Mitochondria as a Target for Future Diabetes Treatments

Franziska Thimm,[1] Marten Szibor.[2]

Abstract

Diabetes mellitus is rapidly becoming the world's most dangerous serial killer. Type 1 diabetes (T1D) is a currently incurable autoimmune disease marked by progressive, and eventually exhaustive, destruction of the insulin-producing pancreatic beta cells. Type 2 diabetes (T2D) describes the combination of insulin resistance in peripheral tissue, insufficient insulin secretion from the pancreatic beta cells, and excessive glucagon secretion from the pancreatic alpha cells. T1D as well as severe cases of T2D are treated with insulin replacement, which can merely be considered as life support for the acute phases of the disease. Islet replacement of insulin-producing pancreatic beta cells represents a potential treatment method for both insulin-depleted diabetes (T1D) and insulin-resistant diabetes (T2D) and may shift diabetes management from life saving measures to a cure. One of the key challenges in islet transplants is the generation of reactive oxygen species (ROS) and the associated oxidative stress, which restricts graft longevity. A major leak of ROS takes place during oxidative phosphorylation at mitochondrial electron transport chain (ETC). Additionally, hyperglycemia-induced superoxide ($O_2 \bullet -$) production has been linked to the development and progression of diabetic complications, both macrovascular and microvascular. Decreasing ROS in diabetic patients may prevent the incidence of long term diabetes complications. This review provides an overview of the role of mitochondria in diabetes, introducing them as a possible target for future treatment of diabetes.

Keywords: Reactive Oxygen Species, Mitochondrial DNA, Diabetes Mellitus, Electron Transport, Oxidative Phosphorylation (Source: MeSH, NLM).

Introduction

About the author: *Franziska Thimm is currently a 4th year medical student at the University of Latvia, Rīga, Latvia of a 6 year program. She is also a former Editor-in-Chief of the European Medical Student Association's (EMSA) official magazine EuroMeds.*

The definite etiology of type 1 diabetes (T1D) is still obscure, but considered to root in a mixture of genetic predisposition and environmental factors, leading to continued autoimmunity.[1] Five percent of diabetics have this type of diabetes.[2] In Type 2 diabetes (T2D), peripheral tissue develops a resistance against insulin.[3] T2D has been linked to "metabolic syndrome", which is defined by the International Diabetes Federation (IDF) as central obesity with two of the following: elevated blood pressure, elevated fasting plasma glucose, high serum triglycerides, and low high-density cholesterol (HDL) levels (Available from: http://www.idf.org/metabolic-syndrome, updated 2014 Oct 23; cited 2015 Jan 21). According to the Centers for Disease Control and Prevention (CDC), one out of three people will develop T2D in their lifetime.[2]

The aims of diabetes management are primarily to save life in the short term and secondarily to prevent the development of diabetic complications in the long term. Both can be achieved by improving glycemic control, aiming for a glycated hemoglobin (HbA1c) between 6.5%-7.0%.[4] For patients with T1D, lifelong insulin replacement therapy marks the therapeutic objective for attaining glycemic control.[5] In T1D, insulin dose varies between 0.4–0.8 UI/kg insulin a day.[6] However, insulin administration may induce hypoglycemic episodes that require hospitalization. The fear of hypoglycemia and the inconvenience of daily insulin injections cause many patients to neglect proper disease management and experience glycemic lability.[7]

For the management of T2D, lifestyle adjustments are considered the mainstay. If glycemic control fails to be obtained by lifestyle changes, treatment may resort to oral hypoglycemic agents (OHA), including biguanides, sulphonylureas, alpha glucosidase inhibitors, and thiazolidinediones. In severe cases, insulin injections are required. If indicated, T2D patients need between 0.2–1.6 UI/kg of insulin a day.[5] The current treatment options available for both types of diabetes merely delay the complications of the disease and do not provide a long-term solution.

