohol, aflatoxin, heterocyclic aromatic amines, and even "overnutrition"), tissue damage (e.g., mechanical, heat, acid), infectious factors (e.g., *Helicobacter pylori*, hepatitis B and C virus, Epstein-Bar virus), inflammatory reactions (e.g., pancreatitis, ulcerative colitis), environmental hazards (e.g., ultraviolet light, ionizing radiation, tobacco smoke, gas exhaust, lead, asbestos), and biochemical changes (e.g., hyperglycemia, dyslipidemia) are recognized as generating excess ROS [35]. There is an age-dependent increase in the fraction of ROS and other free radicals that may escape the cellular defense mechanisms and exert damage to cellular constituents including DNA, RNA, lipid, and proteins. During aging, the redox equilibrium between oxidants and antioxidant defenses gradually deteriorates in favor to more ROS and tissue damage occurs. ROS play important roles in regulating a variety of cellular functions and act as secondary messengers in the activation of specific transcription factors including NF-κB and activator protein-1 (AP-1) within a certain local concentration range; however, excessive production of ROS is harmful to cells *via* the same means. Once excess ROS production induces transcription factor activation (e.g., NF-κB, AP-1), the harmful effects of excess ROS are spread due to gene activation for TNF-α, IL-1β and other cytokines [36]. Furthermore, any signal or stimulus that triggers over-production of ROS may induce the opening of the membrane permeability transition pore in mitochondria with the release of cytochrome c and other apoptogenic factors, which ultimately lead the cell into dysfunction, apoptosis, and/or necrosis [37].

It is presumed that, initially ROS production reduces the transcription of endothelial NOS (eNOS), the constitutive NOS enzymes providing NO under physiologic circumstances, while activating iNOS which causes almost a 1,000-fold higher NO production than eNOS does. Inducible NOS is predominantly expressed in inflammatory cells such as macrophages, although epithelial cells from affected tissues also express iNOS. Intensified expression of iNOS has been detected in virtually all cell types tested including macrophages, fibroblasts, chondrocytes, osteoclasts, and epithelial cells and results in the production of large amounts of NO in animals and patients with inflammatory diseases [38-41]. The level of iNOS expression is well correlated with the degree of inflammation. The controversy arises from observations reporting both cytotoxic and cytoprotective effects of NO. In cases where NO was found cytotoxic, it was ques-

tioned whether NO, directly or indirectly, or through the formation of more reactive species such as the peroxynitrite anion ($ONOO^-$) exerted these effects. The combination of elevated NO plus excess $\cdot O_2^-$ with the formation of high levels of $ONOO^-$ is the proverbial intracellular "devil's triangle" (Fig. **1**). Essentially any pathophysiologic process caused exclusively by oxygen-derived free radicals could presumably be alleviated by conventional antioxidants such as vitamin E and C and/or intracellular enzymatic antioxidants such as SOD, CAT and GSH-Px. Once iNOS is activated, however, because of NO's affinity for the $\cdot O_2^-$, neither enzymatic nor pharmacologic levels of conventional antioxidants are able to compete with NO for $\cdot O_2^-$; as a result, high $ONOO^-$ levels follow [14]. In case of chronic hyperglycemia and/or dyslipidemia, highly activated iNOS could readily shift the molecular destruction from oxidative to nitro-oxidative damage leading to a situation where conventional antioxidants are less efficient in reducing damage.

