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## Biological Significances, Diagnostic Values and Therapeutic Approach of Proinsulin Connecting Peptide

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### ABSTRACT

In the past years, proinsulin-connecting peptide (C-peptide) considered a biologically inactive peptide. Recently it has been demonstrated that this peptide exert insulin-independent biological effects. This review provides a summary of recent research and discusses some beneficial and detrimental effects of C-peptide. The binding of C-peptide with cell membrane inducing signal transduction through G-Protein Coupled Receptors (GPCR). This resulted in phospholipase-C (PLC) activation that provokes an increase in  $Ca^{2+}$  and diacylglycerol (DAG) leading to activation of many cellular signaling. The treatment of diabetic patients in particular type 2 with C-peptide resulted in improved glucose metabolism as well as blood flow. So that renal functions in addition to nerve functions are get well again. C-peptide and insulin are secreted in equimolar amounts; therefore the measurement of C-peptide permits the quantitation of insulin secretion. Due to the longer half life of C-peptide than insulin it regard as a good indicator for endogenous insulin secretion. C-peptide is sensitive to physiological degradation therefore, several strategies are taken to improve the bioavailability for C-peptide. These strategies include amino acids modification, endogenous carrier conjugation, liposome incorporation and peptidase inhibition. These data indicated that C-peptide is biologically active peptide and has many functions in treatment of diabetic associated complications. Further, studies in particular *in vivo* are required to explore the exact roles of C-peptide the treatment of diabetic associated complications as well as cardiovascular disease.

**Key words:** Proinsulin, insulin, C-peptide, signal transduction

### INTRODUCTION

Similar to secretory proteins, insulin is translated as preproinsulin that carries a signal peptide which directs the preproinsulin to the interior of the endoplasmic reticulum (ER). In the ER, the signal sequence of preproinsulin at N-terminal is cleaved and the resulting proinsulin. In the Golgi complex proinsulin is packaged into secretory granules and converted to insulin then C-peptide by proprotein convertases (PPCs). These products are stored in secretory vesicles until C-peptide and insulin are co-secreted in equimolar amounts (Mares-Guia *et al.*, 2006). After cleavage of the C-peptide, mature insulin is formed in the  $\beta$ -granules and is stored in the form of zinc-containing hexamers until secretion (Goodge and Hutton, 2000).

C-peptide plays a functional role in proinsulin folding, linking A and B chains of the insulin moiety and thus helping to provide optimal orientation of sulfhydryl groups for intramolecular disulfide bond formation. Additionally, this peptide helps in the maintenance of the C-peptide/insulin A-chain junction structure in proinsulin, which is a recognition site for PPCs.

Furthermore, C-peptide acts as molecular guardian, i.e. proinsulin folding is facilitated by an intramolecular chaperone-like action of this peptide (Chen *et al.*, 2001) and also, C-peptide regulates the kinetic folding pathway of proinsulin (Qiao *et al.*, 2003).

Early studies with C-peptide failed to report any therapeutic effects for C-peptide. Conversely, In the last few years C-peptide has insulin-like actions, therefore treatment of Type 1 diabetic (T1D) with this peptide resulted in improved renal function, increased blood flow, augmented glucose utilization, as well as improved nerve function. Therefore, C-peptide plays important roles in the treatment of diabetic neuropathy. Furthermore, C-peptide administration associated with improvement in sensory nerve functions. These effects may be attributed to C-peptide activates insulin receptor, phosphatidylinositol 3-kinase (PI-3-K), mitogen-activated protein kinase (MAPK) and glycogen synthase kinase-3(GSK3), Na<sup>+</sup>/K<sup>+</sup>-ATPase and endothelial nitric oxide synthase (eNOS) (Hills and Brunskill, 2009).

