C-peptide replacement therapy as an emerging strategy for preventing diabetic vasculopathy

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Lack of C-peptide, along with insulin, is the main feature of Type 1 diabetes mellitus (DM) and is also observed in progressive β-cell loss in later stage of Type 2 DM. Therapeutic approaches to hyperglycaemic control have been ineffective in preventing diabetic vasculopathy, and alternative therapeutic strategies are necessary to target both hyperglycaemia and diabetic complications. End-stage organ failure in DM seems to develop primarily due to vascular dysfunction and damage, leading to two types of organ-specific diseases, such as micro- and macrovascular complications. Numerous studies in diabetic patients and animals demonstrate that C-peptide treatment alone or in combination with insulin has physiological functions and might be beneficial in preventing diabetic complications. Current evidence suggests that C-peptide replacement therapy might prevent and ameliorate diabetic vasculopathy and organ-specific complications through conservation of vascular function, as well as prevention of endothelial cell death, microvascular permeability, vascular inflammation, and neointima formation. In this review, we describe recent advances on the beneficial role of C-peptide replacement therapy for preventing diabetic complications, such as retinopathy, nephropathy, neuropathy, impaired wound healing, and inflammation, and further discuss potential beneficial effects of combined C-peptide and insulin supplement therapy to control hyperglycaemia and to prevent organ-specific complications.

1. Introduction

C-peptide and insulin are co-secreted in equimolar amounts into the circulation from the pancreatic β-cells of Langerhans.1–4 In the past two decades, the physiological function and potential protective role of C-peptide in diabetes have been actively investigated.3,5 Numerous studies, including the Diabetes Control and Complications Trial and experimental studies, have described the beneficial role of C-peptide in diabetic complications in animal models and Type 1 diabetes mellitus (T1DM) patients.3,6–11 These beneficial effects have been attributed to C-peptide’s ability to prevent or ameliorate diabetes-induced vasculopathy.3,6–12 C-peptide has been shown to protect against diabetic vascular dysfunction and micro- and macrovascular damages.6,12–14 The vasoprotective mechanisms of C-peptide involve the maintenance of vascular function, as well as the prevention of endothelial cell death,6,12 microvascular permeability,13 inflammation,14–16 and neointima formation.17

Diabetic complications are the result of secondary systemic damage caused by chronic hyperglycaemia, and they are a substantial cause of diabetes-related morbidity and mortality.18 The therapeutic management of hyperglycaemia is the primary intervention for preventing diabetic complications, which is generally achieved by regular insulin supplement therapy in T1DM, treatment with oral hypoglycaemic agents in early T2DM, and administration of insulin along with oral hypoglycaemic agents in late-stage T2DM. To date, efforts against glycaemic control alone have not been entirely successful in preventing long-term diabetic complications, and alternative therapeutic strategies targeting aetiological factors for diabetic complication pathogenesis are required to prevent organ-specific damage.19–24 Although two major factors (insulin deficiency and subsequent hyperglycaemia) contribute to the development of diabetic complications, C-peptide deficiency is suggested to be the third major factor because of beneficial effects of C-peptide against diabetic complications.3,6–8,10,12–14,19,25 In this review, we present the current understanding of C-peptide conservation of vascular function and its potential physiological and protective effects against the development of diabetic vasculopathy and organ-specific complications, and we describe future clinical prospects of combined C-peptide and insulin supplement therapy in controlling metabolic (or hyperglycaemic) memory defects.

2. C-peptide deficiency in DM and pathological consequences

Human C-peptide and insulin are the post-translational cleavage products of proinsulin that are produced in the β-cells of the pancreatic islets of Langerhans and secreted into the circulation.1–4 It has been more than four decades since Steiner and colleagues36–38 described...
the structure of proinsulin and suggested that the connective peptide (C-peptide) between insulin chains A and B plays an important role in insulin biosynthesis and processing (Figure 1). After the signal peptide of pre-proinsulin is removed in the rough endoplasmic reticulum, proinsulin is post-translationally modified in the Golgi apparatus and the secretory granules of pancreatic β-cells, resulting in the production of insulin and C-peptide (31 amino acids). After the structure of human C-peptide was elucidated, immunoassays have been developed to detect blood or urine C-peptide levels, which are useful in the clinical management of patients with diabetes.

