Effects of C-Peptide and / or Insulin Administration on Motor Nerve Conduction Velocity in Diabetic Rats

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Abstract: Objective: The present investigation was done to study the effects of exogenous administration of c-peptide and / or insulin on motor nerve dysfunction in experimentally induced diabetic rats. Methods: Rats were rendered diabetic by intraperitoneal injection of streptozotocin (60 mg/kg body weight). Diabetic rat groups were subjected to exogenous injection of insulin either alone or in combination with c-peptide for 6 weeks. Fasting blood samples were collected from all rat groups at 15 days intervals for monitoring the blood glucose level. At the end of the experiment, dissection of sciatic nerve - gastrocnemius muscle unite preparation was performed in control and treated rats (5 rats / group) to measure motor nerve conduction velocity. Results: revealed that the treated diabetic rats with c-peptide combined with insulin was associated with an obvious improvement in motor nerve conduction velocity of affected rats as compared with their injection alone. In Conclusion: the injectable combination between c-peptide and insulin could be considered as an prophylactic tool to prevent or minimize the incidence of motor nerve dysfunction usually associated with diabetic patients that needs a further clinical investigations.

Key words: Diabetes • Insulin • C-Peptide • Nerve Conduction Velocity

INTRODUCTION

Diabetes is a disease of increasing worldwide public health importance. There are two general types of diabetes mellitus; Type-I and type - II diabetes [1]. Diabetic polyneuropathy (DPN), the most common microvascular complication of diabetes mellitus, is a group of disorders that affects both types of diabetic patients [2]. However, it was noticed that it could progresses more rapidly with type-I diabetes than in type-II diabetes [3,4].

C-peptide is a 31 amino acid residue peptide, which originates from the mid-portion of proinsulin, corresponding to the segment between the insulin A- and B-chains[5]. Several studies on c-peptide-deficient diabetic patients and rats have provided evidence of physiological effects of c-peptide, suggesting that c-peptide deficiency might contribute to the development of late diabetic complications [6].

C-peptide replacement of type -I diabetic rats was found to prevent acute and chronic metabolic, functional and structural changes that separate Type-I diabetic polyneuropathy from its type - II counterpart suggesting that c-peptide deficiency plays a pathogenic role in type -I diabetic polyneuropathy and it was found that if the peptide is given within one week after the onset of Diabetes, it prevents the development of nerve conduction velocity (NCV) deficits and completely prevents thermal hyperalgesia as well as degeneration and loss of unmyelinated fibers [7-9].

The present study was aimed to clarify the effects of exogenous administration of c-peptide and / or insulin on motor nerve conduction Velocity in experimentally induced diabetic male rats.

MATERIALS AND METHODS

Animals: This experiment was conducted in the Department of Biology (Physiology Laboratory), University College, Umm Al-Qura University, Makkah, saudia Arabia during the period of September 2012 to June 2013. Fifty Sprague-Dawley male rats of average weight 200 g were used. Diabetes was induced...
by intraperitoneal injection (IP) of 60 mg/kg body weight of streptozotocin. Diabetes was verified by testing blood samples for hyperglycemia (>300 mg/dl), 48 h postinduction. Forty rats having blood glucose level exceeding 300 mg/dl were considered as diabetic rats. The institutional ethical committee approved the research procedures used in this study. Experimental rats were divided into the following groups:

- Group I / control group / Non-Diabetic rats : (n=10) they were daily injected subcutaneously (SC) with 0.1 ml of isotonic saline and twice weekly with IP injection of 1 ml isotonic saline for 6 weeks.
- Group II / diabetic non – treated rats: (n=10) they were daily injected (SC) with 0.1 ml of isotonic saline and twice weekly with IP injection of 1 ml of isotonic saline for 6 weeks.
- Group III / diabetic rats treated with insulin: (n=10) they were daily injected SC with 2U insulin (Mixtard, Novo Nordisk) for 6 weeks.
- Group IV / diabetic rats treated with c-peptide: (n=10) They were injected IP twice weekly with 20 ng/kg body weight of c-peptide (Monobindinc. Lake Forest, USA) for 6 weeks.
- Group V / diabetic rats treated with insulin and c-peptide: (n=10) they were injected with c-peptide and insulin as in insulin group and c-peptide group for 6 weeks.

