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Dipeptidyl Peptidase-IV (DPP-IV) Inhibitory Activity of Parotid Exudate of *Bufo melanostictus*

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ABSTRACT

Type 2 diabetes arises as a result of ß-cell failure combined with concomitant insulin resistance. Glucagon-like peptide-1 is a gastrointestinal hormone that is released postprandially from the L cells of the gut and exerts a glucose- dependent and direct insulinotropic effect on the pancreatic ß cell. Which activate adenylate cyclase and enhances insulin secretion. GLP-1 is rapidly degraded by DPP-IV to GLP-1(9-37) amide following release from gut L cells. GLP-1 directly enhances glucose-dependent insulin secretion via an increase in ß-cell cAMP. Dipeptidyl peptidase IV (DPP-IV) is a plasma membrane glycoprotein ectopeptidase. In mammals, DPP-IV was widely expressed on the surface of endothelial and epithelial cells and highest levels in humans have been reported to occur in the intestine, bone marrow and kidney. Inhibiting DPP-IV reduces its rapid degradation of GLP-1, increasing circulating levels of the active hormone *in vivo* and prolonging its beneficial effects. The IC_{50} value of parotid exudate was found to be 9.4 µg/ml. The maximum % inhibition (61.8) was showed at a concentration of 12µg/ml. Parotid exudate through inhibition of DPP-IV, improves glucose tolerance and enhances insulin secretion. DPP-IV inhibitors are a novel class of oral hypoglycemic agents with a potential to improve pancreatic beta cell function and the clinical course of type 2 diabetes.

KEY WORDS: DPP-IV, GLP-1, parotid exudate, type 2 diabetes.

INTRODUCTION

Type 2 diabetes is characterized by peripheral insulin resistance and progressive failure of pancreatic ß cell function that leads to inadequate insulin secretion (1). Glucagon-like peptide-1 (GLP-1) is a gastrointestinal hormone that is released postprandially from the L cells of the gut (2) and exerts a glucose- dependent and direct insulinotropic effect on the pancreatic ß cell. It acts via specific receptors, which activate adenylate cyclase and enhances insulin secretion (3). It also enhances glucose uptake in peripheral tissues (4), control of gastric emptying, antroduodenal motility (5) and gastric acid secretion (6). These combined effects improve glucose tolerance and are the rationale for evaluating the peptide's therapeutic potential in the treatment of diabetes mellitus (7).Therefore GLP-1(7-37) is actively being evaluated as a therapy for diabetes mellitus. Its exogenous administration to nondiabetic and type 2 diabetic subjects results in lowering blood glucose (8). GLP-1 is rapidly metabolized by the enzyme dipeptidyl peptidase- IV (DPP-IV) to release the N-terminaldipeptide Tyr1-Ala2, giving rise to the major degradation fragment GLP-1 (9-37) (9). This N-terminally truncated peptide lacks biological activity and possibly serves as a GIP receptor antagonist *in vivo* (10). Inhibiting DPP-IV reduces its rapid degradation of GLP-1, increasing circulating levels of the active hormone *in vivo* and prolonging its beneficial effects.

The venom secretions of the parotid gland of the *Bufo* species are known to posses several bioactive compounds and have been used as folk medicine by

Chinese and Japanese physicians for centuries (11). The glandular secretions are known to be secreting a variety of compounds which are species specific (12).The skin secretions and cutaneous glands of amphibians were shown to contain several bioactive peptides with significant pharmacological actions on smooth muscle as well as an cardiac muscle (13). Recently, a derivative of the nonmammalian peptide, exendin-4 has been act as a specific and competitive antagonist at the GLP-1 receptor (14). It was isolated from the venom found in the saliva of a poisonous lizard *Heloderma suspectum*. Exendin-4 shows 53 % sequence homology to GLP-1 but is resistant to the actions of DPP-IV and it has much longer plasma half-life than GLP-1.In view of these findings, DPP-IV inhibitors are considered to be a novel class of potential drugs for the treatment of type 2 diabetes. Therefore, we attempt to evaluate the DPP-IV inhibitory activity of parotid exudate of Indian Toad, *Bufo melanostictus*.

MATERIALS AND METHOD

Materials: Dipeptidyl peptidases-IV (enzyme) and glycine - proline P- nitroanilide (substrate) were purchased from Sigma, St. Louis, MO, USA. Tris HCL and phosphate buffers (pH7.6) were purchased from E. Merck Ltd, Mumbai, India.

Animals: The toad *Bufo melanostictus* Schneider collected from the vicinity of Kakatiya University, Warangal, and Andhra Pradesh. It was identified and authenticated by Prof. Lakshimipathi, Department of Zoology, Kakatiya University, Warangal. Parotid glands were gently pressed with the help of forceps to stimulate the release of secretions from the gland by an adopted method (15). The average amount of secretions (100 mg / 600gm body weight of frog) obtained from a single frog. These secretions were collected in ice jacketed containers, weighed and stored at -80^{0}C until analysis. This secretion collection method has been cited in many publications in the past.

