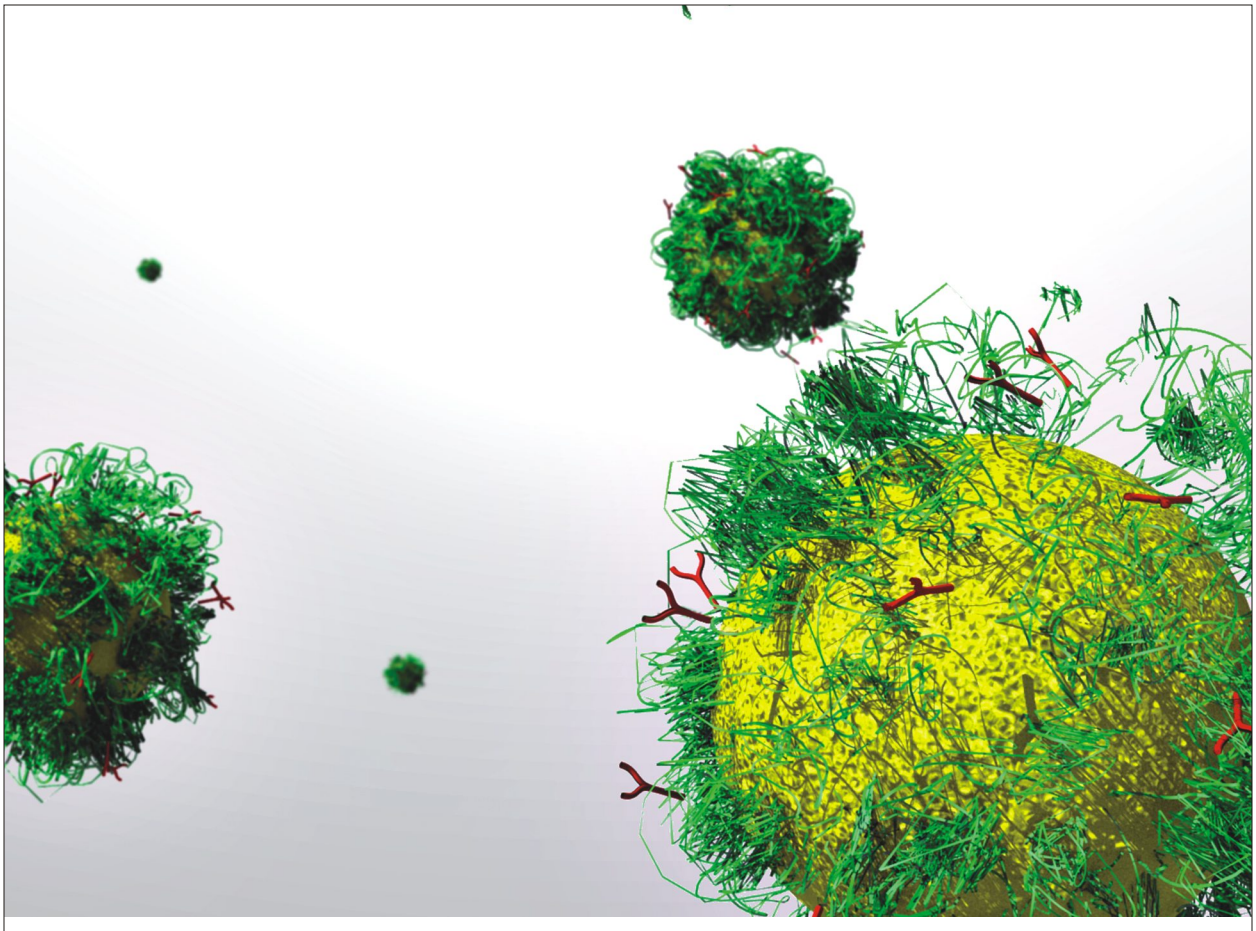

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Role of C-Peptide in Development of Diabetic Neuropathy [DN] Animal Model

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ABSTRACT

Diabetes is an increasingly common disorder which causes and contributes to a variety of complications which are often associated with neuropathic condition. Diabetic neuropathy is a group of disorders and as such the most common chronic complication affecting both type1 and type 2 diabetic patients. Diabetic research in preclinical studies has emerged as a promising area in the recent times. The current models used in the study for understanding diabetic complications are not efficient enough for producing reliable results about neuropathy. The role of C-peptide in development of neuropathy is clear and can be used as a tool to study neuropathy and even the molecular pathways can be studied by using it. Here with the help of collected data it is concluded that DN model could be developed with the help of C-peptide which may be used as standard model in diabetic neuropathy.