The lack of C-peptide, along with insulin, is the main feature of Type 1 DM and is also observed in progressive β-cell loss in later stage of Type 2 DM. DM is a group of chronic metabolic diseases characterized by hyperglycaemia resulting from defective insulin secretion, insulin action, or both, and it is becoming a global epidemic. Insulin deficiency corresponding to C-peptide-deficient state is the aetiology of T1DM, which accounts for 5 – 10% of the diabetic population. Among the subclasses of T1DM, Type 1A results from cell-mediated autoimmune attacks on β-cells, whereas Type 1B is less frequent and is seen in patients of Asian and African descent with unknown aetiology of β-cell loss who present with varying degrees of insulin deficiency and residual β-cell production. Although T2DM is a state of insulin resistance to relative insulin insufficiency, it may progress to a late-stage insulin- and C-peptide-deficient state due to pancreatic β-cell demise, which might necessitate exogenous insulin and C-peptide supplement therapy.

Altered metabolic pathways during hyperglycaemia induce the development and progression of organ-specific complications in both T1DM and T2DM. Hyperglycaemia and subsequent metabolic changes seem to develop into diabetic complications due to progressive damage and dysfunction of blood vessels termed as vasculopathy or angiopathy. Hyperglycaemia also induces abnormalities in blood flow, vascular permeability, angiogenesis, inflammation, and vascular occlusion and cardiac dysfunction, which can result in organ-specific cellular apoptosis, renal failure, amputation, and neuronal damage. The consequences of hyperglycaemia damage are commonly attributed to a C-peptide-deficient state, and therapeutic strategies using anti-hyperglycaemic...
treatment and/or insulin are not sufficient to prevent diabetes-related morbidities. These lines of evidence suggest that diabetic complications may be developed or enhanced, at least in part, by C-peptide deficiency along with insulin insufficiency during a hyperglycaemic state. C-peptide replacement during hyperglycaemia due to β-cell insufficiency may be beneficial in the prevention or amelioration of various diabetic complications including neuropathy, nephropathy, retinopathy, cardiovascular disease, and impaired wound healing (Table 1). However, both C-peptide and insulin have anti-inflammatory effect and induce Na+/K+-ATPase activation and nitric oxide (NO) production. Synergistic effects of insulin and C-peptide have been reported compared with insulin alone in normalizing renal function, arteriolar dilation, preventing nodal and paranodal degeneration, and improving myocardial vasodilation and left ventricular function in T1DM. Thus, C-peptide and insulin combinatory therapy would be beneficial for preventing diabetic complications because insulin can be used to achieve normoglycaemia, while C-peptide supplementation may protect against vasculopathy and organ-specific complications.

3. Recent advances on the beneficial roles of C-peptide against diabetic vasculopathy

Deficient supplies of C-peptide and insulin in the circulation seem to play an essential role in vasculopathy. Diabetic vasculopathy is a defect in blood vessels caused by hyperglycaemia, and it leads to organ-specific complications. Two major classes of organ-specific diseases caused by diabetic vasculopathy are microvascular complications, including retinopathy, nephropathy, and neuropathy, and macrovascular complications, including cardiovascular diseases such as myocardial infarction and cerebrovascular disease manifesting as stroke. Here, we will discuss recent progress in the investigation of potential C-peptide ability to protect against diabetic vasculopathy and organ-specific complications (Figure 2). In particular, we will highlight the beneficial effect of C-peptide against diabetic vasculopathy by conserving vascular function, preventing vascular damage, and maintaining vascular structure and homeostasis.