Islet replacement of insulin-producing pancreatic beta cells represents a potential treatment method for both insulin-depleted and insulin-resistant diabetes.[8] Following islet transplantation the patients benefit not only from a decrease of hypoglycemic events, but also spectacular improvement of HbA1c levels and stabilization of fasting blood glucose, without exposing themselves to the risk of major surgery as in whole pancreas transplantation.[8] A study by Barton and colleagues compared the efficacy of allogenic islet transplantation in T1D patients in different periods between 1999 and 2010.[9] They analyzed 677 T1D patients who had received islet transplants, with the aim to examine the differences in transplant efficacy between the early (1999-2002), mid (2003-2006), or recent (2007-2010) transplant era. Three years following the islet replacement, insulin independence improved from 27% in the early era to 37% and 44% in the mid and recent eras, respectively, at 36 months after transplantation. Barton and colleagues suggested that the

[1]Medical Student, University of Latvia, Latvia.
[2]Mitochondrial Gene Expression and Disease Group, University of Helsinki, Finland.

Correspondence:
Franziska Thimm
Address: Raiņa bulvāris 19, Rīga, LV-1586, Latvia.
Email: franziska.thimm@gmail.com

improvement of graft survival can be linked to more adequate means for preventing islet rejection.[9] One of the key challenges in islet transplantation is the generation of reactive oxygen species (ROS) and the associated oxidative stress.[9] Oxidative stress occurs due to an imbalance between the intracellular free radical production and the cellular antioxidant defense mechanisms in the transplanted islets, which can lead to cell death. The cellular antioxidant defense mechanism comprises, among others, vitamins and minerals that are hence administered as supplements after organ transplantation.[10] But why not directly target the chief generator of oxidative stress in our cells? Our mitochondria constitute the major ROS generator, as ROS are released as a byproduct of oxidative phosphorylation in the electron transport chain (ETC) of the inner mitochondrial membrane (IMM).

This review provides an overview of the correlation between mitochondria and diabetes, introducing them as a target for future treatment of diabetes.

Search Strategy and Selection Criteria

A literature search was performed using MEDLINE MeSH terms "reactive oxygen species", "alternative oxidase", "mitochondrial DNA", "diabetes mellitus", "respiratory chain" and "oxidative phosphorylation". A total of 9807 articles were found. Following filtering based on selection criteria (publication within last 20 years, English language, and the condition that

at least 4 of the given terms must be in the same article), 7 articles remained and an additional 13 articles were retrieved from references. This review follows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Statement.[11]

The Powerhouse of the Cell

Mitochondria are of prokaryotic origin and now the powerhouse in eukaryotic cells. The term was coined by a pioneer in modern cell biology, Philip Siekevitz, and reflects a critical role of the organelle, which is energy generation by aerobe degradation of nutrients.[10] Mitochondria are enclosed by a double membrane system, the outer mitochondrial membrane (OMM), and the inner mitochondrial membrane (IMM), each reflecting its function through its respective structure.[12]

Metabolic energy is derived through a process known as oxidative phosphorylation. The proteins required for this process are embedded in the IMM. The surface area that is required to accommodate the proteins that participate in this process is provided by the configuration of the IMM into crista (**Figure 1**).[12]

Substrate oxidation and adenosine triphosphate (ATP) production [phosphorylation of adenosine diphosphate (ADP)] are coupled and commonly referred to as "oxidative phosphorylation".[12] During oxidation, electrons are transferred from redu-