### **PREPROINSULIN AND PROINSULIN**

The insulin is the example for peptides that are processed from larger precursor molecules. The insulin is synthesized as a preprohormone molecular weight (11,500 Dalton), the hydrophobic 23-amino-acids are leader sequence directs the molecule into the interior of ER and then is removed. Proinsulin molecular weight (9,000 Dalton) is transferred to Golgi apparatus of pancreatic islet  $\beta$ -cells. During the process of secretory granules maturation, proinsulin is cleaved by endopeptidases [proprotein convertase-1 (PPC1) and proprotein convertase-2 (PPC2)] into insulin molecular weight (6000 Dalton) and C-peptide molecular weight (3,000 Dalton). In comparison with the insulin, proinsulin has low biological potency, low affinity for insulin receptors and prolonged half life in circulation. Proinsulin/insulin levels are finely regulated during development, since an excess of the protein interferes with correct morphogenesis and is deleterious for the embryo (Hernandez-Sanchez *et al.*, 2006).

### **THE PROINSULIN CONNECTING PEPTIDE**

C-peptide is the 31-amino-acid peptide that connects the A and B chains the insulin precursor molecule, therefore it named as connecting peptide. Cleavage of C-peptide from proinsulin leads to exposure of the C terminus of the insulin  $\beta$ -chain to subsequent conformational changes required for the binding of insulin to its receptor. C-peptide is secreted in a 1:1 ratio with insulin; it has a longer plasma half-life 20-30 min compared with 3-5 min for insulin.

### **SIGNAL TRANSDUCTION BY C-PEPTIDE**

The mechanisms by which C-peptide signals are transduced remain incomplete clear. But several studies reported that C-peptide binds specifically to target cells membranes through G-Protein-coupled Receptor (GPCR) activate phospholipase C (PLC). Therefore the intracellular level of diacylglycerol (DAG) is increase and this activates P-I-3-K which increase inositol trisphosphate (Hills and Brunskill, 2009).

Moreover administration of C-peptide at physiological concentrations provokes a prompt elevation of intracellular Ca<sup>2+</sup> concentrations (Shafqat *et al.*, 2002). This leads to stimulation of eNOS in renal tubular and endothelial cells (Wallerath *et al.*, 2003). Moreover, C-peptide stimulates phosphorylation of protein kinase-C (PKC) (Zhong *et al.*, 2005).

Additionally, exposure of cell to C-peptide induced the activation of one or more components of the MAPKs cascade in a concentration-dependent manner (Kitamura *et al.*, 2003). MAPKs increase

transcription factor expression, apoptosis and increased expression of eNOS. In addition to C-peptide has also been found to mimic the effects of insulin in muscle cells (Grunberger *et al.*, 2001). Stimulation of the MAPK pathway also results in increased Na<sup>+</sup>/K<sup>+</sup> ATPase activity and activation of various transcription factors.

### **DIAGNOSTIC VALUES OF C-PEPTIDE**

C-peptide is a measure of endogenous insulin secretion; the normal fasting level is (1-4 ng mL<sup>-1</sup>) which may be rises after meals. C-peptide can be measured in urine and urinary concentrations correlate with those in the blood. The half-life of C-peptide in the circulation is five times longer than that of insulin. Therefore, C-peptide levels are a more stable indicator of insulin secretion. As compared to insulin C-peptide levels in peripheral venous blood are about 5-6 times greater than insulin levels. In addition, the C-peptide assay distinguishes endogenous from injected insulin. Moreover, the clinical indications for C-peptide measurement include diagnosis of insulinoma and differentiation from factitious hypoglycemia. As well, C-peptide measurement is used in follow-up of pancreatectomy and evaluation of viability of islet cell transplants. It has been suggests that C-peptide of insulin may provide a more direct tool for assessing relative energetic condition.

Unlike insulin, C-peptide does not undergo significant clearance by the liver, meaning that it is probably a better indicator of pancreatic insulin secretion than circulating insulin itself. Additionally, C-peptide is typically used for clinical diagnosis of insulin resistance and other metabolic disorders, as well as it can also be used to quantify relative energy balance. Ellison and Valeggia (2003) used urinary C-peptide measures to track longitudinal changes in energy balance during the postpartum period in human females, tying resumption of cycling to C-peptide thresholds. Deschner *et al.* (2008) found that C-peptide levels correlated with body mass changes during a 2-week period of caloric restriction, as well as during the subsequent period of recovery.