"Devil's Triangle"; Changing the Nature of Oxidative Stress

A vital ubiquitous molecule, NO, has numerous roles in regulating physiological processes including functions as an intracellular messenger and as an autocrine/paracrine agent influencing blood flow, glial and motor neuron activity, and regulating smooth muscle relaxation. After the discovery that the endothelium-derived relaxing factor (EDRF) called molecule was NO, it became a leading substance of research. In the last two decades, NO was found to have the ability to be a toxic molecule which damages and even kills cells when produced in excess. In the presence of excess $\cdot O_2^-$ and plentiful NO, this latter vital molecule shows its dark side. Neither $\cdot O_2^-$ nor NO is particularly toxic by themselves because there are efficient means to minimize their accumulation [14]. Thus, $\cdot O_2^-$ is rapidly removed by SOD with the isoenzymes of this molecule being located in the cytoplasm (SOD1), mitochondria (SOD2), and extracellular (SOD3) compartments. NO normally is rapidly removed by its quick diffusion through tissues into red blood cells [42], where it is rapidly converted to nitrate by a reaction with oxyhemoglobin. This limits the biological half-life of NO *in vivo* to less than a second. However, when both $\cdot O_2^-$ and NO are generated within a few molecular diameters of each other, they combine spontaneously to form the $ONOO^-$ in a diffusion-limited reaction. Basically, every time NO and $\cdot O_2^-$ collide, they form $ONOO^-$. No enzyme is required to form $ONOO^-$ since no enzyme can possibly catalyze such a rapid reaction. NO is the only known biological molecule that reacts faster with $\cdot O_2^-$ and is produced in such high concentrations that it outcompetes endogenous levels of SOD; hence, the creation of the "devil's triangle" (Fig. **1**). Consequently, from a biological viewpoint, the reaction of $\cdot O_2^-$ with NO to form $ONOO^-$ is inevitable. Evidence suggests that during the early stages of hyperglycemia, reduced NO availability exists [43] although iNOS is highly activated. Any of several antioxidants may have beneficial effects in these acute situations including acute hyperglycemia, ischemia-reperfusion, myocardial infarction, etc. However, in case of chronic oxidative stress, once iNOS is totally activated, ordinary antioxidants provide little protection due to massive $ONOO^-$ generation. It is well known that iNOS is induced *de novo* by various stimuli including hyperglycemia which leads to the production of large amounts of NO and inevitably $ONOO^-$.

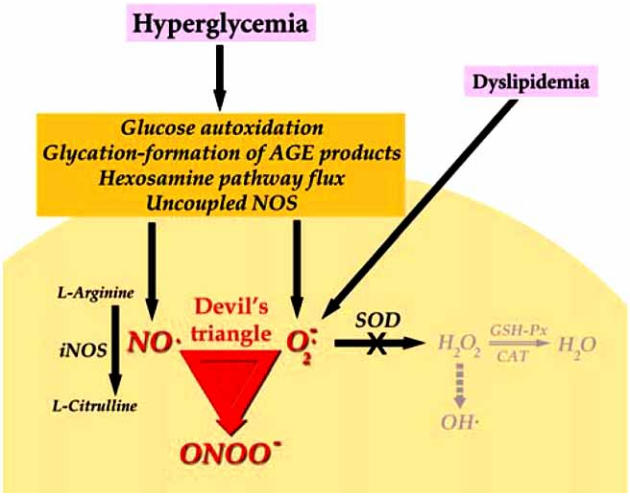

Fig. (1). Organization of the so-called "devil's triangle" within a targeted cell.

Hyperglycemia causes excess $\cdot O_2^-$ production and NO via several means. Normally, $\cdot O_2^-$ can be readily dismutated to H_2O_2 by intracellular enzymatic antioxidants. In presence of ample $\cdot O_2^-$ and iNOS-derived NO, the biochemically inevitable coupling of the two molecules produces vast amounts of $ONOO^-$; under these conditions SOD cannot scavenge $\cdot O_2^-$. In the early stages of oxidative stress, if iNOS is not activated, several conventional antioxidants including vitamin E and C diminish the damage via scavenging $\cdot O_2^-$. However, once $\cdot O_2^-$ and NO became highly elevated conventional antioxidants are much less effective in reducing the resulting molecular damage.