Figure 1. The Mitochondrial Respiratory Chain.
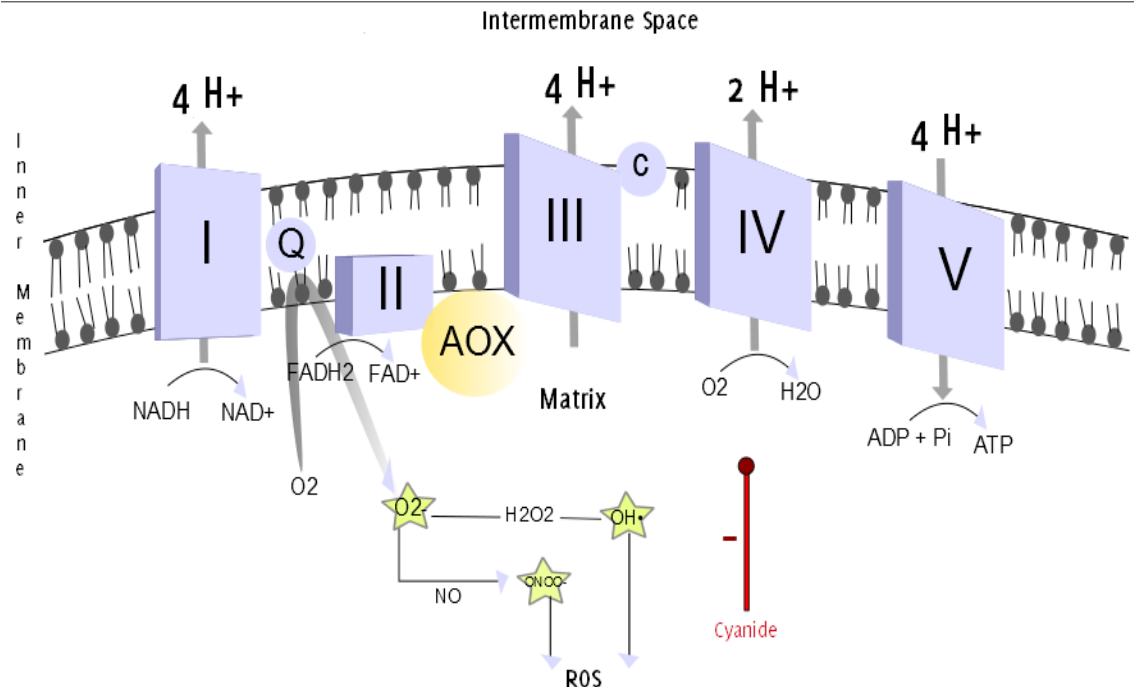
Legend: The mitochondrial respiratory chain. The efflux of protons from the mitochondrial matrix into the intermembrane space establishes an electrochemical gradient. The back movement of protons down the electrochemical gradient is utilized by complex V/ATP Synthase (a proton channel) to phosphorylate ADP, and produce ATP. Oxidation and phosphorylation are coupled. The uncoupling of the processes can be achieved when uncoupling substances allow protons to pass through the IMM through an alternative pathway, without passing through complex V.[13-14] This can be realized by the uncoupling protein *thermogenin*, which is present in brown adipose tissue. This alternative flow results in thermogenesis, the production of heat, rather than the ATP generation.[15] In neonates, *thermogenin*-containing brown adipose tissue makes up to 5% of the body mass and is located primarily on the back. This can be explained evolutionarily, as the infant's back was exposed to the environment during breast feeding and at higher risk of hypothermia.[16] AOX provides an alternative pathway for electrons passing through the respiratory chain, directly converting O_2 into H_2O, bypassing complexes III and IV. *Antimycin* and *cyanide*, inhibitors of complex III and complex IV, respectively, are therefore ineffective during activation of AOX. Superoxide ($O_2\bullet-$) anions are produced when Coenzyme Q donates unpaired electrons to molecular oxygen.[17] The reaction between $O_2\bullet-$ and NO produces peroxynitrite ($ONOO\bullet-$). Hydrogen peroxide (H_2O_2) is converted into a hydroxyl radical ($OH\bullet$). Both $O_2\bullet-$ and $ONOO\bullet-$ are powerful oxidants.[18]

ced coenzymes (nicotinamide adenine dinucleotide in its reduced form, NADH, and flavin adenine dinucleotide in its reduced form, FADH2) via the ETC to molecular oxygen as a final electron acceptor. The "chemiosmotic hypothesis" of oxidative phosphorylation postulates that during the electron movement, released energy is used by complexes I, III, and IV to facilitate the transport of protons from the matrix to the intermembrane space (IMS).[19]

The proton movement establishes an electrochemical proton gradient (delta Psi, $\Delta\Psi$) (180-190 mV negative to the cytosol),[13] that is subsequently equalized by H+ movement through a gateway of the IMM – "ATP synthase". Like in a watermill, the "molecular unit of currency" or ATP is generated by the movement of protons down their electrochemical gradient through ATP Synthase.[14]

Oxidative Stress in Diabetes

Oxidative stress makes up two crucial puzzle pieces of diabetes. First, hyperglycemia-induced long-term complications of diabetes occur due to excessive production of ROS.[20] Second, if scientists are successful in decreasing ROS generation after pancreatic islet replacement, prolonged graft survival could be ensured and diabetes treatment would shift from being life sustaining to achieving a cure.