**Sampling and Techniques:** Individual fasting blood samples were collected at 9 am from rats of all experimental groups under ether anesthesia every 15 days by orbital sinus puncture. Measurement of fasting plasma glucose level was performed by using Accu-Check meter, Roche Diagnostics, GmbH, Germany, for monitoring of diabetic condition. At the end of experimental time, dissection of sciatic nerve - gastrocnemius muscle unite preparation and the motor nerve conduction velocity (NCV) test were performed for 5 rats / group as described previously [10]. The animals were anesthetized with 30/2.5 mg/kg ketamine /xylazine to prevent discomfort. Body temperature was monitored with a dermal temperature probe and maintained at 32°C with a warming lamp during NCV. Body temperature was maintained at 37°C after NCV using a warming pad to ease animal stress from anesthetic. The nerve studies last less than 30 min per rat. The electrodes are cleaned with 70% alcohol between animals to maintain pathogen-free status.

**Statistical Analysis:** Data were analyzed using "ANOVA" test to analyze the differences among groups using general linear model procedure (SAS). Level of significance used in all results was (p< 0.05).

**RESULTS**

Data illustrated in Fig. 1 and 2 indicate that blood glucose concentrations were significantly elevated all over the experimental period in untreated rats as compared with control rats (p < 0.05). However, blood glucose level in rats treated with either c-peptide or insulin alone or in combination was significantly decreased comparing with those of untreated rats at 4th and 6th weeks of experiment (p< 0.05). Motor nerve conduction velocity was significantly decreased in untreated rats as compared with those of control one (p< 0.05). Moreover, motor nerve conduction velocity of rats treated with either c-peptide or insulin or both was significantly increased comparable with those of untreated rats (p<0.05). However, combination treatment with c-peptide and insulin was associated with higher significant improving effect for the motor nerve conduction velocity when compared with those treated with insulin alone (p<0.05).

![Fig. 1: Effect of insulin and / or c-peptide administration on blood glucose level (mg/dl) in streptozotocin induced diabetic rats.](image1)

![Fig. 2: Effect of insulin and / or c-peptide on motor nerve conduction velocity (m/s) of sciatic nerve of control and diabetic rats after 6 weeks of diabetes induction.](image2)
DISCUSSION

Data revealed that blood glucose level was decreased in all treated groups, either diabetic treated groups or subgroups of untreated diabetic group across experimental periods. The elevated blood glucose level in such diabetic rats may be due to the effect of streptozotocin which selectively damage of pancreatic β-cells resulting in hyperglycemia [11,12]. However, insulin treatment was associated with reduction in blood glucose level in streptozotocin diabetic rats which may be attributed to the regulatory role of insulin in glucose homeostasis in the body through suppressing hepatic glucose production and accelerating glucose utilization in liver and peripheral tissues, resulting in decreased plasma glucose level [13,14].

The obtained results indicated that c-peptide treatment, either alone or combined with insulin was associated with a reduction in blood glucose level. These may be attributed to the stimulatory effect of c-peptide on glucose utilization through stimulation skeletal muscle glucose transport by an unknown mechanism which is cAMP-inhibitable and not dependent on the insulin receptors or the activation of tyrosine kinase or Phosphoinositide 3-kinases (PI3-kinase) activation in diabetes [15,16] or may be due to the capability of c-peptide of stimulating 3-O-methyl glucose transport in incubated human muscle strips in a level dependent manner [17]. Moreover, the capability of c-peptide on phosphorylating the insulin receptors and insulin receptor substrate 1 (IRS-1) and inhibits protein tyrosin phosphorylation in L6 myoblasts may be also further included [18].

Additionally, c-peptide treatment was found to induce a marked improvements in nodal, paranodal and axonal structural changes and increased repair activity. c-peptide was found to mimic insulin effects in myoblasts and neuroblastoma cells by increasing autophosphorylation of the insulin receptor, stimulation of phosphoinositide 3-kinase (PI3-kinase) and phosphorylation of MAP-kinase [19].

Further more, c-peptide administration was found to elicit a normalization of the early gene response in the injured sciatic nerve and partially corrects the expression of neurotrophic factors or of dorsal root ganglia neuroskeletal proteins [20]. Also, human c-peptide administration in diabetic rats prevented the decrease in caudal motor nerve conduction velocity (MNCV) and Na⁺-K⁺-ATPase activity in the sciatic nerve and improved sciatic nerve conduction velocity [21].

Additionally, Many authors have suggested that c-peptide deficiency plays a pathogenic role in type 1 diabetic polyneuropathy and it was found that if the peptide is given within one week after the onset of diabetes, it prevents the development of nerve conduction velocity (NCV) deficits and completely prevents thermal hyperalgesia as well as degeneration and loss of unmyelinated fibers [7-9]. The obtained results agree well with such suggestion.

Conclusively, c-peptide administration, either alone or in combination with insulin for the diabetic patients could be considered a promising approach for minimizing the possible risk of nerve dysfunction commonly associated with diabetes that needs a further clinical investigations.

REFERENCES