DPP-IV inhibitory method: This assay method is based on ELISA for the determination of product formed by penultimate proline cleaving activity of the enzyme (16).

Enzyme + Substrate ⟶ *Gly- Proline + P-Nitroanilide (Dipeptidyl peptidase- IV) (Gly-Pro-pNA)*

Incubation of the enzyme dipeptidyl peptidase-IV (280 µl) with parotid exudate (different conc of 2, 4, 6,8 and 10 µg/ml in 5% DMSO) at 30^{o}C followed by addition of this reaction mixture to the substrate Gly-pro-pNA (90µl) that was equilibrated at 30^{o}C for 2 min. The enzyme cleaves the substrate penultimate proline and releases p- nitroanilide will be reduced in the presence of inhibitor. Optical density was measured for 2 hours at different time interval 30, 60, 90 and 120 min at 385nm. The activity of molecule expressed in terms of % inhibition.

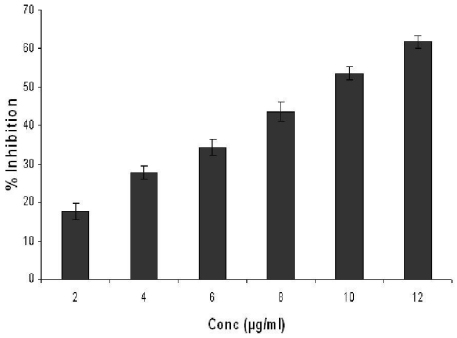
% Inhibition == {(1- Vi/Vo)} *100

Where Vi and Vo are values with and without inhibitor respectively.

RESULTS

Picture of Bufo *melanostictus* frog species was showed in figure 1. IC_{50} value of parotid exudate shows a dose dependant inhibition on dipeptidyl peptidase-IV represented in figure 2. The IC_{50} value of parotid exudate was found to be 9.4 µg/ml. The maximum % inhibition (61.8) was showed at a concentration of 12µg/ml.

Figure 1: Picture of Bufo melanostictus frog species.



Values expressed as mean ± SD (n = 6)

Figure 2: Effect of parotid exudate Indian toad (bufo melanostictus) on DPP-IV Inhibition.

DISCUSSION

Some potent DPP-IV inhibitors reported are dipeptide boronic acid derivatives (IC_{50} = 15 nM). These proline boronic acid derivatives are reversible, slow-binding inhibitors; however, they display poor stability in weakly basic buffer due to an intramolecular

cyclization between the N-terminal amino group and the boronic acid (17). The cyclized compounds are inactive *in vitro* but are active *in vivo* because the cyclization is reversible in acidic conditions. The other DPP-IV inhibitor FE-999011 suppresses plasma DPP-IV activity for 12 hours after a single oral dose (18). Chronic treatment with this compound in the Zucker diabetic fatty (ZDF) rat postponed the development of diabetes with a twice-daily dose delaying the onset of hyperglycemia by 19 days. These results suggest that this compound may be useful in preventing the progression from impaired glucose tolerance to Type II diabetes. In our present study, IC_{50} value of parotid exudate was found to be 9.4 µg/ml.

The tryptophan derivative TSL -225 derived from a natural product lead, shows only moderate activity (IC_{50} = 5.7µM) (19). Another cyclic peptide has been reported as an irreversible DPP-IV inhibitor with good potency (IC_{50} = 3 nM) and this inhibition lasts for several hours (20). Some other series of isoquinoline derivatives, the 4 -ethoxycarbonyl analog being the most active (IC_{50} = 0.32 µM) and fluoroolefin derivative is an irreversible inhibitor with moderate potency (Ki = 188 nM) (21) and good stability (t½ = 103 h at pH 7.6). It was reported that the fluoroolefin mimics an amide bond (22). Vildagliptin is a selective, reversible, competitive inhibitor of dipeptidyl peptidase IV enzyme (23). It has been shown to reduce HbA1c, fasting plasma glucose levels, prandial glucose levels, and prandial glucagon secretion and to improve ß-cell function (24).

CONCLUSION

Parotid exudate through inhibition of DPP-IV, improves glucose tolerance and enhances insulin secretion. The present investigation is only preliminary, which indicates that some constituents, one or more seem to have DPP-IV inhibitory activity. Further investigations are required to isolate, purify and characterize the chemical constituents of parotid exudate. DPP-IV inhibitors are a novel class of oral hypoglycemic agents with potentials in improving pancreatic beta cell function and the clinical course of type 2 diabetes.

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