EVALUATION OF ANXIOLYTIC AND ANTIDEPRESSANT ACTIVITY OF SITAGLIPTIN AND LINAGLIPTIN IN RATS

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Abstract

Glycemic control remains fundamental to the management of diabetes, especially for preventing complications. Incretins and DPP-IV inhibitors have a physiological role in glucagon and insulin secretion, insulin biosynthesis, and in modulating Beta cell mass and function. It is well known, that patients with diabetes have an increased prevalence of anxiety and depression as compared to non-diabetic population. Recent studies have reported the anxiolytic and anti-depressant nature of GLP-1 and its analogues. However, no satisfactory reports are available for the role of DPP-IV inhibitors in the same. Since DPP-IV inhibitors like Sitagliptin and Linagliptin are currently widely used as treatment interventions for Type 2 diabetes, the study aimed at investigating the anxiolytic and antidepressant activity of these drugs in animal models of anxiety and depression. Anxiolytic activity was evaluated in Light Dark Box model and Elevated Plus Maze (EPM) model, while anti-depressant activity was evaluated in Despair Swim Test model in rats. Results showed that, Sitagliptin and Linagliptin have a significant anxiolytic and antidepressant activity. Further work on chronic models can give an in-depth idea about the possible mechanistic actions of these drugs in anxiety and depression. Additionally, the study may also provide a better understanding of the pleotropic role of Incretins and DPP-IV inhibitors. This will help in designing optimum treatment strategies for the delay or prevention of complications in such metabolic disorders and in turn may lead to better compliance and effective achievement of therapeutic goals.

Keywords: Anxiety, Depression, Diabetes mellitus, DPP-IV Inhibitors, Incretins.

Introduction

Diabetes mellitus is emerging as one of the leading metabolic disorders in both developed and developing countries [1-2]. It is estimated that almost 285 million people are currently suffering from diabetes worldwide and the number
is expected to rise to 438 million by the year 2030 [3]. Glycemic control remains fundamental to the management of diabetes, especially for preventing complications [4-5]. Incretins and DPP-IV inhibitors have been an important addition to the treatment possibilities of diabetes mellitus. Incretins comprise of hormones secreted into the GIT, Glucagon like Peptide-1 (GLP-1) and Gastrointestinal Peptide (GIP). They have a physiological role in glucagon and insulin secretion, insulin biosynthesis, and possible role in increasing Beta cell mass and function [6, 7]. It has been observed through studies that apart from pancreas, GLP-1 receptors are also expressed in extra-pancreatic tissues like the gastrointestinal tract, the brain, heart, kidneys and lungs [6,7,8].There have been numerous reports of pleotropic effects of incretins on CNS, GIT, liver, Immune System and Inflammation [9,10]. Studies that have been conducted in recent years provide evidence for an impaired cerebral glucose metabolism in CNS disorders [11]. In recent years, perturbed brain glucose metabolism and decreased insulin sensitivity have been proposed as a possible link between the increased glucocorticoid action and the formation of the changes in the brain [12-13]. Studies also show that chronic unpredictable mild stress (CUMS) increases HPA axis activity which disturbs glucose and lipid metabolism and evokes insulin resistance in peripheral tissues of high fat-fed rats [15]. Hence, diabetes, anxiety and depression have shown a profound correlation according to many reports [13-17]. Studies show that people with diabetes are almost twice as likely to suffer from anxiety and depression compared to general population,[18] but this often remains unrecognized and thus untreated [19]. When these conditions co-exist, the risk of developing co-morbidities, complications, patient suffering and associated costs escalate alarmingly. In light of the above, the role of GLP-1 and DPP-IV has been hypothetised in correlation to the HPA axis and hence stress [20,21]. There are very few reports of role of GLP-agonists in anxiety and depression [22]. There are some recent reports on anxiogenic and antidepressant effects of GLP-1 [23]. Despite the correlation of Incretins and DPP-IV inhibitors, the role of DPP-IV inhibitors in stress and related disorders has not been studied significantly. Also, there are no substantial studies done for the evaluation of anxiolytic and antidepressant activities of DPP-IV inhibitors. However their role in anxiety and other psychological complications associated with diabetes needs to be evaluated further. Hence the study aims at evaluation of anxiolytic and antidepressant activity of DPP-IV inhibitors, Sitagliptin and Linagliptin in rats.

