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REVIEW

Pathological consequences of C-peptide deficiency in insulin-dependent diabetes mellitus

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Abstract

Diabetes is associated with several complications such as retinopathy, nephropathy, neuropathy and cardiovascular diseases. Currently, insulin is the main used medication for management of insulin-dependent diabetes mellitus (type-1 diabetes). In this metabolic syndrome, in addition to decrease of endogenous insulin, the plasma level of connecting peptide (C-peptide) is also reduced due to beta cell destruction. Studies in the past decade have shown that C-peptide is much more than a byproduct of insulin biosynthesis and possess different biological activities. Therefore, it may be possible that C-peptide deficiency be involved, at least in part, in the development of different complications of diabetes. It has been shown that a small level of remaining C-peptide is associated with significant metabolic benefit. The purpose of this review is to describe beneficial effects of C-peptide replacement on pathological features associated with insulin-dependent diabetes. Also, experimental and clinical findings on the effects of C-peptide on whole-body glucose utilization, adipose tissue metabolism and tissues blood flow are summarized and discussed. The hypoglycemic, antilipolytic and vasodilator effects of C-peptide suggest that it may contribute to fine-tuning of the tissues metabolism under different physiologic or pathologic conditions. Therefore, C-peptide replacement together with the classic insulin therapy may prevent, retard, or ameliorate diabetic complications in patients with type-1 diabetes.

Key words: C-peptide; Diabetes; Insulin; Nephropathy; Neuropathy

Core tip: In type-1 diabetes, in addition to decrease of endogenous insulin, the plasma level of connecting peptide (C-peptide) is also reduced due to beta cell destruction. Therefore, it may be possible that C-peptide deficiency be involved in the development of diabetic complications such as retinopathy, nephropathy, neuropathy and cardiovascular diseases. In this paper, beneficial effects of C-peptide replacement on pathological features associated with type-1 diabetes

are described. Also, experimental and clinical findings that support the hypoglycemic, antilipolytic and vasodilator effects of C-peptide are discussed.

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INTRODUCTION

Diabetes mellitus is still an increasing health problem in both developing and developed countries. World Health Organization reported (August, 2011) that, 346 million people have diabetes worldwide and 3.4 million patients died from diabetes-related complications in the year 2004. Diabetes is generally classified into two main types: insulin-dependent diabetes mellitus [type-1 diabetes (T1D)] which is a state of insulin deficiency because of destruction of islet beta cells, and non-insulin-dependent diabetes mellitus [type-2 diabetes (T2D)] which is characterized by resistance to the action of insulin[1].

Poor control of diabetes is associated with several complications such as nephropathy, retinopathy, neuropathy and cardiovascular diseases[1]. Currently, insulin is the main used medication for management of T1D[2]. Even though early-onset complications may be controlled by insulin therapy, it remains difficult to achieve normal glycemic control and late-onset complications occur in many of diabetic patients[3,4]. In addition to decrease of endogenous insulin, the level of connecting peptide (C-peptide) is also reduced in the plasma of patients with T1D due to autoimmune destruction of beta cell[5]. Although for many years C-peptide has been considered as a byproduct of insulin biosynthesis, data from several lines of studies reveals the beneficial actions of C-peptide replacement in prevention of metabolic changes and structural alterations in T1D[6]. Therefore, one cannot rule out the possibility that C-peptide deficiency may also be involved, at least in part, in the development of some pathological features associated with T1D. This article reviews the current understanding of biological effects of C-peptide and the beneficial actions of C-peptide replacement on preventing or ameliorating the T1D-related complications.

C-PEPTIDE SYNTHESIS AND SECRETION

In pancreatic beta cells, proinsulin is transferred in vesicles from rough endoplasmic reticulum to Golgi apparatus, where the vesicles are directed into a regulated secretion. During this transition of vesicles, three peptidases participate in proinsulin posttranslational processing to generate insulin and C-peptide[7]. First, proinsulin is cleaved by prohormone convertase type 2 at the A-chain/C-peptide junction or by prohormone convertase type 1/3 at the B-chain/C-peptide junction. Then, carboxypeptidases H removes two pairs of amino acids located at both cleaved junctions providing the *des* forms of proinsulin (*des*-31,32 and *des*-64, 65). Finally, endopeptidase type 1/3 and type 2 recognizes des-64,65 proinsulin and des-31, 32 proinsulin, respectively, leading to release of insulin and C-peptide from proinsulin. C-peptide facilitates the correct folding of proinsulin to allow form two disulfide bridges between A- and B-chains of insulin and therefore plays an essential role in biosynthesis of insulin[6,8]. In most species only one form of proinsulin has been described. However, in rats and mice two proinsulin isoforms I and II have been found[9].