3.1 C-peptide conservation of vascular function

Several studies in diabetic patients and animals support the notion that C-peptide treatment alone or in combination with insulin has physiological functions and may be beneficial in protecting organ-specific vascular outcomes in DM. In patients with T1DM, microvascular blood flow and coronary vasodilatory function are altered despite intensive insulin therapy and good metabolic control. C-peptide infusion achieved significant improvement in vascular blood flow in skin microvessels by increasing capillary blood cell velocity. C-peptide administration also normalized the reduced blood flow and capillary diffusion capacity, and it increased oxygen and glucose uptake in exercising skeletal muscles of DM patients. Moreover, C-peptide improved adenosine-stimulated myocardial blood flow, left ventricular ejection fraction, and stroke volume in diabetic patients to levels similar to those in healthy controls. Administration of C-peptide with insulin to T1DM patients, compared with insulin alone, normalized glomerular function and improved metabolic control.

C-peptide improvement of human vascular blood flow in T1DM is likely to be mediated through a mechanism involving endothelial NO production and erythrocyte Na+/K+-ATPase activation. NO-mediated vasodilatation of C-peptide was enhanced in rat skeletal muscle arterioles in the presence of insulin, suggesting a beneficial effect of insulin and C-peptide co-treatment. C-peptide-mediated NO release inhibits leukocyte–endothelial interaction in rats, which is associated with decreased endothelial surface expression of the adhesion molecules P-selectin and intercellular adhesion molecule-1 (ICAM-1). It is demonstrated in rat aortic endothelial cells that C-peptide-induced NO release from endothelial cells is mediated by enhanced endothelial nitric oxide synthase (eNOS) protein expression through an extra-cellular signal-regulated kinase 1/2 (ERK1/2)-dependent mechanism. However, the mechanism(s) behind the cell type-specific roles of C-peptide remain(s) to be explored.

Erythrocyte Na+/K+-ATPase activity decreased in T1DM patients and C-peptide infusion normalized the decreased Na+/K+-ATPase activity, which indicates that C-peptide deficiency leads to diabetic complication through compromised Na+/K+-ATPase signalling. Na+/K+-ATPase is essential for maintaining cell volume, membrane potential,
and calcium concentrations, and its reduction in diabetes decreases erythrocyte deformability by increasing intracellular sodium concentration and subsequently increasing blood viscosity.\(^{50}\) NO production increases \(\text{Na}^{+}/\text{K}^{+}\)-ATPase activity and contributes to vasorelaxation.\(^{69-71}\) Thus, C-peptide activation of erythrocyte \(\text{Na}^{+}/\text{K}^{+}\)-ATPase might restore vascular function and blood flow in DM; however, the mechanism linking eNOS-dependent NO production and \(\text{Na}^{+}/\text{K}^{+}\)-ATPase activation in endothelial cells and vascular function remains poorly understood.

In contrast to the beneficial effects of C-peptide in T1DM and late stage of T2DM, the effects of C-peptide in T2DM are controversial. Patients with insulin resistance and early T2DM exhibited elevated levels of C-peptide and an increased propensity to develop a diffuse and extensive pattern of arteriosclerosis.\(^{72,73}\) High levels of C-peptide induced recruitment of inflammatory cells and their migration into the subendothelial layer.\(^{60,74}\) C-peptide also had a stimulatory effect on proliferation of vascular smooth muscle cells and kidney human mesangial cells,\(^{60,75}\) while other study demonstrated C-peptide prevention of rat aortic smooth muscle cell proliferation in high glucose condition.\(^{76}\) Thus, further studies are necessary to elucidate the precise function and action mechanism of elevated levels of C-peptide in early T2DM.

### 3.2 C-peptide protection against vasculopathy: an emerging potential therapeutic strategy

#### 3.2.1 Retinopathy

Retinopathy is one of the major microvascular complications induced by diabetes and is the leading cause of blindness in adults, and it develops in nearly all T1DM adults and in >60% of patients with T2DM.\(^{77}\) A retrospective analysis of the Diabetes Control and Complication Trial in T1DM and a cross-sectional study in T2DM revealed that C-peptide levels were associated with reduced incidences of microvascular complications (retinopathy and nephropathy), indicating that preserving \(\beta\)-cell function or C-peptide supplement therapy is important.\(^{7,78}\)