Key words: C-peptide, diabetic neuropathy, animal model

INTRODUCTION

The major complexicity of diabetes is neuropathy (DN) which occurs in approximately 60% of diabetic patients (Vincent and Feldman, 2004). Diabetic peripheral neuropathy is the most common complication of long-standing Diabetes Mellitus (DM) and its much-feared sequel, the diabetic foot, is responsible for most diabetes-related hospitalizations (Stevens *et al*., 1998). DN has been considered the most common and earliest complication of long-term hyperglycaemia (Brownlee, 2005; Bruning *et al*., 2000). On the other hand, evidence is assuring that insulin deficiency rather than hyperglycaemia contributes to the development of DN (Pierson *et al*., 2002, 2003; Schmidt *et al*., 2003, 2004). Sensory disturbances in the feet and hands are usual neurological complications of diabetes mellitus. They consist of 'negative' symptoms (hypesthesia, anaesthesia) or 'positive' symptoms (paraesthesia, dysesthesiae, pain) (Mackel and Brink, 2003). Another distinct possibility for causing abnormal neural impulse generation or conduction is changes in axonal ionic conductance, secondary to metabolic changes in diabetic nerve (Quasthoff, 1998). Thus, the aim of this study is to review the development of DN model with the help of C-peptide and its use to compare DN in animal.

IMPORTANCE OF ANIMAL MODEL FOR DIABETES STUDY

Evaluation of animal research is usually done by three general criteria: the generation of knowledge, reproducible study, the relevance of the study and the predictive validity of clinical pain

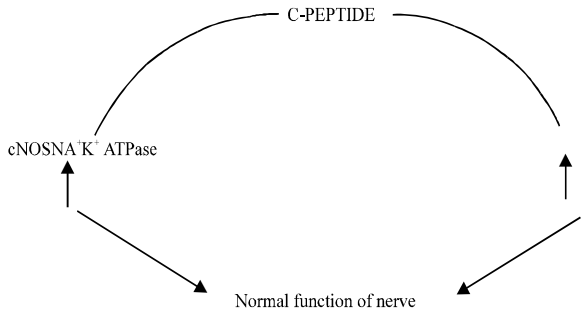

Fig. 1: Normal functioning of nerves

states. Animal models provide pivotal systems for preclinical studies of neuropathic pain which serve as an experimental basis for mechanistic investigations and testing new therapeutic interventions (Colleoni and Sacerdote, 2010). Diabetic rodents develop a number of neurochemical electrophysiological and behavioural disorders that have prompted the use of these animals as models for studying DN (Calcutt, 2004). Animal model have been developed to investigate the pathogenesis of DN and evaluate potential therapeutic agent. No model is perfect and no one would suggest that diabetic rat can replicate the human condition fully (Hounsom and Tomlinson, 1997).

Role of c-peptide in DN model development: Proinsulin c-peptide was first described in 1967 in connection with the discovery of the insulin biosynthesis (Steiner *et al*., 1967). C-peptide consists of 30-35 amino acids, depending on the species in question. It is the hydrophilic property of C-peptide which prevents the aggregation of the insulin molecule in the secretary granules (Wei *et al*., 1995). In recent years, it became clear that hyperglycaemia is not the sole culprit in the development of diabetic complications. Increasing attention is being paid to insulin and C-peptide deficiencies. Both insulin and C-peptide exert a number of metabolic, neuroprotective and anti-apoptotic effects (Pierson *et al*., 2002, 2003; Li *et al*., 2002, 2003). C-peptide has been shown to bind to the surface of various cells such as neuronal, endothelial, fibroblast and renal tubular, innanomolar concentrations to a receptor that is likely G-protein-coupled. The signal activates Ca^{2+} dependent intracellular signaling pathways such as MAPK, plc and PKC, leading to upregulation of a range of transcription factors as well as eNOS and $NA^{+}K^{+}$ ATPase activities (Wahren *et al*., 2000). Also C-peptide has been reported to have anti-inflammatory effects and aid repair of smooth muscle cell (Hills and Brunskill, 2008; Luppi *et al*., 2008) (Fig. 1).