Glucose metabolism uses the Krebs cycle to generate NADH and FADH2, which donate electrons to complex I and complex II, respectively. These electrons are subsequently passed down the ETC to molecular oxygen as a final electron acceptor, a

Figure 2. Hyperglycemia: The Role of Protein Kinase C Activation in Diabetes Complications.[21]

Legend: Hyperglycemia-induced $O_2\bullet-$ generation in the mitochondria leads to the accumulation of glyceraldehyde-3 phosphate and its derivative DAG. Elevated intracellular DAG levels stimulate the PKC-mediated generation of VEGF, an angiogenesis factor that promotes neovascularization of retinal vessels. Unfortunately, the newly formed vessels are highly friable and would often rupture, resulting in hemorrhages that obscure the vision and destroy the retina (proliferative diabetic retinopathy). On the other hand, elevated levels of VEGF are responsible for simultaneously rising amounts of TGF-ß that spurs glomerular proliferation and sclerosis, eventually resulting in diabetic nephropathy (ROS = Reactive oxygen species; DAG = Diacylglycerol; PKC = Protein kinase C; VEGF = Vascular endothelial growth factor; TGF-ß = transforming growth factor beta).

process which facilitates proton movement across the IMM. Glucose laden, diabetic cells exhibit a higher rate of glucose oxidation in the Krebs cycle, triggering more NADH and FADH2 being shoved into the ETC and ultimately establishing a higher $\Delta\Psi$ across the IMM. Eventually the electrical gradient will reach a threshold, and the electron transfer in complex III will be inhibited, resulting in the backup of electrons to coenzyme Q. Coenzyme Q then donates unpaired electrons to molecular oxygen, thereby generating the free radical $O_2\bullet-$.[22]

Under the influence of superoxide dismutase (SOD), $O_2\bullet-$ can become a weaker ROS, hydrogen peroxide (H_2O_2).[23] However, when $O_2\bullet-$ reacts with nitric oxide (NO•) it may also be converted into the more active ROS peroxynitrite (ONOO-) (*Figure 1*).[20] Both $O_2\bullet-$ and ONOO•- are powerful oxidants.[23]
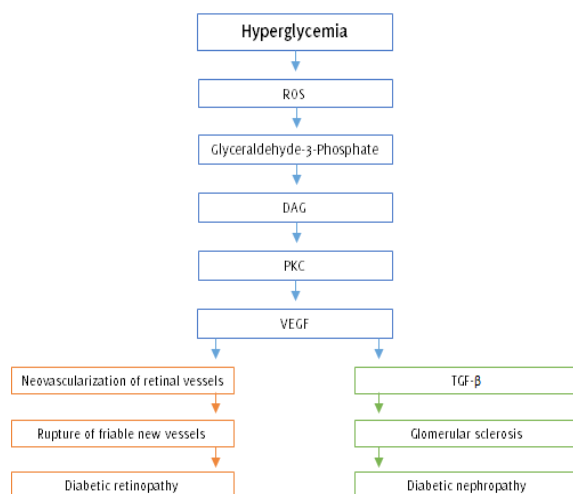
Furthermore, hyperglycemia-induced $O_2\bullet-$ generation in the mitochondria decreases the activity of glyceraldehyde-3 phosphate dehydrogenase (GAPDH), an enzyme that serves as the catalyst for the sixth step of glycolysis and is essential for glucose production from glycogen breakdown (glycogenolysis).[20,24] The inhibition of GAPDH results in the accumulation of glyceraldehyde-3 phosphate, and the activation of the hyperglycemia pathways described hereafter in detail [protein kinase C (PKC) pathway; advanced glycation end-products (AGE) pathway].[20]

The PKC pathway is initiated by the presence of a derivative of glyceraldehyde-3 phosphate, diacylglycerol (DAG). Excessive intracellular DAG stimulates PKC, a kinase protein that induces vascular endothelial growth factor (VEGF) expression in non-vascular cells.[25] VEGF is an essential angiogenesis factor and key to neovascularization. The VEGF-mediated neovascularization of the retina is a process known to play role in diabetic retinopathy. In diabetic retinopathy, the genesis of friable vessels that frequently rupture results in hemorrhages that obscure the vision.[26] VEGF also increases levels of transforming growth factor beta (TGF-ß), which is profibrinogenic and accelerates progression to glomerular sclerosis and hypertrophy, contributing to diabetic nephropathy (*Figure 2*).[27]