### **BIOLOGICAL SIGNIFICANCES OF C-PEPTIDE**

#### **C-PEPTIDE AND GLUCOSE METABOLISM**

The biological activity of C-peptide was the stimulation of glucose transport in human skeletal muscle in dose-dependent manner. The supra-physiological concentrations of C-peptide prolonged the hypoglycemic actions of insulin in diabetic rats in comparison with normal rats. Likewise, physiological concentrations C-peptide were found to significantly enhance the utilization rates of glucose (Wahren *et al.*, 2000).

The effects of C-peptide on glucose metabolism have also been examined in short-term studies in T1D patients. The infusion of C-peptide display a 25% increase in glucose turnover. C-peptide receptor is fully saturated at 0.9 nmol L<sup>-1</sup> of the molecule concentration and there is no further increase in glucose metabolism when C-peptide levels were more elevated. Enhancement of whole-body sugar utilization under these conditions due to increased muscle uptake rather than reduced hepatic synthesis. The hyperglycemia is associated with increase the glycation of protein particularly hemoglobin and albumin (Mahesh and Brahatheeswaran, 2007). Moreover the same study added that the improvement of insulin secretion and minimize the protein glycation. The administration of C-peptide and insulin to T1D patients decrease proteins glycation as indicated by a significant lowering of glycated albumin and glycated hemoglobin (HbA1c) (Wilhelm *et al.*, 2008).

#### **C-PEPTIDE AND CIRCULATORY FUNCTION**

Nitric Oxide (NO) is one of the vasodilator molecules, which regulate the blood flow; such molecule plays an important role in regulation of cardiovascular, nervous and immune systems

functions (McGrowder and Brown, 2007). NO activity is low in diabetes, either due to increasing the destruction of NO by oxidative stress or by decreasing NO production from L-arginine by endothelial NO synthase (Harisa *et al.*, 2009). The supplementation of C-peptide at physiological level resulted in an increase of microvascular blood flow. These effect is related to stimulation of endothelial NO release by the activation of Ca<sup>2+</sup> calmodulin-regulated eNOS. There several studies demonstrated that C-peptide restore the circulatory function through NO dependent mechanisms.

Forst *et al.* (2008) reported that the vascular effect of C-peptide, indicating its role in the regulation of microvascular blood flow. Moreover, inhibition of NOS by certain inhibitors completely abolished the vasodilator response to C-peptide. The same study reported that C-peptide was shown to affect microvascular blood flow and to improve nerve or renal function in animal models of T1D and in humans with T1D. Moreover, C-peptide supplementation was shown to increase microvascular blood flow and to enhance the recruitment of capillaries in isolated kidneys of the rat. Moreover, C-peptide induced a concentration dependent dilation of skeletal muscles arterioles isolated from rats muscle.

### **C-PEPTIDE AND DIABETIC RENAL FUNCTION**

The elevated blood sugar level associated with several events including activation of polyols pathway as well as increase of Reactive Oxygen Species (ROS) formation (Abo-salem *et al.*, 2009). Moreover, activation of PKC, secretion of transforming growth factor- $\beta$  (TGF- $\beta$ ), altered expression of cyclin kinases, increased matrix proteins and decreased matrix-degrading enzymes and metalloproteinases (Gnudi *et al.*, 2003). Additionally, uncontrolled hyperglycemia associated with increased glycosylation of proteinases inhibitors like  $\alpha$ -1-antitrypsin leading to decrease its function. This is also one of the factors responsible for diabetic complications in kidneys and other organs (Naderi *et al.*, 2006). These events are responsible for histopathological changes associated with diabetic nephropathy.

New signaling roles for C-peptide have recently been discovered with evidence that it can amehorate complications of T1D. So that C-peptide can be used as a potential therapy for diabetic nephropathy (Hills *et al.*, 2010a, b).The beneficial effect of C- peptide in diabetic nephropathy has been proved in a series of studies. Firstly, insulin treatment in patients with newly diagnosed T1D improves but often fails to normalize renal hyperfiltration. Secondly the patients with T2D who have maintained endogenous insulin and C-peptide secretion generally do not develop glomerular hyperfiltration.

