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Review Article

Redefining C-peptide: Therapeutic Potential in Mitigating Diabetic Microvascular Complications

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Abstract: This review examines the changing insights into C-peptide and its potential for therapeutic actions against the backdrop of diabetic microvascular complications. C-peptide, formerly regarded as just a byproduct of insulin production, has recently surfaced as a bioactive peptide with important physiological effects. Findings indicate that C-peptide has anti-inflammatory and antioxidant capabilities, strengthens glucose utilization, and raises insulin sensitivity. Trial results for C-peptide replacement therapy show hope for treating diabetic neuropathy and nephropathy conditions. The article reviews the influence of C-peptide on cells, focusing on interactions with cell surface receptors and the stimulation of signaling molecules inside cells. C-peptide's role in generating nitric oxide, activating Na+K+ATPase, and improving the flow of blood in microvessels is emphasized. The review explores how C-peptide affects red blood cell deformability and its potential capabilities in reducing multiple diabetic complications. Besides, the article examines current research into C-peptide analogs that have a long-lasting effect and clinical trials that assess C-peptide replacement therapy. ErsattaTM, a new generation of weekly dosage disease-modifying replacement peptides, is seen as a promising advance in the treatment of chronic diabetes complications. Overall, this review stresses the increased acceptance of C-peptide as a powerful agent for the management of microvascular complications in diabetes, giving hope for superior results in patients with both type 1 and potentially type 2 diabetes.

Keywords: C-peptide, Na+-K+-ATPase, Diabetes.

INTRODUCTION

Described for the first time in 1967, C-peptide is a 31-amino acid polypeptide resulting from insulin biosynthesis in pancreatic beta cells and having a molecular mass of 3600 [1]. During insulin biosynthesis, endopeptidases present in the endoplasmic reticulum cleave proinsulin into insulin and C-peptide. These are packaged into secretory granules within the Golgi, and when the beta cell is stimulated, it releases into the portal blood in equal molar proportion [2]. In non-diabetics, the concentration of plasma C-peptide ranges from 0.9 to 4 ng/mL [3]. While type 1 diabetics will usually manifest significantly reduced, or no C-peptide at all, those diagnosed with diabetes type 2 may reveal significantly reduced or normal C-peptide levels. An

insufficiency of C-peptide may result in microvascular issues, exacerbating neuropathy, retinopathy, and nephropathy. Since the 1990s, research findings have demonstrated that C-peptide is a bioactive peptide with vital physiological effects. Studies that involved C-peptide injections for type 1 diabetics indicated improvements in regional blood flow as well as complications from diabetes. New research has augmented our insight into C-peptide, clarifying its wider significance in diabetes management. Findings suggest that C-peptide has both anti-inflammatory and antioxidant capabilities, which may provide defense against vascular damage for diabetic individuals. Moreover, it has demonstrated an ability to boost glucose utilization and to improve skeletal muscle insulin sensitivity, which contributes positively to metabolic regulation. Trials concerning C-peptide replacement therapy have shown encouraging results, particularly in the arena of diabetic neuropathy and nephropathy [4]. Also, fresh C-peptide analogs with lengthened half-lives are in development, thus presenting novel therapeutic options. The assessment of C-peptide levels has become an essential method for measuring remaining beta cell function in people with diabetes and for telling apart type 1 from type 2 diabetes [5]. This progress illustrates the growing role of C-peptide in both treating and administering diabetes, along with its potential uses in future therapeutic strategies.

C-peptide binding to cell surface receptors by radioligand

The first investigation into the connection between C-peptide and cell membranes emerged in 1986, revealing specific binding at nanomolar concentrations with receptors on a variety of cell surfaces such as neuronal, endothelial, fibroblast, and renal tubular that are coupled to G proteins [5]. The rat C-peptide carboxy terminal pentapeptide, EVARQ, has been found to evoke 100% of the activity characteristic of intact C-peptide. The rest of the molecule was completely without activity. For the proper activation of signaling pathways, conserved glutamic acid residues at positions 3, 11, and 27 and helix-promoting residues in the N-terminal segment of C-peptide are fundamental [6].