Increase of blood glucose leads to secretion of an equimolar amount of insulin and C-peptide into the portal circulation[7]. However, the liver rapidly uptakes insulin because of single pass effect and only 50% of insulin reaches to the systemic circulation with a half-life about 4 min. On the other hand, C-peptide is primarily metabolized by kidney and has a circulating half-life about 30 min which is the reason for its higher plasma concentration than insulin[5,7]. The level of C-peptide in fasting and postprandial conditions varies between 0.3-1 nM and 1.5-2.5 nmol/L, respectively[10].

Since C-peptide and insulin are secreted from beta cells in equimolar concentrations, measuring serum C-peptide is an estimate of residual beta cell function and can be used to differentiate between patients with T1D and T2D[6]. However, the mean C-peptide concentration is higher in diabetic patients with renal diseases insufficiency compared with those have normal renal function. Therefore, in sever renal failure, the serum C-peptide assay is unreliable for assess residual beta cells[6,11,12].

BIOLOGICAL EFFECTS OF C-PEPTIDE

Effects of C-peptide on glucose utilization

Experimental studies on diabetic rats showed that C-peptide prolongs the hypoglycemic effect of insulin[13] and increases whole-body glucose utilization[9,14,15]. The glucose lowering effect of C-peptide was also investigated in human. Hoogwerf *et al*[16] have shown no effect by C-peptide on blood glucose level in healthy subjects or patients with T1D. However, Johansson and coworkers demonstrated that infusion of physiological concentrations of C-peptide to patients with T1D augments whole body glucose utilization by approximately 25%[17]. Also, Oskarsson *et al*[18] showed that C-peptide hasten the insulin-induced hypoglycemia in diabetic patients. Activation of glucose metabolism by short time C-peptide infusion in healthy controls and in patients with T1D was also reported by Wilhelm *et al*[19].

The augmented whole body glucose utilization is most probably a result of increased muscle glucose uptake rather than inhibition of hepatic gluconeogenesis[20]. In normal rats, we observed that adipose tissue glucose consumption was not affected by C-peptide[21]. Direct

examinations under *in vitro* condition confirmed that C-peptide stimulates the rate of glucose transport to muscle strips obtained from healthy subjects or patients with T1D[22]. Also, Zierath *et al*[23] showed that C-peptide dose-dependently increases glucose uptake into human skeletal muscle through a mechanism shared partly with insulin. Although the exact pathway involved in this effect of C-peptide is still unknown, incubation of isolated muscle strips with a cAMP analogue abolishes the C-peptide-stimulated glucose transport.

Regarding metabolic actions of C-peptide, it should be considered that although this peptide at low physiological concentrations mimics insulin effects, however in the presence of high level of insulin (*e.g.,* in the postprandial condition) the concomitant elevated level of C-peptide may blunt the insulin's peripheral effects[21,24]. It is possible that high levels of C-peptide induce a desensitization processes which may be recovered after a period of its absence.

Effect of C-peptide on adipose tissue

Soon after discovery of C-peptide, Solmon *et al*[25] examined the effects of pork and beef C-peptide on adrenocorticotropin-induced lipolysis in rats, but no significant effects were found. Subsequently, Yu and coworkers tested the effect of supraphysiological concentrations of porcine C-peptide on the lipolysis in isolated adipocytes from rats and found an insignificant antilipolytic effect[26]. Using an *ex-vivo* organ culture method, we observed a similar insignificant reduction in basal lipolysis of rat retroperitoneal adipose tissue[21]. Because it has been reported that some effects of C-peptide appear only in diabetes condition[9,27,28], we examined whether C-peptide alters lipolysis in diabetic rats. Our data showed that C-peptide like insulin significantly inhibits isoproterenol-stimulated lipolysis[29]. Therefore C-peptide may act, conditionally, as an antilipolytic hormone and may be involved in fine-tuning of lipid metabolism.