IN VIVO MODEL OF DIABETES MELLITUS

Streptazotocin [STZ] induced type 1 diabetes: STZ an antibiotic obtained from *Streptomyces achromogenes*, is composed of 1-methyl-1-nitrosourea and glucose (Mughal *et al*., 2010). STZ is an antimicrobial agent and has also been used as a chemotherapeutic alkylating agent (Sharma and Richard, 2000; Rees and Alcolado, 2005; Serreze and Leiter, 1994). The half life of STZ is more as compared to alloxan and also the hypoglycaemic duration is longer, so the DN study is mainly carried out on this model. STZ is taken up into the β-cell and it splits into glucose and methylnitrosourea. This compound is having the alkylating property due to which fragmentation of DNA and destruction of β-cell occurs (Hattangady and Rajadhyaksha, 2009). STZ is taken up by pancreatic β cells via glucose transporter GLUT-2. A reduced expression of GLUT-2 has been found to prevent the diabetogenic action of STZ (Herr *et al*., 1967; White, 1963). The STZ

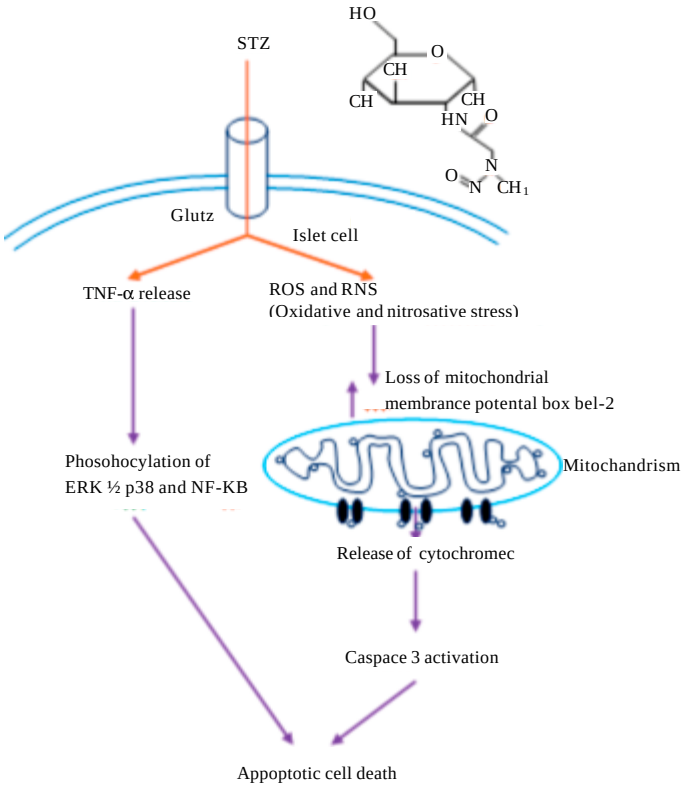

Fig. 2: Mechanism of streptazotocin inducing Diabetes mellitus (White, 1963)

itself restricts GLUT-2 expression *in vivo* and *in vitro* when administered in multiple doses. When STZ enters into the cell and does not come out through the GLUT-2 receptor (Schein *et al*., 1967). STZ is attached as a carrier molecule to the 2 carbon of glucose of methyl nitrosourea moiety and it selectively accumulate in pancreatic β-cell (Schein *et al*., 1974). The STZ targets mitochondrial DNA and impairs signallingfunction . STZ is a Nitric Oxide (NO) donor and NO was found to bring about the destruction of pancreatic islet cells, and it was proposed that this molecule contributes to STZ-induced DNA damage (Lenzen, 2008; Schnedl *et al*., 1994). The participation of NO in the cytotoxic effect of STZ was confirmed in several experiments (Thulesen *et al*., 1997; Wang and Gleichmann, 1995) (Fig. 2).

When DN is induced through the STZ, there is no such reference standard available to compare the neuropathic complication. This is difficult study and judgement of the extent to which neuropathy is caused by STZ could not be determined, Hence here c-peptide is used which is comparatively simpler.