Moreover, previous study investigated the possibility that C-peptide may regulate renal functional alterations, such as GFR, in diabetes. It has been founded that C-peptide administration was accompanied by a significant fall in GFR, whereas the GFR of controls did not change. Extending these observations in T1D, the same investigators demonstrated that there is a 6% reduction in GFR and a 55% reduction in proteinuria after 1 month of C-peptide and insulin administration compared with insulin administration alone. Similarly, 3 month study in microalbuminuric T1D patients, C-peptide administration was associated with a reduction in urinary albumin excretion (Johansson *et al.*, 2000). These findings in patients have also been paralleled in animal studies. In STZ rats with early diabetes, physiological levels of C-peptide attenuate glomerular hypertrophy, glomerular hyperfiltration and proteinuria (Huang *et al.*, 2002).

In other study with an angiotensin-converting enzyme inhibitor, C-peptide was like effective captopril in reducing glomerular hyperfiltration in STZ rats. After 4 weeks STZ diabetic rats given C-peptide, these kidney functional improvements were accompanied by preserved glomerular structure with significantly reduced hypertrophy and matrix accumulation (Samnegard *et al.*, 2004). In STZ diabetic mice, C-peptide also reduced urinary albumin excretion, together with glomerular expression of the pro-fibrotic cytokine, TGF- $\beta$  and type IV collagen. When mouse glomerular podocytes were exposed to C-peptide, TGF- $\beta$  induced expression of mRNAs for type IV collagen and plasminogen-activator inhibitor 1 (PAI1) were blocked (Maezawa *et al.*, 2006).

Moreover, in isolated glomeruli from alloxan-induced diabetic mice have revealed that C-peptide constricts afferent glomerular arterioles when applied via the vessel lumen (Nordquist *et al.*, 2008). Administration of C-peptide prevented TNF- $\alpha$ -mediated apoptosis in opossum proximal tubular cells (Al-Rasheed *et al.*, 2006), suggesting a protective role of C-peptide in the progression of diabetes-related kidney disease.

### **C-PEPTIDE AND NERVE FUNCTION**

Many factors including, genetic factors, hyperglycemia, oxidative stress, activation of polyols pathway and formation of advanced glycosylation end products are involved in diabetic polyneuropathy (DPN). The lacking of C-peptide in T1D patients might exert an important role in the development of neuropathy. The nerve abnormalities in T1D animals, who lack C-peptide, includes impairment of nerve Na<sup>+</sup>/K<sup>+</sup>-ATPase and eNOS activities, resulting in intra-axonal sodium accumulation and reduced endoneurial blood flow (Sima, 2003). Gradually, structural changes appear, involving axonal atrophy and characteristic nodal and paranodal abnormalities that contribute to the progressive deterioration of nerve conduction velocity.

In contrast, hyperglycemia is the primary pathogenetic factor in T2D while C-peptide levels in the normal range. The functional and structural abnormalities of the peripheral nerves are less marked and show a different pattern, including milder axonal degeneration and no or only minimal nodal and paranodal abnormalities (Murakawa *et al.*, 2002). Thus, the lack of C-peptide in T1D contributes to the development of the more severe nerve dysfunction and structural abnormalities.

Studies in diabetic animals have suggested that C-peptide may prevent neuronal dysfunction by improving endoneurial blood flow and nerve Na<sup>+</sup>/K<sup>+</sup>-ATPase activity (Stevens *et al.*, 2004). Zhang *et al.* (2007) reported that C-peptide administration dose-dependently improved nerve conduction velocities. The protective effect of C-peptide was accompanied by beneficial alterations in a variety of neurotrophic factors and receptors and was most marked if the agent was continuously administered by an osmo-pump (Kamiya *et al.*, 2006).