Effects of C-peptide on circulation

Patients with T1D show reduced tissues blood flow despite intensive insulin therapy and good management of glucose control[30]. C-peptide has been shown to enhance blood flow of kidney[17], nerve[31], skeletal muscle[32], myocardium[30,33] and skin[34]. The vasodilator effect of C-peptide is mediated by stimulation of nitric oxide release from endothelial cells[35-37]. Wallerath *et al*[35] reported that physiological postprandial concentration of C-peptide is able to activate endothelial nitric oxide synthase (eNOS) and stimulating nitric oxide production. Forst *et al*[38] showed that intravenous infusion of C-peptide to patients with T1D increases plasma concentration of cGMP, as an index of nitric oxide activity[38]. This finding is in agreement with earlier report that in diabetic rats the C-peptide induced glucose utilization is sensitive to eNOS inhibition[9].

OTHER BIOLOGICAL EFFECTS OF C-PEPTIDE

Interaction with insulin

In the presence of C-peptide, insulin hexamers in solution becomes undetectable. Also, subcutaneous injection of an insulin and C-peptide mixture to diabetic patients accelerates the increase of insulin levels in plasma and in comparison with injection of insulin alone utilizes more glucose. Therefore, it seems that C-peptide increases disaggregation of insulin by binding to insulin oligomers and thereby enhances the availability of monomeric (biologically active form) insulin[39].

Protection of endothelium

It has been reported that C-peptide is able to inhibit leukocyte-endothelium interaction induced by thrombin or by NG-nitro-L-arginine methyl ester. This effect of C-peptide may be important in protection of vasculature against inflammatory disorders such those observed in T1D[40].

EFFECTS OF C-PEPTIDE ON DIABETIC COMPLICATIONS

Effects on diabetic nephropathy

Different aspects of the diabetic renal pathogenic abnormalities can be improved by C-peptide in T1D (Figure 1). In diabetic rats, C-peptide decreases urinary protein excretion[41-43], reduces glomerular hyperfiltration rate and restores the renal functional reserve[42-44]. These beneficial effects have also been demonstrated in insulin-dependent diabetic patients[17,45]. In a clinical study, patients were administered insulin alone or in combination with C-peptide by subcutaneous infusion pump for 4 wk. While combination therapy led to decrease of glomerular filtration rate and protein excretion after 2 wk, the insulin alone was ineffective[45]. Johansson *et al*[46] extended the period of C-peptide therapy to 3 mo and reported a significant decrease in the rate of protein excretion in patients receiving combination of insulin and C-peptide. In line with these findings, Zerbini *et al*[47] found a decreased C-peptide/creatinine ratio in the plasma of T1D patients with nephropathy when compared with those without albuminuria[47]. Microscopic examinations have showed that in diabetic rats, C-peptide reduces the hypertrophy of mesangial matrix in glomeruli of the kidneys[48]. Several mechanisms are postulated for beneficial effects of C-peptide on renal function including inhibition of apoptosis, increase of Na^+, K^+-ATPase activity and interaction with the signaling pathway of growth factors[49,50]. Activation of the key signaling molecules such as phospholipase C and protein kinase C followed by phosphorylation of extracellular-signal-regulated kinase and c-Jun N-terminal kinase have been shown in human renal tubular cells treated with