Alloxan (AXN) induce type 1 diabetes: AXN (2,4,5,6-pyrimidinetetrone) was originally isolated in 1818 by Brugnatelli and was named in 1838 by Wöhler and Liebig. AXN induces diabetes by inhibition of the glukokinase and glucose sensor of β-cell and further produce the necrosis of the β-cell. The main mechanism of AXN is in presence of intracellular thiol, especially glutathione which generates reactive oxygen species in a cyclic reaction. During each redox cycle a small amount of "compound 305", is formed which is an AXN-GSH adduct that is non toxic (Karunanayake *et al*., 1976; Kroncke *et al*., 1995). Several experimental studies have demonstrated

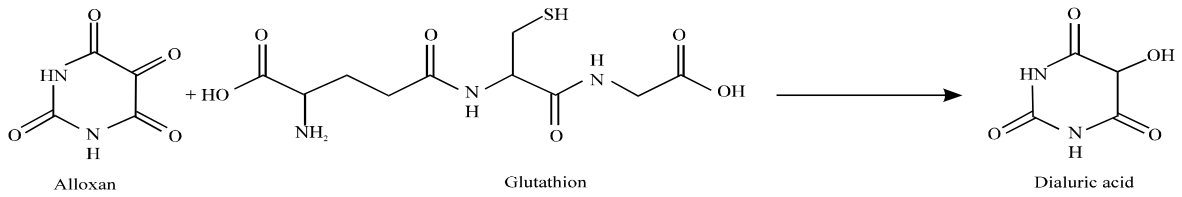

Fig. 3: Synthesis of Dialuric acid from Alloxan

that AXN evokes a sudden rise in insulin secretion in the presence or absence of glucose which appeared just after AXN treatment (Morgan *et al.*, 1994; Turk *et al.*, 1993). AXN exhibits a high affinity to the SH-containing cellular compounds. Reduced glutathione (GSH), cysteine and protein-bound sulfhydryl groups (including SH containing enzymes) are very susceptible to its action (Chueh and Lin, 2012) (Fig. 3).

When AXN reacts with cytosolicglutathione there is formation of dialuric acid. The auto oxidation then results in formation of superoxide radical and hydrogen peroxide. This diluric acid is further iron catalysed reaction and there is formation of OH radical. And this hydroxyl radical is responsible for the death of β-cell. The fragmentation of DNA takes place in the beta cells exposed to AXN that causes DNA damage, which stimulates poly ADP-ribosylation, a process participating in DNA repair. Antioxidants like superoxidedismutase, catalase and the nonenzymatic scavengers of hydroxyl radicals have been found to protect against AXN toxicity (Weaver *et al.*, 1979). In this model it is very difficult to achieve neuropathy because of short half life of AXN. And also AXN causes very rapid destruction of b-cell. The half life of AXN could be increased with the help of an antioxidant which ultimately leads to DN. This DN is then compared with the c-peptide treated animal.

CURRENT STATUS OF DIABETIC NEUROPATHY MODEL

Rats with STZ-induced selective pancreatic islet b-cell injury provide a well-known STZ-hyperglycemic (STZ-HG) rat model of over type 1 diabetes which is also widely used in pre-clinical studies of diabetic neuropathy (Gorus *et al.*, 1982; Szkudelski *et al.*, 1998). The Non-obese Diabetic (NOD) mouse is a genetic model that closely resembles the human type 1 diabetes, involving CD4+ and CD8+ T cell-dependent autoimmune destruction of pancreatic β-cells (Lachin and Reza, 2012). The NOD mice also produce the neuropathic complication. The most extensively used *in vitro* models are primary culture of dorsal root ganglia neurons and the neuroblastoma cell line. Here, the advantages and disadvantages of *in vitro* systems used as models of DN was critically reviewed. This summarizes such *in vitro* models and their effectiveness in the study of DN (Lenzen and Munday, 1991).