During the course of diabetes, retinal overexpression of VEGF stimulates ROS generation and stress fibre formation, and interrupts vascular endothelial (VE)-cadherin-based adherens junction in retinal endothelial cells, leading to vascular hyperpermeability and macular oedema.\(^{79-83}\) Our recent study demonstrated the potential retinoprotective role of C-peptide against VEGF-induced microvascular leakage in the retina of diabetic mice through inhibiting ROS generation, stress fibre formation, VE-cadherin disruption, and vascular permeability.\(^{13}\) In T1DM patients, C-peptide administration with insulin, but not with insulin alone, prevented blood-retinal barrier leakage.\(^{19}\) Increased extracellular matrix (ECM) protein deposition and capillary basement membrane thickening also contribute to diabetic retinopathy development. Notably, overexpression of oncofoetal fibronectin in diabetic BB/Wor rat was normalized by C-peptide treatment.\(^{84}\) C-peptide’s protective role against endothelial apoptosis may also prevent retinal endothelial cell and pericyte loss during retinal microangiopathy. Further studies are required to elucidate the role of C-peptide in retinal angiogenesis, ECM deposition and basement membrane thickening, vascular permeability, and endothelial cell and pericyte loss.

#### 3.2.2 Nephropathy

A number of studies demonstrate C-peptide beneficial effects on diabetes-induced renal dysfunction. Diminished glomerular function
due to microvascular dysfunction is an important feature of renal failure in diabetes. In T1DM patients, administration of both C-peptide and insulin, but not of insulin alone, improved glomerular function and metabolic control by reducing glomerular hyperpermeability, 19,64 In cultured renal micro-VE cells, C-peptide prevented hyperglycaemia-induced ROS generation by normalizing mitochondrial complex 1 activity. 85 Glomerular hyperfiltration and protein leakage is reduced by C-peptide replacement therapy in diabetic rats. 86-89 In vitro and in vivo studies using rodent diabetic models revealed that the renoprotective role of C-peptide is mediated through Na\(^+\)/K\(^+\)-ATPase activation. mRNA and protein levels of Na\(^+\)/K\(^+\)-ATPase α1-subunit are markedly decreased in the medullary collecting ducts of diabetic rats, and these changes were significantly prevented by chronic C-peptide supplement therapy. 52 However, insulin supplement therapy alone failed to normalize Na\(^+\)/K\(^+\)-ATPase expression. Similar to the full-length C-peptide, the C-peptide fragment EVARQ (corresponding to rat C-terminal pentapeptide) protected against DM-induced glomerular hyperfiltration and stimulated Na\(^+\)/K\(^+\)-ATPase activity. 51,86 C-peptide also prevented glomerular hypertrophy by suppressing collagenase IV-dependent mesangial matrix expansion in a DM rat model. 90 Renal tubulointerstitial fibrosis is considered a crucial etiology leading to kidney failure due to the profibrotic effect of transforming growth factor-β1 (TGF-β1)-induced epithelial–mesenchymal transformation, and it is attenuated by retinoic acid and hepatocyte growth factor. Gene expression profiling of human renal proximal tubular cells (PTC) line (HK-2) cells revealed that C-peptide antagonizes the profibrotic effects of TGF-β1 via up-regulation of retinoic acid- and hepatocyte growth factor-signalling pathways. 91 C-peptide reversed TGF-β1-stimulated morphological alterations (associated with epithelial–mesenchymal transformation), including increased vimentin expression, decreased E-cadherin expression, and cytoskeletal re-arrangement in HK-2 cells, by blocking Type I and II TGF-β1 receptors. 92 C-peptide also prevented TNF-α-induced apoptosis of opossum kidney PTCs by activating nuclear transcription factor (NF)-κB. 93 Furthermore, C-peptide and insulin treatment of the opossum kidney cells also induced activation of peroxisome proliferator-activated receptor-γ (PPAR-γ), which is implicated in the regulation of adipogenesis, inflammation, and lipid and glucose metabolism. Further studies are required to elucidate these in vitro findings in diabetic animals and patients.