Additionally treatment with C-peptide in addition to insulin was accompanied by improved autonomic function (Johansson *et al.*, 2000). Moreover, C-peptide treatment resulted in significant and substantial improvements in neuropathic parameters as determined by both neurophysiological measurement and clinical examination (Ekberg *et al.*, 2007). In the presence of insulin, C-peptide has been shown to exert antiapoptotic effects on neuroblastoma cells and to increase the production of NF- $\kappa$ B (Li *et al.*, 2003). In addition to C-peptide has been suggested to be beneficial in diabetic neuropathy (Sima *et al.*, 2004) by promoting neuronal development, regeneration and cell survival. Furthermore C-peptide prevents neuronal apoptosis in T1D and it induces neurite outgrowth and cell-growth of the neuroblastoma cell line (Li *et al.*, 2003; Li and Sima, 2004). In neurons, C-peptide may play a pro- or antiapoptotic role, depending on the cell type and the state of the cell, in addition

to its causal role in vascular disease (Denk *et al.*, 2000). C-peptide in replacement doses stimulates nerve Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, increases endoneurial blood flow and stimulates neurotrophic factors, resulting in improved nerve conduction velocity and prevention of nerve structural changes (Wahren *et al.*, 2007).

### **C-PEPTIDE AND ERYTHROCYTE FUNCTIONS**

Induction of glucose oxidation by hyperglycemia is responsible for oxidative stress resulted in increased lipids peroxidation as well as proteins oxidation leading to cellular damage. The oxidative stress has been implicated in erythrocytes damage in diabetes (Serdevi *et al.*, 2007). The Na<sup>+</sup>/K<sup>+</sup>-ATPase controls many of cellular functions, like, cell volume, free calcium concentrations and membrane potential. The erythrocytes activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase is attenuated under diabetic conditions (Vague *et al.*, 2004). Although there are tissue specific differences in the regulations of Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, hyperglycemia and diabetes are predominantly characterized by a decrease in Na<sup>+</sup>/K<sup>+</sup>-ATPase activity. This would result in an increase in intracellular calcium concentration and an increased vascular tone, promoting the development of vascular complications in diabetes mellitus. The C-peptide restores the activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase in diabetic rats. The incubation of erythrocytes from T1D patients with C-peptide normalized erythrocyte Na<sup>+</sup>/K<sup>+</sup> ATPase activity (Djemli-Shipkolye *et al.*, 2000). Furthermore, infusion of C-peptide was found to improve erythrocyte Na<sup>+</sup>/K<sup>+</sup>-ATPase activity in T1D patients (Forst *et al.*, 2000). The improvement of erythrocyte Na<sup>+</sup>/K<sup>+</sup>-ATPase is associated with improved rheological properties of the blood and increase the vascular blood flow (Forst *et al.*, 2009).

### **C-PEPTIDE AND INFLAMMATION**

C-peptide elicits insulin-independent biological effects on a number of cells proving itself as a bioactive peptide with anti-inflammatory properties (Haidet *et al.*, 2009). NO from inducible NOS plays important roles in regulation of many inflammatory and immunity processes. Moreover, NO regulation is altered in diabetic and inflammatory states (Marx *et al.*, 2004). C-peptide has been shown to increase intracellular Ca<sup>2+</sup>, in smooth muscle cells (Chakrabarti *et al.*, 2004) and aortic endothelial cells, thereby inducing NO production by eNOS and inducible nitric oxide synthase (iNOS) (Tsimaratos *et al.*, 2003). The protective effect of C-peptide against myocardial ischemia-reperfusion has been mediated through the release of NO (Young *et al.*, 2000). The same study demonstrated that C-peptide reduces polymorphonuclear cell adherence to vascular endothelium in isolated ischemic and reperfused rat hearts.

The impaired release of NO from the vascular bed will up-regulate adhesion molecules on endothelial cells, thereby increasing leukocyte-endothelium interactions. In addition to a single injection of C-peptide decreased the expression of endothelial cell adhesion molecules on the rat microvascular endothelium, leading to reduced leukocyte adhesion as well as transmigration in mesenteric venules. This attenuation of leukocyte-endothelial interactions is mediated by an increase in eNOS synthesis and subsequent release of NO (Scalia *et al.*, 2000). Overall, these reports suggest that administration of C-peptide in physiological doses exerts anti-inflammatory effects.