Thence, in case of chronic oxidative stress such as chronic hyperglycemia, dyslipidemia, tobacco smoking, prolonged drug use including doxorubicin, cisplatin, or cyclophosphamide which are known to produce oxidative damage [44] conventional antioxidants become ineffective. Since iNOS-derived NO plays a major role in the development of chronic nitro-oxidative stress, many clinical trials have failed to show beneficial effects of vitamin E and/or C in several conditions [30, 31]. The relation, however, between hyperglycemia and free radicals is well established and a number of prospective studies indicate that long-term glycemic control is an important predictor of both microvascular disease and macrovascular complications [30, 45]. Thus, a novel molecular approach to protect the vascular wall, endothelial cells and β-cells in diabetes is needed.

Progression of Nitro-Oxidative Stress in Cells: How $ONOO^-$ is Harmful

Once $ONOO^-$ forms, it can act through two distinct ways; first it has direct toxic effects leading to lipid peroxidation, protein oxidation and DNA damage. The second mechanism involves the induction of several transcription factors including NF-κB and AP-1 leading to cytokine-induced chronic inflammation (Fig. **2**). Well known activators of these transcriptional factors are cytokines and microbial products, e.g., bacterial lipopolysaccaride. Free radicals and $ONOO^-$, how-

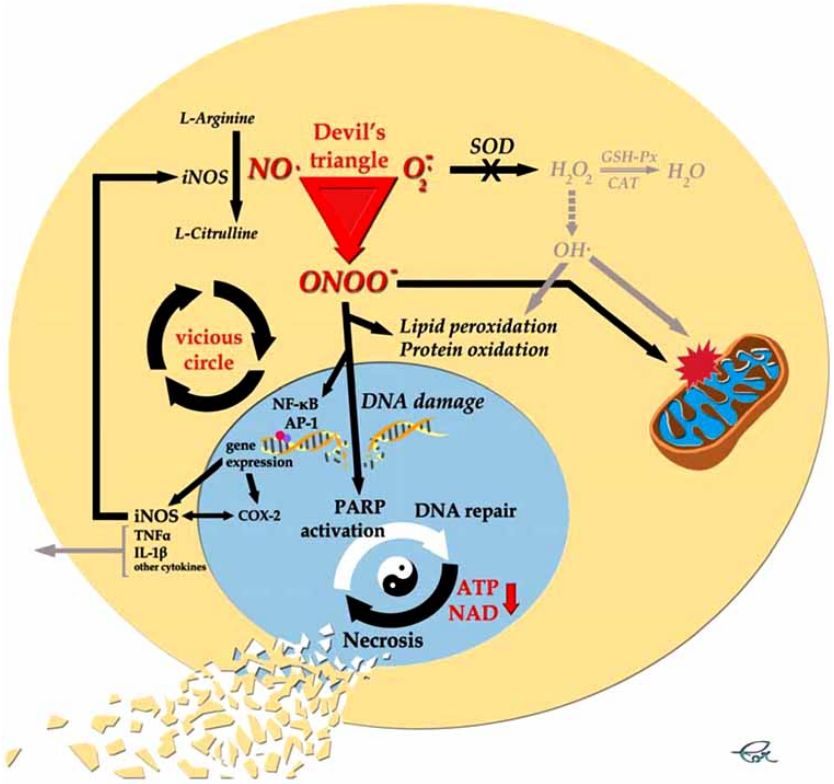

Fig. (2). Consequences of the "devil's triangle" within cell.

Once $ONOO^-$ is formed, cellular stress is transformed from oxidative only to nitro-oxidative. $ONOO^-$ exerts its harmful effects directly and indirectly. It causes activation of transcriptional factors leading to pro-inflammatory gene expression. During this process, nitro-oxidative stress also involves an inflammatory response. Interactions between transcriptional factors and pro-inflammatory products lead to a vicious cycle of damage. The cytokines spread the inflammatory signals through the circulation. Unless excess $^{\cdot}O_2^-$ and iNOS-derived NO production are terminated (e.g., normalizing blood glucose levels), this mechanism continues to propagate damage within cell. Moreover $ONOO^-$ directly damage all macromolecules including lipids, proteins and DNA. $ONOO^-$-induced DNA damage is sensed by DNA repair enzymes, in particular PARP. In presence of severe genomic damage, overactivation of PARP causes cellular NAD^+ and ATP depletion by attempting a repair process. This drives cells into an energy crisis eventually leading to necrosis. This futile mechanism, so-called "suicide hypothesis" of PARP activation, is reportedly involved in many diseases related to nitro-oxidative stress. Since the mitochondrion has its own DNA and PARP enzyme, this pathophysiologic process also takes place within the mitochondrion. It is well known that, both oxygen and nitrogen-based radicals are prone to directly damage this organelle. Consumption of the majority of NAD^+ by PARP also slows the rate of glycolysis and mitochondrial respiration, and eventually leads to cellular dysfunction and death.