3.2.3 Neuropathy
Several studies in T1DM patients and animal models suggest C-peptide beneficial effects on diabetic neuropathy by enhancing neuronal function and endoneurial blood flow. In patients with DM, the duration and degree of hyperglycaemia are important determinants of microvascular complications, including neuropathy, which leads to chronic pain, numbness, and substantial diabetic morbidity. Apart from intensive glycaemic control, no other evidence-based treatments are available to ameliorate or prevent diabetic neuropathy, and more than half of patients with good glycaemic control still develop this complication. 22,94 A randomized, double-blinded, placebo-controlled study demonstrated that a 3-month C-peptide replacement therapy administered to T1DM patients with early signs of diabetic neuropathy ameliorated nerve dysfunction. 95 This effect was achieved by improving sensory nerve conduction velocity (80% correction), which was accompanied by improved vibration perception. A 6-month regimen of C-peptide replacement therapy also improved sensory nerve function in patients with early stage diabetic neuropathy. 96 Similarly, C-peptide improved polyneuropathy in diabetic BB/Wor rats by preventing Na\(^+\)/K\(^+\)-ATPase defects, nodal and paranodal degeneration, and axoglial dysfunction and increasing nerve fibre regeneration. 97-100 In diabetic BB/Wor rats, C-peptide delivery using osmotic pumps attenuated nociceptive neuropathy by preventing the decline in levels of nerve growth factor (NGF) receptor, insulin receptor, and insulin-like growth factor 1 receptor in dorsal root ganglia, as well as by increasing sciatric levels of NGF; however, daily subcutaneous injection has marginal effects. 35,101 Oxidative stress and neurovascular dysfunction are considered to contribute to diabetic neuropathy in experimental animal models. C-peptide treatment improved sciatic blood flow and vascular conductance in diabetic rats, and these effects were partially reversed by eNOS inhibitors, indicating that C-peptide protection is at least partially mediated through NO-sensitive vascular mechanism. 102,103 Since various studies have supported the beneficial effect of C-peptide against diabetic neuropathy, 35,65,95–98,101 C-peptide might be able to mitigate diabetic neuropathy during intensive insulin therapy of diabetic patients (T1DM and late T2DM).

Cognitive failure due to hippocampal neuronal loss and diabetic encephalopathy was observed in T1DM rats and was prevented by C-peptide replacement. 34 C-peptide protection against hippocampal neuron death and cognitive dysfunction is achieved by preventing apoptosis related to oxidative stress and NGF receptor p75-, and poly(ADP-ribose) polymerase-, apoptosis-inducing factor-, and caspase-dependent apoptosis. Additionally, the effects of C-peptide were investigated in human neuroblastoma SH-SY5Y cells; together with insulin, C-peptide exerted a synergistic effect on cell proliferation and decreased apoptosis in high glucose-stimulated cells. 105

3.2.4 Impaired wound healing
DM is one of the major contributors of impaired wound healing. 106,107 Impaired wound healing can result in chronic open wounds, infections, ulcers, and even amputation. 108–110 Wound healing is a dynamic and interactive process involving angiogenesis, coagulation, inflammation, tissue formation, and tissue remodelling controlled by growth factors. 111–113 Recently, we reported the protective function of C-peptide against impaired wound healing in diabetes. 14 C-peptide normalized diabetes-impaired wound healing by inhibiting inflammation and stimulating angiogenesis in streptozotocin-induced diabetic mice, demonstrating that C-peptide replacement may be to therapy for impaired angiogenesis and delayed wound healing in diabetes. C-peptide activation of angiogenesis was mediated through activation of extracellular signal-related kinase 1/2 and AKT, as well as NO formation. 14 However, additional investigations are required to elucidate the mechanism(s) for C-peptide prevention of hyperglycaemia-induced impaired wound healing.