Another derivative of glyceraldehyde-3 phosphate, methylglyoxal, activates the AGE pathway.[22] The hyperglycemia-induced AGE pathway generates AGEs by non-enzymatic glycation of proteins. AGE production is irreversible, and circulating AGEs contribute as a deteriorating factor to vascular complications of diabetes patients.[25]

Circulating AGEs are involved in the trapping of albumin, low-density lipoprotein (LDL), immunoglobulins, and complement components. This procoagulating effect of AGEs is further increased by its function to deactivate NO•, which leads to the loss of the vasodilatory effect of NO. Besides, AGEs function as procoagulants themselves through increasing platelet adhesion and, at the same time, decreasing fibrinolysis. Also, AGE molecules are capable of binding to RAGE receptors present on both macrophages and mesangial cells of the kidney. This binding stimulates the release of cytokines and growth factors, which results in inappropriate cell proliferation, collagen synthesis, and fibrosis in the glomeruli. Finally, AGEs spur lipid oxidation, which increases oxidative stress and favors inflammation.[28]

Many experiments demonstrated that the inhibition of AGEs slow down the developments of diabetic retinopathy in laboratory rats.[29] Hammes and coworkers treated 26-week-old Wistar rats with aminoguanidine, an inhibitor of AGE formation. The induction of diabetes was achieved with an injection of streptozotocin in 0.05 M sodium citrate. The administration of aminoguanidine was initiated 2 weeks later. Advanced glycosylation-specific fluorescence enabled the scientists to visualize the amount of accumulated AGEs in the animals' retinal vessels in the 26th and 75th week. While no morphological changes and 170 ± 15 fluorescence absorbance units were observed in the healthy animals after 75 weeks, the diabetic rats had a high degree of neovascularization with friable vessels and 440 ± 20 fluorescence absorbance units. The retinal vessels of those diabetic animals that had received aminoguanidine injections were less affected by these morphological changes, and glycosylation product-specific fluorescence measured was only 220 ± 13.[30] Although aminoguanidine has been under development as a drug by the pharmaceutical company Alteon, clinical trials have been abandoned since 1998.[31]

The inhibition of GAPDH also results in the accumulation of the first and second glycolytic metabolites, glucose and fructose 6-phosphate. Glucose is reduced by aldose reductase into sorbitol, which is further oxidized by the enzyme sorbitol dehydrogenase into fructose. This so-called polyol pathway leads to a decrease of reduced NADPH.[26] NADPH is a crucial cofactor in redox reactions throughout the body, including the synthesis of myoinositol in the kidneys (*Figure 3*). Myoinositol deficiency has been shown to be present in laboratory animals with induced diabetes as well as the sciatic nerve from deceased diabetic patients.[26]

Myoinositol is particularly important for the normal function of nerves. Already in 1987 it has been suggested by Salway and colleagues that myoinositol may prove valuable in preventing or delaying diabetic neuropathy. Neuropathy is marked by a slowed conduction velocity in peripheral and autonomic nerve fibers. Salway and his team, who had administered 500 mg myoinositol twice a day to seven different diabetes patients over a time span of two weeks, had observed an increased amplitude of the action potential of three different nerves (two in the lower extremity, one in the upper extremity). They suggested the possible value of myo-inositol in diabetes treatment in the future.[30]

In excess, ROS can activate several stress-sensitive intracellular signaling pathways (NF-kB, p38 MAPK, JNK/SAPK, and hexosamine) that induce gene expression. The products of these genes are involved not only in the development of diabetes complications, but also the development of insulin resistance.[32,33] For example, hyperglycemia-induced overproduction of $O_2\bullet$ diverts fructose-6-phosphate to the hexosamine pathway. The end product of the hexosamine pathway, UDP-N-acetylglucosamine is capable of glycating intracellular proteins, including transcription factors that alter gene expression (Available from: http://www.thepharmaletter.com/article/alteon-may-drop-pimagedine-in-niddm, updated 2014 Dec 21, cited 2015 Jan 21). This suggests that antioxidants could play a role in delaying and/or preventing the development of diabetes complications and insulin resistance, thereby immensely altering the pathophysiology of T2D.