ever, can also trigger this signaling cascade [46]. Once activated, this cascade causes the release of the abovementioned cytokines which induces widespread inflammation. The hyperglycemia [47, 48] and dyslipidemia-induced [49] inflammatory response also uses this pathophysiologic mechanism [36, 50]. During this process, several adhesion molecules and monocyte chemoattractant proteins are also involve [51] widening the inflammatory response and vascular events. Evidence supports the claim that over-expression of pro-inflammatory cytokines including TNF-α plays a role in the pathophysiology of insulin resistance [52, 53], atherosclerosis [53] and chronic diabetic complications [52].

A direct toxic effect of $ONOO^-$ at the site of its production involves an intriguing process which decides the fate of cells. $ONOO^-$ is per se not a radical but is a powerful nitrosating agent. It can directly react with target biomolecules *via* one or two-electron oxidations [54, 55]. $ONOO^-$ interacts with and covalently modifies all major types of biomolecules including membrane lipids, thiols, proteins and DNA [46, 56]. In addition, $ONOO^-$ can yield the hydroxyl ($^{\cdot}OH$) and nitrogen dioxide ($^{\cdot}NO_2$) radicals (less than 30% yield). Although this is a minor process in biology [57], $^{\cdot}OH$ is a potent oxidant and it has been reported to oxidize relevant targets like amino acids (tyrosine, phenylalanine, histidine), sugars and lipids [55, 58]. The generation of $ONOO^-$ also decreases the availability of NO for G-protein stimulation and vasodilatation, thus further contributing to endothelial dysfunction leading to elevated blood pressure, insulin resistance and diabetes. In addition, $ONOO^-$ can inhibit SOD as well as other antioxidant molecules and systems, which leads to positive feedback cycles of intracellular oxidant generation and increased radical damage [59]. $ONOO^-$ activates matrix metalloproteinases (MMPs) [60, 61] and triggers the expression of selectins and cellular adhesion molecules, *via* enhancing of NF-κB activation [59], thereby promoting pro-inflammatory responses.

The mutagenic properties of $ONOO^-$-induced modified products have also been determined [62, 63]. Several studies

have shown that NO itself does not induce DNA single-strand breaks *in vitro* in plasmid DNA [64, 65], whereas exposure of plasmid DNA to preformed $ONOO^-$ [66] or NO plus $^{\cdot}O_2^-$ generated concurrently [67] induces DNA strand breaks. Single strand breakage can be induced by treatment with very low concentrations of $ONOO^-$ indicating that this agent is a potent inducer of this type of damage to DNA [68]. DNA cleavage caused by $ONOO^-$ was observed at almost every nucleotide, with a small preference for guanine residues. Furthermore, it has been reported that $ONOO^-$ can inactivate several enzymes that are critically involved in the repair of DNA damage including Fpg (formamidopyrimidine DNA glycosylase) [69] and its functional homolog OGG1 (8-oxoguanine DNA glycosylase) [70], MGMT (O6-methylguanine-DNA methyltransferase) [71] and DNA ligase [72]. These observations suggest additional pathways by which $ONOO^-$ may be associated with not only elevated DNA damage but also impairment of DNA repair capacity [73]. Accumulation of DNA damage induced by $ONOO^-$ can activate the p53 gene, which is an important DNA damage-response molecule, leading to cell cycle arrest and/or apoptosis to avoid proliferation of damaged cells. However, under certain conditions, excess $ONOO^-$ may instead inhibit the functions of p53 [42, 74, 75].