3.2.5 Blood disorders, inflammation, and glucose uptake
Haematological and haemorheological disorders are important factors contributing to vasculopathy in DM by obstructing blood flow and function in the target tissue, leading to organ-specific complications, such as diabetic nephropathy. 114,115 Administration of C-peptide to diabetic animals and patients with T1DM has been shown to be beneficial against haematological disorders. Diabetes-induced impairment of NO release in microvessels and erythrocyte deformability alters blood rheology. C-peptide increases endothelial NO production and prevents impaired erythrocyte deformability to improve vascular blood flow in T1DM patients. 115,116 C-peptide-mediated activation of erythrocyte Na\(^+\)/K\(^+\)-ATPase is also involved in improving erythrocyte deformability. Furthermore, C-peptide treatment also restores
Na+/K+-ATPase activity in erythrocytes of insulin-treated T2DM patients; however, enzyme activity was unaltered in controls and T2DM patients who were treated with oral hypoglycaemic drugs. Diabetic patients also develop coagulation disorders due to enhanced platelet aggregation and the increased synthesis of coagulation factors, including plasminogen activator inhibitor 1 (PAI-1). C-peptide was shown to exhibit an anti-thrombotic effect in the microcirculation of diabetic mice; however, this effect was reversed by co-treatment with insulin, which suggests that further confirmatory studies are necessary. Still, the evidence indicates that C-peptide improves microvascular blood circulation via eNOS-mediated NO formation and erythrocyte Na+/K+-ATPase activation.

Diabetes can create a proinflammatory microenvironment, largely due to ROS and advanced glycation end product (AGE) formation and progression to micro- and macrovascular complications. Recently, C-peptide administration at resuscitation following haemorrhagic shock was shown to ameliorate the inflammatory response in lungs of non-diabetic rats by reducing IL-1, IL-6, macrophage inflammatory protein-1, and cytokine-induced neutrophil chemoactantant-1. C-peptide reduced adhesion of lipopolysaccharide-stimulated U-937 monocytes to human aortic endothelial cells in hyperglycaemic conditions. C-peptide also exhibited a beneficial effect on impaired wound healing by inhibiting inflammation and stimulating angiogenesis in diabetic mice with T1DM. These observations demonstrate that C-peptide in the physiological concentration range exerts an anti-inflammatory effect through its anti-inflammatory properties. However, high levels of C-peptide induced recruitment of inflammatory cells into the subendothelial layer and stimulated proliferation of vascular smooth muscle cells. Further studies are required to reconcile these opposing findings at high C-peptide concentrations and to understand the role of C-peptide in early T2DM.

Insulin stimulates systemic glucose uptake and utilization to maintain glucose homeostasis; however, C-peptide might be also involved in glucose uptake and metabolism. C-peptide-stimulated glucose transport was reported in human skeletal muscle strips from non-diabetic subjects and T1DM patients, and the effect was mediated through an insulin receptor-independent mechanism. It was also reported that acute infusion of C-peptide into diabetic patients markedly increased glucose uptake and normalized capillary diffusion capacity, blood flow, and microvascular function in the exercising forearm. Consistent with this finding, infusion of rat C-peptide increased glucose utilization in a streptozotocin-induced diabetic rat using an insulin clamp. C-peptide stimulates insulin signalling in L6 myoblasts and myocytes by activating insulin receptor tyrosine kinase and Insulin receptor substrate 1 and also by inhibiting protein tyrosine phosphatase activity, which suggests an insulinomimetic effect.

The stimulatory effect of C-peptide on glucose uptake and utilization might be explained by C-peptide interaction with insulin. C-peptide interacts with insulin and islet amyloid polypeptide by charge interactions. This chaperone-like action prevents aggregation of insulin during storage and secretion in the pancreas, which attributes to enhanced physiological insulin effects in Type 1 and late Type 2 diabetic patients. C-peptide replacement therapy demonstrated no significant effect on the blood glucose level in streptozotocin-induced diabetic mice or Type 1 diabetic patients. Thus, it is possible that C-peptide might indirectly affect on glucose uptake and utilization by enhancing insulin effects through formation of its bioactive monomers.