In contradiction, C-peptide has also been reported to possess proinflammatory properties. C-peptide improves dermal wound healing associated with an increased number of leukocytes adherent to the endothelium (Langer *et al.*, 2002). Also, it has been reported that

C-peptide co-localize with macrophages and monocytes in artery specimens from diabetic subjects and to co-localize with and act as a chemoattractant for lymphocyte and monocytes in early atherosclerotic lesions (Walcher *et al.*, 2004).

Furthermore, C-peptide has been shown to stimulate the transcription of inflammatory genes, such as cyclooxygenase-2 (COX-2), via the activation of a PKC/NF- $\kappa$ B signaling pathway in certain type of fibroblasts (Kitazawa *et al.*, 2006). It is concluded that, the role of C-peptide in the regulation of inflammation still unclear, so that further studies are needed in order to determine C-peptide has beneficial or detrimental function in inflammatory process.

### **C-PEPTIDE AND ANGIOGENESIS**

There are indications that diabetes induces the up-regulation of oncofetal fibronectin in the retina (Khan *et al.*, 2004), a substance believed to be involved in angiogenesis and normally not found in mature tissue. Increased retinal expression of oncofetal fibronectin in diabetic rats is completely prevented by C-peptide treatment (Chakrabarti *et al.*, 2004). The C-peptide normalize diabetes-induced oncofetal fibronectin up-regulation in diabetic retinas, this suggesting that the important role of this peptide in the development of microangiopathy (Chakrabarti *et al.*, 2004).

### **C-PEPTIDE AND ATHEROSCLEROSIS**

The previous studies have demonstrated that the administrated of C-peptide has contradictory effect on atherosclerosis. Firstly, C-peptide prevent vascular dysfunction in diabetic rats as well as possess antiproliferative effects on vascular smooth muscle cells (SMC) which indicate that treatment with C-peptide may delay progression of atherosclerosis. The administration of C-peptide in concentration range from 1 to 100 nM appears to suppress hyperglycemia induced hyperproliferation of aortic SMC. The antiproliferative effects of C-peptide on vascular SMC are mediated through the inhibited expression of the platelet-derived growth factor- $\beta$  (PDGF- $\beta$ ) receptor and increased phosphorylation of MAPKs (Kobayashi *et al.*, 2005).

In contradiction, there are another study reported that C-peptide may be acts as a mitogen by the induction of vascular SMC proliferation, this finding suggesting proatherogenic activity of this peptide. Moreover in certain cell lines, C-peptide (1 nM) has been shown to stimulate the PKC/NF- $\kappa$ B signaling pathway (Kitazawa *et al.*, 2006). The PI-3 pathway is implicated in the pathogenesis of diabetic endothelial dysfunction and atherosclerosis and also this pathway has been shown to be increased by C-peptide (Brownlee, 2001; Kitamura *et al.*, 2001), implicating a synergistic effect of C-peptide and TNF- $\alpha$  in aggravating diabetes-associated complications. These data suggest that C-peptide is involved in regulation of cell proliferation and apoptosis on multiple levels. Conversely, C-peptide appears to have predominantly antiproliferative effects in SMC.

### **C-PEPTIDE AND INSULIN RESISTANCE**

The metabolic abnormality of this disease include many factors such hyperglycemia, hyperinsulinemia, advanced glycation end products and dyslipidemia with low level of good cholesterol (Huq, 2007). These metabolic alterations have been demonstrated that to stimulate SMC proliferation (Indolfi *et al.*, 2001).