Alternative oxidase (AOX)

Alternative oxidase (AOX) is an integral mitochondrial membrane enzyme which is mainly found in sessile organisms. It is capable of limiting the overproduction of mitochondrial ROS. However, this alternative pathway bypasses several proton-pumping steps, decreasing the pH and electrical gradients, thereby reducing the ATP generation. During the course of evolution, AOX has been lost from fast-moving organisms (including humans), maybe due to the slight reduction in ATP production.[34]

By bypassing the respiratory chain, AOX confers resistance to cyanide and other inhibitors of the respiratory chain. Sessile animals, deep-sea organisms which are exposed to a hostile environment, thereby benefit from AOX and are still endowed with it.[34]

AOX provides a bypath of the ETC, thereby decreasing the $O_2\bullet$- generation and suppressing the infliction of oxidative damage on the cell. In the lab, AOX has been safely expressed in flies and human cells without eliciting any unwanted physiological side effects.[34]

Figure 3. The Polyol Pathway in Hyperglycemia.[35]
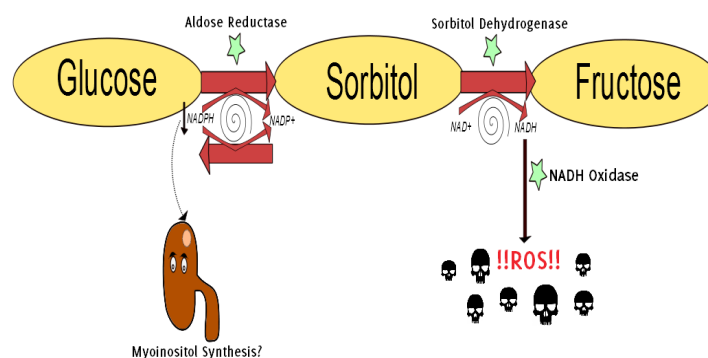
Legend: Hyperglycemia stimulates the aldose reductase-mediated reduction of glucose into sorbitol (a polyol, hence polyol pathway). Aldose reductase utilizes cofactor NADPH, which results in a depletion of the antioxidant glutathione (GSH) and increases intracellular oxidative stress.[31] Besides, NADPH is a crucial cofactor in redox reactions throughout the body, including the synthesis of myoinositol in the kidneys. Myoinositol deficiency has been identified in diabetic patients.[36] Sorbitol is further oxidized to fructose. The generated NADH is subsequently converted by NADH oxidase into ROS and, therefore, acts as a booster for oxidative stress on the cell.[37]

Review

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In 2013, *C. intestinalis'* (a sessile sea squirt) AOX gene was successfully expressed in mouse embryos with the help of germ line lentiviral transduction and subsequently passed down to the next generation.[38] This so-called MitAOX mouse could be crossed with selective lines of diabetic mice, for example the non-obese diabetic (NOD) mice, an animal model for T1D. The resultant diabetic mice should have AOX incorporated in their genome. This AOX incorporation could be helpful to investigate hyperglycemia-induced overproduction of $O_2\bullet-$ and its role in the development of diabetic complications.

Additionally, it has been suggested that AOX mitochondrial targeting sequence could be delivered with the aid of a viral vector, especially since recent data clearly points to the longevity and safety of viral vectors.[34] This injectable AOX has the potential to allow for therapeutic application in many disorders with marked overproduction of $O_2\bullet-$.[39]

Oxidative stress is influencing the allograft outcome during the peritransplantation period of kidney transplant.[38] In a study that Morales-Indiano and colleagues conducted on 131 patients with end stage renal disease (ESRD), both diabetic and non-diabetic patients had similar oxidative stress levels before kidney transplantation. However, measured oxidative stress was significantly higher in the diabetic patients after the intervention. In order to effectively measure oxidative stress, Morales-Indiano and colleagues determined and measured oxidative stress markers both prior to and at 120 days after grafting. The markers used to measure the generation of oxidative stress included anti-oxLDL antibodies (oxLDLab) and oxidized LDL. The poorer allograft function in diabetics was attributed to elevated HbA1C, which promotes oxidative stress.[17]

In future, AOX injections may be administered to decrease hyperglycemia-induced oxidative stress in diabetic patients after transplant, including pancreatic islet transplants.

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