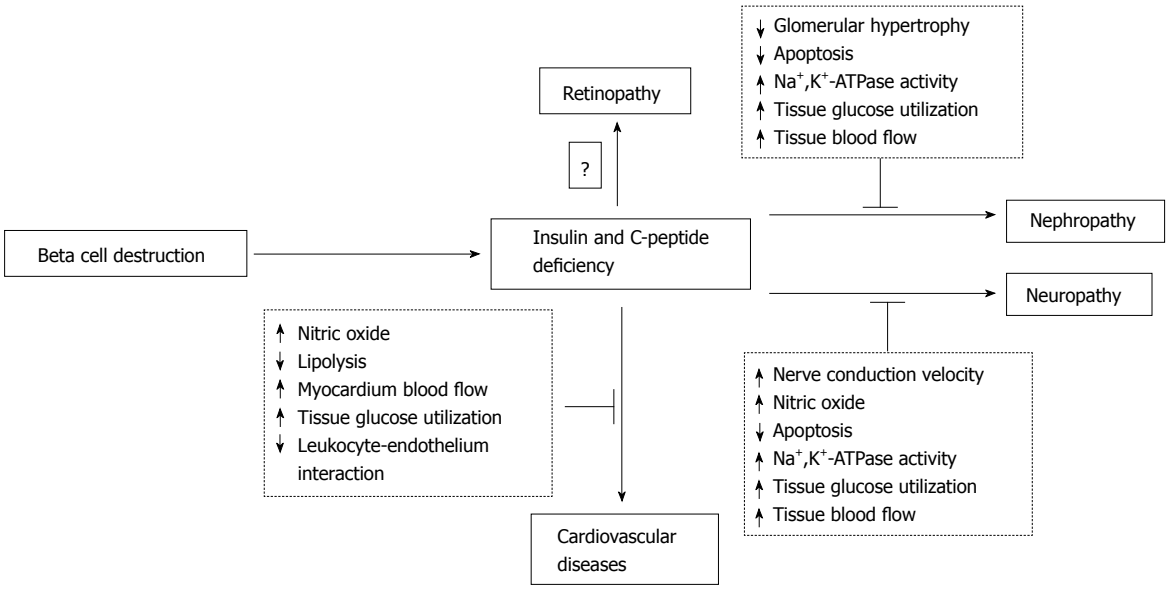

Figure 1 Proposed mechanisms (dashed rectangles) by which C-peptide may prevent, retard, or ameliorate diabetic complications in patient with type-1 diabetes. ↓: Decrease; ↑: Increase.

C-peptide[49]. Regarding beneficial effects of C-peptide, we should emphasis that some of the C-peptide beneficial effects are limited to animals or patients who show very low or missing C-peptide plasma levels[9,27,28]. Therefore, the nephroprotective effect of C-peptide may represent a therapeutic goal for patients with T1D.

Effects on diabetic neuropathy

Accumulating evidence suggests that C-peptide can prevent, retard, or ameliorate neuropathy in T1D (Figure 1)[51-57]. Decreased level of Na^+, K^+-ATPase activity and reduced nitric oxide formation are considered as contributor factors to pathogenesis of diabetic neuropathy. It has been shown that C-peptide prevents the neural Na^+, K^+-ATPase defect and the nerve conduction velocity reduction[52]. In study of Cotter *et al*[31] C-peptide at physiologic doses improved sensory and motor nerve conduction velocity in STZ-induced diabetic rats through increase of nitric oxide release. Ekberg *et al*[58] demonstrated that C-peptide improves vibration perce-ption in patients with T1D[58]. C-peptide also may prevent cognitive dysfunction by its antiapoptotic effect in the brain particularly in the hippocampus[51-54]. The antiapoptotic property was also confirmed by Li *et al*[59] who showed that C-peptide, in the presence of insulin, inhibits high glucose-induced apoptosis in neuroblastoma cells. There are also clinical evidence that autonomic dysfunction can be ameliorated by C-peptide replacement. Infusion of C-peptide to patients with T1D increases the heart rate variability during deep breathing and the heart rate brake index after tilting[55]. In contrast to insulin alone, administration of a combination of C-peptide and insulin improves heart rate during deep breathing in T1D patients[46].

CONCLUSION

According to data presented in this paper, C-peptide is much more than a byproduct of insulin synthesis and has several biological actions such as hypoglycemic, antilipolytic and vasodilator effects. These biological effects suggest that it may act as a hormone to contribute in fine-tuning of the tissues metabolism under different physiologic or pathologic conditions. In T1D diabetes, in particular, it was found that patients who conserve low but sustained secretion of endogenous C-peptide show better metabolic control and less retinopathy, nephropathy and neuropathy than patients who have become fully C-peptide and insulin deficient[56-60]. These beneficial effects are demonstrated only in T1D models. It is possible that in physiological conditions, C-peptide produces its maximum effect and induces some levels of desensitization processes in phosphorylation mediated actions, especially nitric oxide-dependent pathways. Recovering the C-peptide mechanism of action during a period of its absence is in good agreement with the experimental results in T1D models. Therefore, present data suggest the possibility of a clinically applicable role for C-peptide replacement, together with the classic insulin therapy, to prevent, retard, or ameliorate diabetic complications in patient with T1D.

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