A Crucial Step in the Pathogenesis: PARP Activation by $ONOO^-$

$ONOO^-$ induces apoptosis and necrosis in cells. More highly elevated exposure of this agent is associated with necrosis rather than with apoptosis [59, 76]. In this mechanism, activation of the DNA repair enzyme poly(ADP ribose)polymerase-1 (PARP-1), a member of PARP enzyme family, mediates $ONOO^-$-induced necrosis. PARP-1 detects and signals DNA strand breaks induced by a variety of genotoxic insults including ionizing radiation, alkylating agents, oxidants (essentially $^{\cdot}OH$, $ONOO^-$), and free radicals [77-81]. Upon binding to DNA, strand breaks occur and, PARP transfers ADP-ribose units from the respiratory coenzyme nicotinamide adenine dinucleotide (NAD^+) to various nuclear proteins. From a physiological view point, PARP-1 activity and poly(ADP)ribosylation reactions are implicated in DNA repair processes, the maintenance of genomic stability, the regulation of gene transcription, and DNA replication. An important function of PARP-1 is to allow DNA repair and cell recovery under conditions associated with a low level of DNA damage. In case of severe DNA injury, overactivation of PARP-1 depletes the cellular stores of NAD^+, an essential cofactor in the glycolytic pathway, the tricarboxylic acid cycle, and the mitochondrial electron transport chain. As a result, the loss of NAD^+ leads to a marked reduction in the cellular pools of ATP, resulting in cellular dysfunction and cell death *via* the necrotic pathway [59, 76]. This is known as "suicide hypothesis" of PARP activation and seems to be a regulatory mechanism to eliminate cells after irreversible DNA injury (Fig. **2**). A vast amount of experimental evidence has established that the PARP-1 pathway of cell death plays a pivotal role in tissue injury and organ dysfunction in virtually every disease process [46, 82] including hyperglycemia and diabetes [83].

It seems likely that, the "devil's triangle" mentioned above may determine the fate of a cell as well as its inability to function (e.g., endothelial and β-cell dysfunction), mutagenesis, apoptosis (e.g., β-cells), and necrosis leading to clinical manifestations. Based on the proposed mechanism, several steps may be set as pharmacological targets to block the hazards of hyperglycemia: attempting to normalize hyperglycemia with exercise, diet and drugs; reduce excess ROS production; preventing intracellular enzymatic antioxidant depletion; stimulating intracellular enzymatic antioxidants; inhibiting iNOS overactivation; supporting physiologically eNOS-produced NO bioavailability; detoxifying nitrogen-based species; inhibiting (normalizing) NF-κB and AP-1 activation and/or lowering NF-κB binding to DNA; reducing pro-inflammatory cytokines such as TNF-α and IL-1β; limiting adhesion molecule production, e.g., P-selectin and ICAM-1 (intercellular adhesion molecule 1); preventing lipid peroxidation and/or repairing damaged lipids, limiting protein oxidation and nitration; blocking PARP activation, thereby preserving NAD^+ and cellular energy; and reducing DNA damage and/or mediating its repair.

If control of these processes could be accomplished, adjuvant therapeutic drugs that depress hyperglycemia may be less important. Given the harmful potential of diabetic complications, a versatile treatment protocol that includes exercise, diet, sugar-lowering drugs as well as a multifunctional agent with antioxidant, iNOS inhibitory, $ONOO^-$ scavenging properties, along with several beneficial metabolic properties and little or no toxicity may improve the outcome of hyperglycemic patients. One molecule that has many of the actions that may reduce the toxicity of hyperglycemia is the endogenously-produced indolamine, melatonin.