Nonreceptor interactions

The C-peptide midsegment could be active, as it seemingly does not take part in receptor or membrane interactions and does not obstruct the receptor-like interactions within the COOH-terminal segment pentapeptide, EVARQ. The C-peptide used in the study was heterologous and was applied in a concentration surpassing physiological levels (100 nM), and effects from nonreceptor interactions became apparent after 2–3 days [7].

Cellular effects of C-peptide

The activating of G proteins leads to Ca2+-dependent intracellular cascades, namely MAPK, PLCγ, and PKC, which prompts increased intracellular concentration of Ca2+. Ca2+ stimulates both endothelial nitric oxide synthase (eNOS) and Ca2+-calmodulin-dependent protein phosphatase 2B (PP2B). PP2B subsequently switches the inactive phosphorylated Na+-K+-ATPase form into its dephosphorylated, operative state [5].

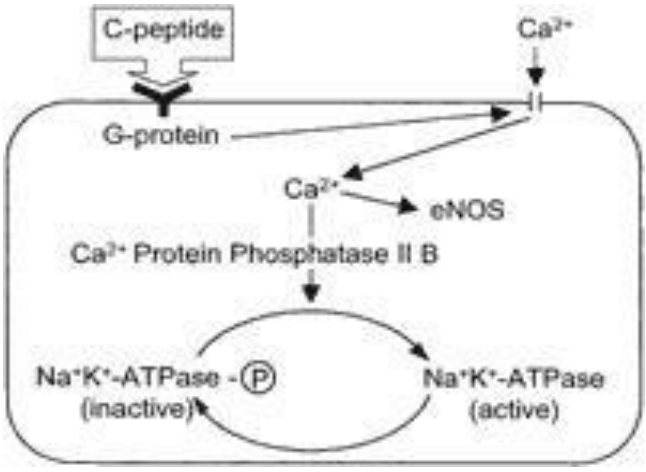

Figure 1: C-peptide binds to cell membrane receptors, activates G protein resulting in an increased intracellular Ca^{2+} concentration and activation eNOS and PP2B and subsequent activation of Na^{+}-K^{+}-ATPase [4]

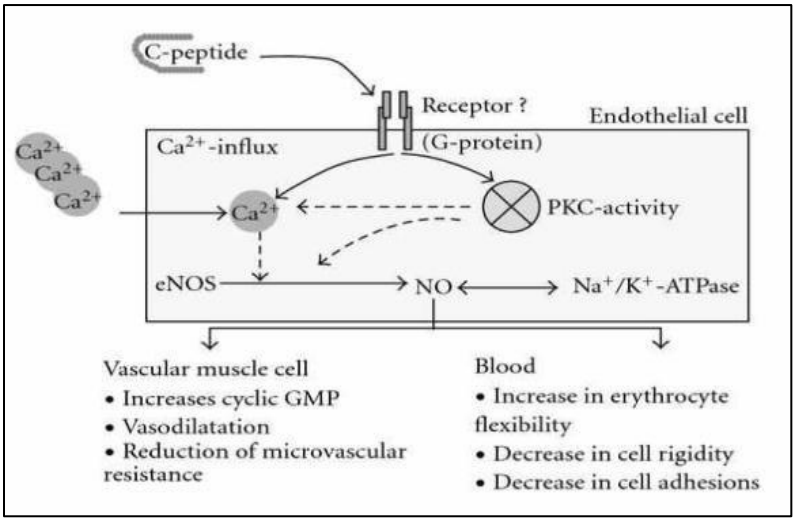

Figure 2: Schematic presentation of the molecular mechanism of C-peptide activity on endothelial cells and microvascular blood flow [8]

The enhancement of mitogen-activated protein-kinase-dependent transcription of endothelial nitric oxide synthase by C-peptide was demonstrated to stimulate NO production in aortic endothelial cells of Wistar rats [9]. It markedly strengthens the release of NO from bovine aortic endothelial cells (BAECs) when intracellular Ca2+ concentration is in the physiological dose range of 1–6 nM [10,11]. The regulation of endothelial eNOS occurs through Ca2+/calmodulin, as is the case with the C-peptide. Under conditions without Ca2+, both the stimulation of the Ca2+ signal and the release of NO were nullified [12]. Subsequently, NO results in vasodilatation as well as a reduction of microvascular resistance.