**Experimental Design**

**Animals**

Wistar albino rats of either sex, weighing 150-250 gm were procured from Zydus Research Centre Ahmedabad. They were housed in groups of six animals each and were fed on standard pellet diet and water ad libitum. Also, they
were maintained in optimum conditions of temperature and humidity, at 25±3 °C and 50±20 % humidity with 12h light/dark cycle. The experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC) and experiments were conducted according to the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). (Protocol No: LJIP/IAEC/14-15/06 )

**Drugs**

Sitagliptin and Linagliptin were procured from local sources. Alloxan monohydrate was used to induce diabetes in animals [24, 25]. Alprazolam was used as the reference standard drug for anxiolytic activity [26-28], while Sertraline was used as a reference standard for anti-depressant activity [29, 30]. Distilled water was used as a vehicle for the drugs. All the chemicals and reagents used were of Analytical Reagent (AR) grade

**Dosage and Administration**

All the solutions were freshly prepared in distilled water before initialising the experiment. Animals were divided into the following groups comprising of 6 animals in each group. Alloxan was administered Intraperitoneally to all groups except normal control, 72 hrs before evaluation of anxiolytic activity. Drugs, namely Sitagliptin, Linagliptin and Alprazolam were given orally, 1 hr prior to the initiation of the study. The doses of Sitagliptin and Linagliptin were optimised in studies performed before the study.

**Group Design**

1. **Anxiolytic Activity.**

**Table 1: Group Design for Anxiolytic activity of Sitagliptin and Linagliptin.**

<table>
<thead>
<tr>
<th>SR.NO</th>
<th>GROUP</th>
<th>TREATMENT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal Control</td>
<td>Vehicle (distilled water)</td>
</tr>
<tr>
<td>2</td>
<td>Disease Control</td>
<td>Alloxan (150 mg/kg IP)</td>
</tr>
<tr>
<td>3</td>
<td>Sitagliptin treated</td>
<td>Sitagliptin (10 mg/kg po)</td>
</tr>
<tr>
<td>4</td>
<td>Linagliptin treated</td>
<td>Linagliptin (3 mg/kg po)</td>
</tr>
<tr>
<td>5</td>
<td>Alprazolam treated</td>
<td>Alprazolam (0.3 mg/kg po)</td>
</tr>
<tr>
<td>6</td>
<td>Sitagliptin+Alprazolam</td>
<td>Sitagliptin (5 mg/kg po) + Alprazolam (0.15 mg/kg po)</td>
</tr>
<tr>
<td>7</td>
<td>Linagliptin+Alprazolam</td>
<td>Linagliptin (1.5 mg/kg po) + Alprazolam (0.15 mg/kg po)</td>
</tr>
</tbody>
</table>
2. Antidepressant Activity

Table 2: Group Design for Anti-depressant activity of Sitagliptin and Linagliptin.

<table>
<thead>
<tr>
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</tr>
<tr>
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<td>Sertraline treated</td>
<td>Sertraline (20 mg/kg po)</td>
</tr>
<tr>
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<td>Sitagliptin+ Sertraline</td>
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<td>7</td>
<td>Linagliptin+ Sertraline</td>
<td>Linagliptin (1.5 mg/kg po) + Sertraline (10 mg/kg po)</td>
</tr>
</tbody>
</table>

Animal Models

1. Light Dark Box Test [31-33]

The apparatus consisted of a Plexiglas box with two compartments (20 cm × 20 cm each), one of which was illuminated, while the other remained dark. Each animal was placed at the junction of the light dark, facing the illuminated compartment. The time spent in illuminated and dark chambers, as well as the number of entries in each space, was recorded for 5 minutes. Sitagliptin (10 mg/kg PO) and Alprazolam (0.3 mg/kg PO) were administered one hour before the study.

2. Elevated Plus Maze Test [34-36]

Based on studies, elevated plus-maze apparatus consists of two open arms, 50×10×40 cm, and two enclosed arms, 50×10×40 cm, with an open roof, arranged so that the two open arms are opposite to each other. The maze was elevated to a height of 50 cm. Drugs, Sitagliptin (10 mg/kg PO) and Alprazolam (0.3 mg/kg PO) were administered one hour before the study.