Insulin alone is a weak mitogen but it can potentiate the effects of other mitogens such PDGF, angiotensin II and thrombin. In insulin resistant, tyrosine phosphorylation of the insulin receptor and signaling via the insulin receptor substrate pathway is impaired resulting in diminished metabolic effects. In contrast, tyrosine phosphorylation of ERK 1/2 MAPK by insulin is maintained



and perpetuated by other growth factors resulting in SMC proliferation and migration. In this context the presence of C-peptide in atherosclerotic lesions from diabetic patients and it is tempting to speculate that C-peptide-induced proliferation of SMC in the setting of insulin resistance and hyperinsulinemia could provide a previously unrecognized mechanism leading to accelerated atherosclerosis and its complications in patients with T2D (Bruemmer, 2006).

Walcher *et al.* (2006) add other mechanisms promoting SMC proliferation under conditions of hyperinsulinemia by listing C-peptide as one of mitogens. C-peptide, secreted simultaneous to insulin activates both the PI-3-K/Akt and ERK1/2 MAPK pathways. Activation of these pathways results in SMC proliferation through phosphorylation of the retinoblastoma protein and cell cycle progression the activated by PI-3-K and ERK1/2 MAPK signaling is the cell cycle, C-peptide increased cyclin D1 expression and subsequently phosphorylation of the retinoblastoma protein as the gatekeeper of G1-S phase cell cycle progression. Based on these observations, C-peptide stimulates SMC proliferation through a Src3 PI-3 kinase/ERK1/2-MAPKdependent progression of the cell cycle.

## **C-PEPTIDE IN TYPE 2 DIABETES**

T2D is associated with insulin resistance and as the disease evolves patients exhibit elevated insulin and C-peptide concentrations. Many T2D patients develop nephropathy and neuropathy in the face of increased circulating C-peptide levels. These high levels of C-peptide in T2D patients and bearing in mind the experimentally derived affinity of C-peptide-binding sites, it is likely that any similar receptor would be fully occupied and potentially down-regulated (Hills and Brunskill, 2009).

Important differences also exist between the complications observed in T1D and T2D diabetes. Neuropathy in T1D progresses more predictably and quickly and is associated with myelin sheath and axonal derangements not present in T1D. With respect to diabetic nephropathy, the predictable evolution of renal disease observed in T1D is less well documented in T2D. In addition, diabetes-specific renal lesions are found in all T1D patients with nephropathy, renal morphology in T2D is much more heterogeneous with prominent arteriosclerosis and ischaemic nephropathy (Ritz and Tarnig, 2001). In T1D nephropathy, chronic hyperglycemia beginning in the first 2 decades of life is usually the only evident cause of kidney disease.

On the other hand, patients with T2D are generally over the age of 40 and have evidence of age-related glomerulosclerosis, together with other propagators of renal disease, such as hypertension, obesity and dyslipidemia. Thus nephropathy in T2D reflects a heterogeneous combination of kidney diseases precipitated by a mixture of mechanisms that may modify and overwhelm the typical renal responses to hyperglycemia and the features of pure diabetic nephropathy (White *et al.*, 2007). Consequently, it has been suggested that, in terms of responses to treatment, T1D and T2D diabetic patients should be considered separately.

Attention has been drawn to a possible role for C-peptide in the development of vascular inflammation and atherosclerosis in T2D diabetes. This concern is based on observations that C-peptide deposits may be found co-localized in early atherosclerotic lesions in T2D patients, but not in similar vascular lesions in non-diabetics (Marx *et al.*, 2004). Subsequent studies demonstrated the presence of chemotactic activity of C-peptide *in vitro* (Walcher *et al.*, 2004). Although, these findings are weakened because circulating C-peptide levels were not measured in the diabetic subjects, they suggest that elevated C-peptide in T2D may contribute to vascular dysfunction.

Other work has also shown that C-peptide may stimulate smooth muscle cell proliferation, but this was contradicted by other authors (Cifarelli *et al.*, 2008). On contrary to these findings C-peptide may be a key mediator in the development of vascular inflammation and atherosclerosis in T2D. Luppi *et al.* (2008) have demonstrated both an anti-inflammatory and potential anti-atherogenic role for C-peptide through a reduction in the expression of several biochemical markers of endothelial dysfunction.