LIMITATION AND POSSIBLE MODIFICATION IN DIABETIC NEUROPATHY MODEL

The DN model does not produce exact condition as human neuropathy. There is no model for reference of DN for comparison purpose. It has been difficult to characterize spontaneous pain in these models, the observed allodynia and hyperalgesia have been considered as acceptable signs of neuropathic pain (Ebelt *et al.*, 2000). A barrier in understanding diabetes-specific factors in cardiovascular disease is the lack of a good rodent model, though a well-coordinated effort in this area may lead to success. Nonetheless, rodent models have their limitations because they inadequately repeat the human condition and have not proven to be useful pre-clinical guides for

Table 1: Level of c-peptide in various diabetic conditions

Type	Level of c-peptide
IDDM (Insulin Dependent Diabetes Mellitus)	Decrese
NIDDM (Non Insulin Dependent Diabetes Mellitus)	Increse
Insulinoma	Drastically incresed
Exogenous insulin	Decrese

drug development. Now the role of C-peptide is clear. The deficiency of c-peptide causes a neuropathic problem. To induce diabetes in animal usually chemical agents such as STZ and AXN are used. They cause necrosis of pancreatic b-cell and which leads totype1 diabetes. In this condition the c-peptide is not produced which facilitate the diabetic neuropathy. External administration of c-peptide, with these chemical agentsis considered as reference group.

Nowadays the outbred animal model which represents human genetics which are much better, may be used over the inbred rodent. The model with multiple aspect of the diabetic condition, rather than reductionist approaches, may produce more accurate model and thus useful for understanding complication with multiple pathogenic mechanism such as diabetic neuropathy.

The existence of large animal models for diabetes is reported but need further development for testing of DN. But the major disadvantage is the high cost of animal model mentioned above and the requirement of sophisticated labs and techniques to carry out experiment on these models. Hence AXN and STZ with c-peptide which induce diabetes are used as reference standard.

MECHANISTIC APPROCH IN DEVLOPMENT OF DIABETIC NEUROPATHY MODEL

Mechanism of c-peptide in neuropathy: The half life of c-peptide is more as compare to insulin. So the c-peptide is mainly used as the diagnostic purpose for production of insulin by **β**-cell (Table 1).

In healthy person equal amount of insulin and c-peptide is produced by **β**-cell. In IDDM the deficiency of insulin as well as c-peptide occurs. The biological action of c-peptide is now clear.It stimulates the Na^+K^+ ATPase and regulation of NO release. When there is deficiency of c-peptide the regulation and production of nitric oxide gets disturbed. Due to this the microngiopathy is produced. And this microangiopathy get differentiated into the retinopathy, neuropathy, nephropathy. This microcirculatory differences produce the complication of neuron is called DN. Now the synthetic C-peptide is injected to a diabetic patient. C-peptide normalizes the immediate early gene response and the expression of neurotrophic factors, their receptors, tubulin, and neurofilaments in dorsal root ganglia neurons, resulting in normalization of axonalcalibre growth and improvement of the elongation of regenerating fibres (Kontinen and Meert, 2003). It has been Recently published that human c-peptide increases nutritive capillary blood flow in type 1 diabetes patients, i.e., patients deficient in proinsulin c-peptide (Sima, 2004). C-peptide binds to a membrane structure, most likely a G-protein coupled membrane receptor, eliciting a rise in intracellular Ca^{2+} concentration and subsequent activation of at least two enzyme systems, Na^+K^+ ATPase and endothelial nitric oxide synthase (eNOS). Both enzyme systems are essential for the normal function of cells and are known to be deficient in diabetes. Na^+K^+ ATPase maintains normal cellular energy status, electrolyte concentrations, and fluid balance. Nitric oxide, its formation being stimulated by eNOS in cells in the blood vessel walls, is of crucial importance for normal blood flow regulation and vascular homeostasis in tissues. It is hypothesized that C peptide replacement in type 1 diabetes will serve to improve energy status and electrolyte balance (Na^+K^+ ATPase effects) and improve blood flow in critical tissues (eNOS effects), thereby preventing or