Effects of C-Peptide on renal Na+K+ATPase

C-peptide rapidly enhances Na+K+ATPase activity within human renal tubular cells through the stimulation of PKC and MAPK [13,14]. Na+K+-ATPase is an important plasma membrane protein complex that manages important cellular functions, including cell volume, free calcium concentrations, and membrane potential [15]. In diabetes, impairment of Na+K+-ATPase activity is related to multiple complications, such as kidney disorders, slowed nerve conduction velocity, retinal dysfunction, endothelial damage, reduced microvascular blood flow, and hyperkalemia [16,17]. The latest studies show that the administration of C-peptide is able to offset the decrease in renal Na+K+ATPase α1-subunit seen in animals with diabetes and in individuals with type 1 diabetes. This also rectifies glomerular hyperfiltration, lessens albuminuria, and blocks glomerular hypertrophy in the beginning stages of diabetic nephropathy [18-20]. Also, C-peptide has shown the ability to improve sodium excretion, which could enhance fluid balance in kidneys afflicted by diabetes. These actions are mediated by direct activation of Na+-K+-ATPase along with indirect pathways tied to nitric oxide synthesis and calcium signaling, reflecting C-peptide's intricate role in preserving kidney function in people with diabetes [21-23].

C-Peptide's effects on red cell deformability and erythrocyte Na+K+ATPase

C-peptide influences blood and improves RBC deformability, reduced cell stiffness, and reduced cell binding. The problems with deformability, membrane rigidity, and RBC aggregation in the capillary bed of patients with diabetes mellitus result in changes in the microvascular blood flow [24-26]. It is possible that reduced erythrocyte deformability in diabetic patients seriously depends on the attenuation of Na+K+-ATPase activity and might be restored not only by insulin but C-peptide as well [27,28].

C-peptide and glucose utilization

The exact role of C-peptide in enhancement of glucose utilization in different models in vitro and in animals, which may be associated with NO activation, seems not to be significant in human subjects and significant only in short-term doses of C-peptide but not in the long term [29].

C-peptide and anti-inflammatory effect

C-peptide also has been reported to have anti-inflammatory effects as well as aid repair of smooth muscle cells [30,31].

Effects of C-Peptide on Microvascular Blood Flow

C-peptide plays a crucial role in regulating microvascular blood flow and enhancing nerve and renal function both in animal experiment models and human patients with type 1 diabetes mellitus [32-34]. Indeed, in these studies the beneficial effects have