### **C-PEPTIDE AS THERAPY FOR DIABETIC COMPLICATIONS**

Peripheral neuropathy is one of the most common complications of T1D and T2D mellitus. It has been demonstrated that several small non-neural peptides possess neurotrophic activity and exert beneficial effects on nervous system function in experimental and clinical diabetes. The C-peptide and islet neogenesis-associated peptide, are derived from pancreatic proteins. Moreover, derivatives of erythropoietin possess similar effect on the nervous system. These peptides are of increasing interest leads to new approaches in the treatment of diabetes-associated neuropathies (Tam *et al.*, 2006).

C-peptide treatment in rats with STZ induced diabetes is accompanied by correction of glomerular hyperfiltration, diminished levels of microalbuminuria and regression of glomerular hypertrophy. Likewise, when C-peptide is administered in replacement doses to patients with T1D, there is significant reduction of both glomerular hyperfiltration and urinary albumin excretion (Johansson *et al.*, 2002).

Effects of C-peptide replacement therapy on functional and structural changes in peripheral nerves have been studied in diabetic rats. C-peptide administration for two months was found to prevent the defect of nerve conduction velocity (Sima *et al.*, 2001). In patients with autonomic nerve dysfunction, increased heart rate variability during deep breathing has been seen following C-peptide administration.

In addition, evidence from a study involving C-peptide therapy in T1D patients without obvious symptoms of neuropathy indicate that three months of treatment results in significant improvement of sensory nerve conduction velocity. The treatment with C-peptide in rats is accompanied by significant improvement in nerve Na<sup>+</sup>/K<sup>+</sup>-ATPase activity and in nerve blood flow. The relevance of the observed C-peptide effects is supported by the fact that both deficient NO formation and reduced levels of Na<sup>+</sup>/K<sup>+</sup>-ATPase are factors of pathogenetic importance for diabetic neuropathy (Johansson *et al.*, 2002). The present data suggest that C-peptide replacement therapy together with insulin therapy in T1D patients may be beneficial in preventing or retarding the development of long-term diabetic complications.

### **IMPROVEMENT OF C-PEPTIDE HALF LIFE**

Proinsulin C-peptide is sensitive to physiological degradation therefore, it has short plasma half life (20-30 min). Several strategies like amino acids modification, endogenous carrier conjugation, liposome incorporation, N-acetylation, polyethylene glycol (PEG) glycation( PEGylation) as well as peptidase inhibition, are taken to improve the bioavailability of these small peptides could be suitable for C-peptide. For example, the plasma half life of glucagon like peptide 1 (GLP-1), a 31-amino-acid gastrointestinal peptide with neurotrophic effects, can be extended considerably by attachment of a polyethylene glycol (PEG) moiety (PEGylation) (Lee *et al.*, 2005), N-acetylation (Liu *et al.*, 2004) or conjugation of GLP-1 to serum proteins such as albumin (Kim *et al.* (2003). However, bioequivalence and biosafety remain principal concerns in such strategies. These strategies may be playing an important role in the prolongation of t<sub>1/2</sub> of C-peptide.

## CONCLUSION

In the last years the study of C-peptide not explores any biological effect for this peptide. In recent times it has been demonstrated that C-peptide elicits insulin-independent biological effect. Insulin produced by biotechnology lacking the C-peptide, the lack of C-peptide may exacerbate diabetes-associated complications. Inflammation and hyperglycemia are major cause in the development of vascular dysfunction in diabetes. Given the anti-inflammatory properties of C-peptide, one may speculate dual hormone replacement therapy with both insulin and C-peptide in patients with T1D may be warranted in the future to decrease morbidity and mortality. C-peptide prevents diabetic neuropathy by improving blood flow, neuronal apoptosis and axonal swelling. An anti-proliferative effect of C-peptide on vascular smooth muscle cells may be preventing atherosclerosis. Further, molecular studies in particular *in vivo* experiments using either infusion or injection of C-peptide in animal models of atherosclerosis or neointimal smooth muscles cell proliferation to further exploit the contribution of C-peptide to cardiovascular disease in T2D.

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