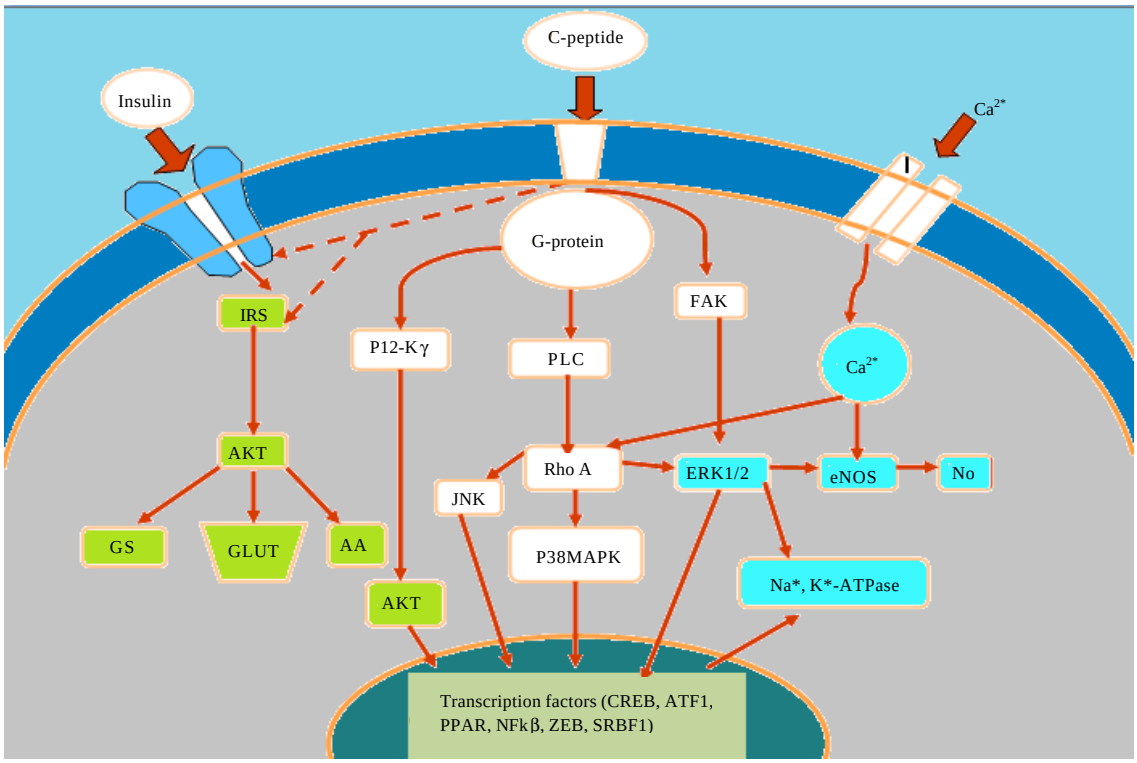

Fig. 4: Molecular mechanism of C-peptide

retarding the development of long-term microvascular complications. C-peptide elicits concentration-dependent stimulation of Na_+ K_+ ATPase activity in a variety of tissues including renal tubular cells, rat sciatic nerve, pancreatic islets, granulation tissue and red blood cells (Forst *et al*., 1998; Sima, 2001; Wahren *et al*., 2000; Forst *et al*., 2000) (Fig. 4).

Animal model with c-peptide: When the c-peptide is injected in diabetic rat prolonged study can be carried out which will focus on the DN problem. In STZ and AXN induced diabetic rat there is rapid degradation of β-cell and animal die in short period. When any antioxidant is given with the inducer with addition of c-peptide mainly treats the DN. The c-peptide treated rat is considered as the reference as compare to the reference substance or drug. In vivo studies in animal models of type 1 diabetes have established that C-peptide administration results in significant improvements in nerve and kidney function. Thus, in animals with early signs of diabetes-induced neuropathy, C-peptide treatment in replacement dosage results in improved peripheral nerve function, as evidenced by increased nerve conduction velocity, increased nerve Na^+K^+ ATPase activity and significant amelioration of nerve structural changes (Turk *et al*., 1993). C-peptide administration in animals that had C-peptide deficiency (type 1 model) with nephropathy improves renal function and structure; it decreases urinary albumin excretion and prevents or decreases diabetes-induced glomerular changes secondary to mesangial matrix expansion (Sima *et al*., 2001; Samnega *et al*., 2001; Samnegard *et al*., 2005; Nordquist *et al*., 2009). C-peptide also has been reported to have anti-inflammatory effects as well as it aids in repair of smooth muscle cells (Mughal *et al*., 2010; Nordquist and Wahren, 2009).

CONCLUSION

C-peptide treated rat may be used as the reference model for developing new drug in DN conditions. The high mortality rate of diabetes related to DN can be overcome by considering the aforesaid discussion. Importance of C-peptide in diabetic model development will provide better treatment patterns in DN studies. The difficulty in development of best, optimized, scalable animal model to DN could be cleaved by C-peptide consideration. All this study can be carried out easily with economic benefits.

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