4. Current evidence for C-peptide receptor and core signalling mechanisms

C-peptide is now recognized as an active biomolecule with clear biological roles, and numerous studies have hypothesized a molecular basis for signal transduction. Although C-peptide may attenuate vascular and neural dysfunction in diabetic rats through non-chiral interaction with membrane lipids, the C-terminal pentapeptide (residues 27–31, EGSLQ) of human C-peptide may have chiral interactions with G protein-coupled receptors (GPCRs). Wahren and colleagues proposed the pertussis toxin-sensitive GPCR as a cell-surface receptor, and the binding occurred at the C-terminal pentapeptide segment of the C-peptide on the surfaces of several cell types. The beneficial effects of C-peptide are inhibited by pertussis toxin pre-treatment. Further evidence showed that C-peptide stimulates GTPγS binding to Gαi in renal tubular cells. In support of Wahren’s finding, Yosten et al. recently provided strong evidence in CATOIII cells, suggesting that GPR146 is a pertussis toxin-sensitive membrane part of the C-peptide signalling complex. Lindahl’s group demonstrated that Rh-labelled C-peptide internalizes in a pertussis toxin-sensitive mechanism in Swiss 3T3 and HEK-293 cells. They also suggested that C-peptide may exert growth factor activity and regulate RNA, with transcriptional effects achieved through its nuclear translocation. In addition, Luppi et al. described a mechanism of C-peptide internalization driven by early endosomes (involving endosomes and lysosomes) using human aortic endothelial and smooth muscle cells. Along with these lines of evidence, C-peptide was found to co-localize and internalize with GPR146, indicating that C-peptide binding to the GPCR and their subsequent co-internalization may be achieved through a receptor-mediated signalling mechanism. However, further studies are needed to elucidate the C-peptide signalling complex that leads to beneficial effects, including the prevention of diabetic complications.

Given the current view of C-peptide biochemistry, this review proposes a core C-peptide signalling pathway (Figure 3) as the mechanism by which C-peptide might be beneficial in the protection of diabetic vasculopathy and organ-specific complications. C-peptide prevents or ameliorates ROS production through inhibiting protein kinase C (PKC)-dependent NADPH oxidase 2 (NOX2) activity, which ultimately attenuates cytosolic and mitochondrial ROS cycling. This effect may reduce AGE formation under hyperglycaemic conditions; however, further studies are required to clarify the direct effect of C-peptide on the AGE pathway. C-peptide-mediated normalization of hyperglycaemia-induced ROS prevents mitochondrial fission and ΔΨm collapse, inhibits intracellular proapoptotic enzyme TGF2, and activates anti-apoptotic protein B-cell lymphoma-2 (Bcl-2) to prevent caspase-dependent endothelial cell apoptosis. Our recent finding demonstrated that C-peptide inhibition of ROS is mediated through AMPKα activation, which regulates downstream apoptotic signalling to prevent endothelial cell death. Hyperglycaemia-induced ROS generation activates NF-κB in vascular cells, and C-peptide abrogates NF-κB transcriptional activity and prevents an inflammatory response by inhibiting the inflammatory mediators monocyte chemoattractant protein-1 (MCP-1), PAI-1, IL-8, ICAM-1, vascular cell adhesion molecule 1, and P-selectin. In addition, NF-κB activation is linked with high glucose-induced endothelial apoptosis, but the direct effect of C-peptide on NF-κB inhibition in endothelial cell apoptosis is not clear. C-peptide regulates the activation of transcription factors, such as PPAR-γ, cyclic-AMP response element-binding protein...
and activating transcription factor 1 (ATF1), and suppresses NF-κB and activator protein-1 (AP-1), thereby controlling gene expression during the course of hyperglycaemia.15,131 Although the C-peptide signalling pathway may provide evidence for the vasoprotective role of C-peptide, additional studies using endothelial cells, VSMCs, diabetic animals, and human subjects are required to elucidate the receptor-mediated signal transduction mechanism.