A MULTIFUNCTIONAL MOLECULE: MELATONIN

Melatonin is secreted from pineal gland during night. This indolamine has a variety of means by which it influences the physiology of the organism; some of these actions are receptor-mediated while others are receptor-independent [84, 85]. Melatonin is a highly lipophilic indole which easily enters all cells. Melatonin has been administered in both physiological and pharmacological amounts to humans and animals, and there is widespread agreement that it is a highly non-toxic molecule [44].

Although it is not a major topic of the current review, a short description of the direct effect of melatonin on hyperglycemia and dyslipidemia may be of interest. Streptozotocin (STZ), an antibiotic produced by *Streptomyces achromogenes*, is the most commonly used agent in experimental animal models of hyperglycemia and diabetes. STZ is a pancreatic β-cell toxin which induces rapid and irreversible necrosis of these cells [86]. The mechanism of STZ-induced β-cell injury involves excessive ROS production, lipid peroxidation, protein oxidation and DNA damage leading to β-cell death. Melatonin reduces blood glucose, HbA_{1c} and plasma lipids in STZ-induced diabetic rats [87]. Melatonin also protects β-cells [88, 89] and several diabetes-affected organs including kidney [90, 91], brain [92], retina [93, 94], and vasculature [95-97] from the damaging effects of STZ [98, 99]. Moreover, melatonin suppresses the hyperglycemia caused by drugs other than STZ [100-102]. There is favorable evidence regarding the beneficial effects of melatonin on endocrine pancreas [102, 103], insulin secretion [102,

104], glucose homeostasis [105] and carbohydrate metabolism [106].

It is well known that adiposity is closely-related to insulin resistance, hyperglycemia, dyslipidemia and metabolic syndrome [107]. Reducing body weight, in particular loosing fat around abdominal region, aids many hyperglycemic and diabetic patients. Melatonin is effective in causing weight loss in adult rats [108, 109] as well as intraabdominal adiposity and total body weight [109]. This result is consistent with melatonin's ability to increase leptin expression by adipocytes [110] and decrease ghrelin levels [111], which is a signal peptide isolated from rat stomach antagonistic to the actions of leptin. Although not yet proven, melatonin may theoretically help patients to obey their diet *via* these two mechanisms. Both diabetic animals and patients have reduced melatonin levels [102], indicating the possible relationship between melatonin and the hyperglycemic/diabetic condition. Circadian rhythm of melatonin may have additional beneficial effects on diabetic patients. Once synthesized during dark period, melatonin is not stored in pineal cells but is quickly released into the bloodstream. Beside the blood melatonin is also present in other body fluids, including saliva, cerebrospinal fluid, bile, semen, amniotic fluid and can easily enter virtually all cells. Therefore, night peak of melatonin may enable the cell repair mechanism during dark period.

Melatonin as a Versatile Antioxidant

There is a very large body of evidence that melatonin is major scavenger of both oxygen and nitrogen based radicals [92, 112-115] including $ONOO^-$ [116-118]. Melatonin has scavenging actions at both physiologic and pharmacologic doses. Not only melatonin but also several metabolites also have the capability to detoxify free radicals and their derivatives [95, 119]. Melatonin also supports several intracellular enzymatic antioxidant enzymes including SOD and GSH-Px [120, 121]. Moreover, melatonin induces the activity of gamma-glutamyl cysteine synthase thereby stimulating the production of another intracellular antioxidant, glutathione [122]. The antioxidative effects of melatonin are documented under hyperglycemic and/or diabetic conditions [122-125] and melatonin is significantly better than other antioxidants in this regard e.g., more effective than vitamin E [123] or garlic oil [126].

Several antioxidants reportedly exhibit SOD and/or GSH-Px preservation properties. These effects are, however, indirect due to their ability to scavenge free radicals and protect the protein from damage. Melatonin, on the other hand, possesses genomic actions and regulates the expression of several genes including those for SOD and GSH-Px. Melatonin influences both antioxidant enzyme activity and cellular mRNA levels for these enzymes under both physiological conditions and during elevated oxidative stress [97]. These two features in a single molecule are unique for an antioxidant and both actions protect against pathologically-produced free radicals during hyperglycemia. As noted above, pancreatic β-cells have naturally lower antioxidant enzyme levels [127]; thus, melatonin supports these cells in two ways, i.e., by scavenging the free radicals produced and by inducing the enzymes involved in metabolizing toxic reactants to innocuous products.