been observed, suggesting that supplementation of C-peptide can be useful for the treatment of diabetic complications. The fact that the vasodilatory effect of C-peptide depends on the NO pathway. C-peptide also enhances the activation of endothelial Nitric Oxide Synthase (eNOS), which was followed by an increase in NO release [35]. This mechanism was further supported when pre-treatment of the vessels with N-monomethyl-l-arginine (L-NMMA), an inhibitor of nitric oxide synthesis, effectively obliterated the vasodilating actions of C-peptide. Recent investigations have also established that C-peptide stimulates Nα+/K+-ATPase as one of the mechanisms of action responsible for its vascular effects. In fact, C-peptide unleashes a nonlinear dose-invariant dilation of the arterioles, between 0.3 and 1000 ng/mL, as evidenced by skeletal muscles and arterioles harvested from the rat cremaster muscles [36]. Such a wide effective range indicates that C-peptide may indeed prove to be therapeutic even at fluctuating physiological levels. One study described an interaction between C-peptide and insulin when it comes to microvascular blood flow. When insulin was added at these low concentrations, it did not produce a vasoactive response on its own but clearly potentiated the response to C-peptide. This finding means that both peptides from the pancreas are permissive in controlling microcirculation and points to the possible advantage of administering insulin with C peptide in diabetes. Supplementation with C-peptide has reported positive influence in several vascular beds in diabetic rats. Human C-peptide, when given photosynthetically in a dose of 2 IU/kg twice daily for five weeks at a plasma level of 9–10 nM in diabetic rats, offered preeminent vascular and neural advantages in STZ-induced diabetics [37]. Diabetic rats demonstrated lesser increments of blood flow within the diabetes, particularly in the anterior uvea, retina, and sciatic nerve, as well as enhanced vascular barrier function by decreased 123I-labeled albumin leakage within the retina, nerve, and aorta. Also, an increase in caudal motor nerve conduction velocity was observed [38]. These effects were not seen in healthy control rats, suggesting that the vascular positive attributes of C-peptide are confined to diabetes. In addition, the improvement in the blood supply was certain impressive, as the endoneurial blood flow augmentation was 57%, vascular conductance was 66% higher, and the motor and sensory nerve conduction velocities were 62% and 78%, respectively. Consequently, it is spotted with the ability to prevent and reverse diabetic neuropathy. These studies will be discussed below. Further, the DCCT trial offered strong support for the clinical use of C-peptide [39]. Compared to Type 1 diabetic patients without measurable C-peptide production by beta cells, patients who maintained normal or near-normal circulating levels of C-peptide had a significantly reduced risk of microvascular complications. This observation has created interest in C-peptide as a therapeutic moiety in the prevention of diabetic complications. Subsequent research has enriched what we know about C-peptide vascular impact, making the compound crucial for managing diabetes complications. Immunologically, C-peptide has been shown to possess anti-inflammatory activity in that it decreases the surface expression of adhesion molecules in endothelial cells, potentially inhibiting vascular inflammation. The study also reveals that it exerts antioxidant action, thus preventing endothelial impairment due to increased oxidative stress [40]. In addition, the angiogenic activity has also been described for C-peptide, providing support for the concept that it stimulates angiogenesis, which is useful for diabetic ulcer healing. C-peptide has been demonstrated to decrease albuminuria and improve GFR in diabetic nephropathy, suggesting renal enlargement. Further, the experimental data shows the neuroprotective actions of C-peptide to be beneficial to the diabetic patient in the area of cognition and thinking ability. These results also point to the benefits of C-peptide for the treatment of other complications of diabetes. Therefore, based on the accumulating evidence indicating that C-peptide improves microvascular blood flow and possibly reduces complications of diabetes, further research into this compound holds great promise for diabetes treatment. Because presently there is no disease-modifying treatment for patients with microvascular complications of type 1 diabetes, the prospects of continuing the research and possible future use of C-peptide therapy for patients with type 1 diabetes may represent a valuable resource for managing such patients in the future [41].

Future prospect

Long-acting C-peptide analogs are still under investigation as potential therapies. These analogs have the potential to maintain relatively stable low physiological concentrations of C-peptide and may represent a new paradigm for therapeutically intervening in microvascular complications of type 1 diabetes. There is debate, however, as to whether this benefit is derived from C-peptide itself or from residual insulin secretion as insulin levels wane in untreated diabetes, but controlled trials are currently under way to assess the use of C-peptide replacement for diabetic complications such as neuropathy, nephropathy, and retinopathy [39]. The consequences of these trials are highly pertinent to the question of the future use of C-peptide in diabetes care. An American pharmaceutical known as Cebix argues that lack of endogenous C-peptide, along with hyperglycemia, plays a critical role in the

development of chronic complications in diabetics. In particular, the company has performed eleven clinical studies in diabetic neuropathy and nephropathy and has treated about three hundred patients with type I diabetes. C-peptide replacement therapy has been shown to enhance nerve blood flow, reduce peripheral and autonomic neuropathy and nephropathy, and improve erectile dysfunction in clinical trials. Weekly dosage disease-modifying replacement peptide (ErsattaTM by Cebix) demonstrates biological activity similar to that of the native peptide and can potentially reverse the chronic complications of the disease. ErsattaTM has received a fast-track category by the FDA for diabetic peripheral neuropathy; it can bring other long-term complications in both type 1 and type 2 diabetic patients in reverse manner as well 42.

CONCLUSION

Numerous biochemical and vascular changes have been elaborated in the past for the pathogenesis, but none of them was amalgamated into common, unified aetiological pathways that result in diabetic complications. From these findings, it appears that impaired microcirculation is central to the DG development of chronic complications of diabetes. In the last few years, much attention has been given to C-peptide because of its stimulative impact on the microcirculation in several tissues, especially the nervous system, kidneys, and retina. At present, there are not many therapeutic interventions available for these complications of diabetes, and probably there is no effective medicine to manage or cure the microvascular effects. With this view, proprietary C-peptide will be the pioneer that will be able to reverse the chronic complications of diabetes. Our expectation is that this drug may assist a lot to the millions of patients with diabetes globally.

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