5. Conclusion and future perspectives

C-peptide is emerging as a potentially beneficial endocrine hormone because it is co-secreted with insulin from pancreatic β-cells, it has a longer circulatory half-life compared with insulin, and it has been shown to exert beneficial positive effects possibly through a pertussis toxin-sensitive intracellular signalling mechanism. C-peptide deficiency is a hallmark of T1DM and the later stages of T2DM, indicating that learning more about C-peptide is clinically important. Tremendous efforts have been invested in the last few decades to understand the physiological benefits of C-peptide and develop therapeutic strategies to reduce diabetic complications. In the present review, we provided an overview of emergent clinical and experimental evidence supporting the beneficial role of C-peptide against diabetic vasculopathy, which is a major complication that leads to diabetes-related morbidity and mortality.

Clinical approaches targeting glycaemic control have not been successful in preventing diabetic complications.132 C-peptide plays
vasoprotective role, and supplemental C-peptide therapy in conjunction with insulin might become an important clinical approach to prevent diabetic vasculopathy. Recent therapeutic strategies against diabetes have focused on glycemic control; however, multiple factors including insulin deficiency, hyperglycemia, and C-peptide deficiency seem to result in the development of diabetic complications. Thus, it is necessary to manage both T1DM and late stage of T2DM with combinatorial therapy for both glycemic control and vasculopathy prevention (Figure 4). In T1DM and late stage of T2DM, insulin can be used to achieve normoglycemia, and C-peptide supplementation may be beneficial in preventing vasculopathy and organ-specific complications. Hyperglycemia-induced ROS production is a crucial pathogenic consequence of diabetes, and a variety of antioxidant therapies have been tested but have only achieved limited clinical benefits.133 Endogenous C-peptide supplement therapy may mimic the benefits of antioxidant treatment. However, patients with insulin resistance and early T2DM exhibit elevated levels of insulin and C-peptide, in contrast to deficiency of the two peptide hormones in patients with T1DM and late T2DM. High levels of C-peptide induce proinflammatory effects in the vasculature and kidneys in early T2DM. Thus, further studies are required to elucidate the differential effects of C-peptide in T1DM and late and early T2DM.

Although hyperglycemia can be intensively monitored to protect individuals from the likely outcome of diabetic vasculopathy, early exposure to hyperglycemia before diagnosis may be sufficient to cause the development of late diabetic complications and the phenomenon referred as metabolic memory or hyperglycaemic memory.24,134 The emergence of metabolic memory suggests that proper metabolic control can reduce ROS and AGEs, which minimizes the development of vasculopathy as a long-term complication.21 Metabolic memory is established in vascular cells due to transient hyperglycaemia, and it induces persistent epigenetic changes that alter the expression of vital proteins associated with diabetic vasculopathy. The major epigenetic modifications by hyperglycaemia are the hypermethylation of histone H3 Lys4, demethylation of histone H3 Lys9, and histone H3 acetylation.134 C-peptide interacts with histone proteins and enhances acetylation of Lys16 of histone 4 in cultured cells.126 Thus, combinatory therapy with C-peptide and insulin might control ROS-dependent hyperglycaemic or metabolic memory defects and might prevent vascular complications and end-stage organ damage.

Although substantial progress has been made towards understanding the role of C-peptide in experimental animal models and diabetic patients, the putative benefits of combinatorial therapy of C-peptide with insulin require further research and clinical trials. Figure 4 depicts the proposed combinatorial therapeutic approach that uses C-peptide and insulin in T1DM and late stage T2DM. With this regimen, insulin can control the glycaemic state to reduce the aggressive progression of diabetic complications, while C-peptide normalizes hyperglycaemia and metabolic memory-induced ROS production, vascular inflammation, and vascular cell damage. The defensive role of C-peptide against ROS generation offers an opportunity to protect the vasculature from

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**Figure 4** A conceptual framework for prospective research and the potential use of combined C-peptide and insulin supplement therapy against diabetic vasculopathy. OHA, oral hypoglycaemic agents.
the recently described vicious circle of metabolic memory, which is an emerging challenge in preventing diabetic complications. This strategy will require understanding the mechanisms of metabolic memory and the beneficial roles of C-peptide in vascular cells, animal models of diabetes, and diabetic patients.

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