Melatonin Counteracts iNOS and $ONOO^-$

In many inflammatory processes including hyperglycemic conditions, $ONOO^-$ rather than oxygen-based radicals is the predominant molecule which decides the fate of cells. Once formed by the coupling NO and $^{\cdot}O_2^-$, $ONOO^-$ cannot be removed or scavenged by vitamin E or C or by other conventional antioxidants. As a multifunctional antioxidant, however, melatonin and its metabolites have unique features over the usual antioxidants including iNOS inhibitory [128-132] and $ONOO^-$ scavenging [112, 116, 133-135] properties. These features of melatonin, apart from direct antioxidative effects, have been documented in STZ-induced hyperglycemia [87, 124] and other circumstances such as colitis [130], liver and lung damage [129], and alkylating agent toxicity [117, 118, 136, 137]. Thus, melatonin is the only medically suitable molecule which has the ability of blocking all sides of the "devil's triangle" (Fig. **3**). Mercaptoethylguanidine [138-140] and ebselen [33, 81, 141] have experimentally been shown to act in a similar manner (e.g., iNOS inhibitor and $ONOO^-$ scavenging) as melatonin, but neither is suitable for human use.

Melatonin has been shown to ameliorate inflammation by blocking transcriptional factors [142], TNF-α and IL-1 [143, 144], *via* several mechanisms [92, 145]. A large body of evidence confirms that these cytokines are capable of inducing formation of free radicals and promoting iNOS activity and transcriptional factor activation within cells. These events inevitably induce a vicious cycle of cellular damage (Fig. **3**). In the case of $ONOO^-$-induced DNA damage, PARP over-activates in an attempt to repair the genome, consumes NAD^+ as a substrate which causes an energy crisis within cells leading to their eventual necrosis. Preservation of NAD^+ and cellular energetics may be helpful for PARP to repair the DNA damage rather than blocking PARP. Melatonin preserves cellular energy production [146, 147] *via* different means including inhibiting iNOS and scavenging $ONOO^-$ and other oxidizing/nitrosating species [92].

Under physiologic conditions, in resting cells, eNOS-derived NO suppresses both iNOS and cyclooxygenase-2 (COX-2) expression by reducing NF-κB translocation into the nucleus. However, it is well documented that high amounts of NO derived from iNOS during inflammatory processes further potentiates COX-2 activity through the NF-κB pathway [148] thereby exaggerating the inflammatory process. This is not unexpected given that $ONOO^-$ directly activates COX-2 as well [149]. In the case of chronic inflammation, inhibition of COX-2 and iNOS (but not COX-2 only) would be beneficial in reducing the severity of inflammation. A recent, intriguing report [150] suggests that neither tryptophan nor serotonin, but only melatonin, inhibits COX-2 and iNOS transcriptional activation. This report is also important since the inhibitory mechanism of COX-2 and iNOS by melatonin is suggested to be an epigenetic action [150].

Accumulating data reveal a close connection between chronic diseases and epigenetic dysregulation [151]. Cancer [152-156], diabetes [157], atherosclerosis [158], hypertension [159], metabolic syndrome [160], and even autoimmune disorders [161] are all possibly related to epigenetic dysregu-

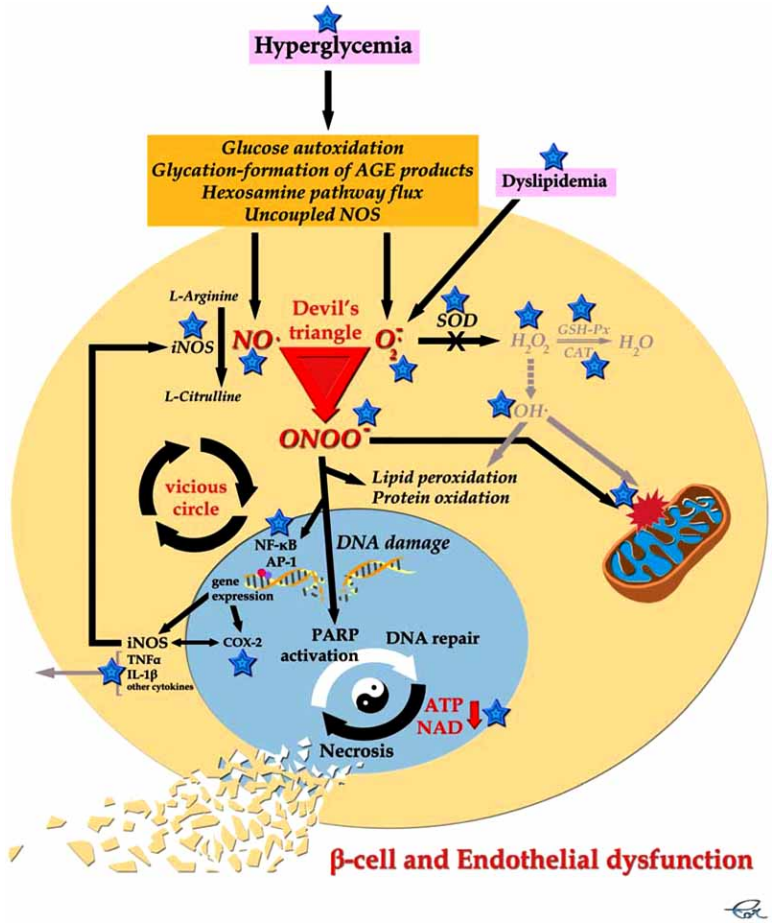

Fig. (3). The overall mechanisms of hyperglycemia-induced cellular dysfunction and necrosis and the sites of melatonin's action (stars).
Melatonin is a unique molecule which possesses beneficial effects at virtually all steps of the pathophysiologic process. There is evidence that melatonin directly decreases blood sugar and lipid levels. It also serves as a multifunctional anti-oxidant by scavenging all types of oxygen and nitrogen-based reactants as well as $ONOO^-$. It activates antioxidant enzymes at expressional level, thereby preventing cellular enzymatic depletion. Melatonin blocks pro-inflammatory cytokine production by reducing NF-κB and AP-1 translocation into the nucleus and directly inhibits iNOS and COX-2. These features break the vicious cycle and alleviate the inflammation. Melatonin preserves cellular energy by several means and has been shown to prevent mitochondrial nitro-oxidative damage.

lation. Presumably, in the near future melatonin will be defined as predominant epigenetic regulator in mammals [162, 163]. Uncertainty over how melatonin can regulate a variety of genes such as antioxidative enzymes, cytokines, and hormones including insulin but always in the right direction (some inhibition, some activation) may be answered by this intriguing mechanism [150, 164].

CONCLUDING REMARKS

Accumulating data suggest that diabetes has become an epidemic in virtually all ethnic groups throughout the world. Chronically-elevated blood glucose is not only important in diabetes but also several other chronic diseases including metabolic syndrome, obesity, and cardiovascular disorders. Any beneficial treatment that limits hyperglycemia and its harmful effects could greatly improve public health. Melatonin, a non-toxic indolamine, shows significant benefits in the treatment of experimental hyperglycemia with its beneficial effects being mediated by a variety of means including as an antioxidant and as an epigenetic regulator. It is important to note that when melatonin is used as a treatment for hyperglycemia, it is essential that it be taken continually over the long term to suppress the cumulative cellular damage that occurs as a consequence of chronically-elevated glucose levels. The positive outcomes of the published studies suggest it is time to consider clinical trials using nature's most versatile molecule [165], melatonin, for inhibition of the hazards of hyperglycemia either by using melatonin as a sole treatment but also in conjunction with anti-diabetic drugs, exercise and diet.

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