The rat was placed in the center of the maze, facing one of the enclosed arms. During a 5 min test period, the number of entries into open and enclosed arms, time spent in the open and enclosed arms, and time spent in centre of the maze were observed. An arm entry was defined as the entry of all four paws into the arm. After each test all the arms were carefully cleaned with 10% ethanol solution.
3. Porsolt’s Forced Swim Test [37]

The behavioural despair test (or Porsolt forced swimming test) is a test, centered on a rodent’s response to the threat of drowning. It is commonly used to measure the effectiveness of antidepressants. Animals are subjected to various trials during which they are forced to swim in a glass cylinder filled with water, and from which they cannot escape. The first trial lasts 15 minutes. Then, after 24-hours, a various trials are performed that lasts 5 minutes each upto 3 hrs. The time that the test animal spends without making any movements beyond those required to keep its head above water is measured. This immobility time is described as passive mobility and the time it spends moving or trying to swim is known as active immobility.

Statistical Analysis: All results were expressed as Mean± SEM or Percentage depending whether the data was parametric or non parametric. Statistical analysis was done using ANOVA followed by Tukey’s multiple comparison tests. Non parametric data was analysed using Wilcoxon’s Signed rank test. P<0.05 was considered to be significant.

Results

1. Light dark box test

The following parameters were evaluated in the light dark chamber model in rats.

A. No of Transitions (Figure 1)

B. ANXIOLYTIC ACTIVITY (Light Dark Box test)

   a. Number of Transitions

![Graph showing number of transitions vs test interval]
**a. Time spent in light chamber**

**Fig 2: Time spent in light chamber (sec) vs Test Interval.**

The number of transitions depicts the exploratory activity of rats, thus indicating the extent of anxiety induced. Results show that the number of transitions reduce significantly with time in both normal control and disease control groups, indicating the onset of anxiety ($p<0.05$). Furthermore, the number of transitions were observed to be higher in Sitagliptin and Linagliptin treated group as compared to disease control group ($p<0.01$). Results also show that the animals treated with a combination of Sitagliptin or Linagliptin with Alprazolam displayed higher number of transitions as compared to Sitagliptin, Linagliptin or Alprazolam alone ($p<0.01$).

**C. Time Spent in Light and Dark chamber (Figure 2 and 3)**

The time spent in light chamber is a direct indicator of extent of anxiety induced in rats. Lesser the time spent, higher is the anxiety induced and vice versa. Results show that the animals in the disease control group spent significantly less time in the light chamber as compared to the normal control group ($p<0.01$). Furthermore, there was a significant difference in the time durations of disease control as compared to Sitagliptin and Linagliptin treated groups ($p<0.01$). Rats treated with Sitagliptin or Linagliptin were observed to be spending more time in the light chamber.
Additionally, animals treated with the combination of Sitagliptin or Linagliptin with Alprazolam showed a higher duration of time spent in light chamber as compared to individual treatment (p<0.05). Along with the above parameters, the time spent in dark chamber is one of the important parameters which show a lack of exploratory drive and thus induction of anxiety. Results show that the disease control group shows a significantly high amount of time spent in the dark chamber as compared with other groups.(p<0.05). Additionally, the rats treated with Sitagliptin or Linagliptin spent a significantly lesser amount of time in dark chamber as compared to disease control animals (p<0.05). Furthermore, the animals treated with Sitagliptin or Linagliptin with Alprazolam showed a significant reduction in time spent in dark chamber as compared to individual treatment groups (p<0.01).

a. Time spent in dark chamber

![Graph 1: Time spent in dark chamber (sec) vs Test Interval]

![Graph 2: Time spent in dark chamber (sec) vs Time Interval]

Fig 3: Time spent in dark chamber(sec) vs Test Interval

2. Elevated Plus Maze Test

A. Time spent in the centre of maze (Figure 4)

Results show that time spent in centre of the maze was significantly low in the disease control animals as compared to treatment groups (p<0.05) indicating a significant onset of anxiety. Additionally, rats treated with Sitagliptin or
Linagliptin showed a significantly higher duration of time spent in the centre of the maze as compared to disease control group (p<0.05). Results also show that animals treated with a combination of Sitagliptin or Linagliptin with Alprazolam showed a significantly high duration of time spent in the centre as compared to individual treatment groups (p<0.01).

2. Anxiolytic Activity (Elevated Plus Maze Model)

   a. Time spent in centre of the maze

![Graph showing time spent in center of maze vs Test Interval](image)

   ![Graph showing time spent in open arms](image)

   The number of entries in open arms in the animals treated with a Sitagliptin or Linagliptin was observed to be significantly higher as compared to disease control group (p<0.01).

B. No of entries in open arms (Figure 5)

The number of entries in open arms indicates the exploratory drive in rats, which directly indicates the extent of anxiety induced in animals. Results show that the number of entries in open arms in disease control animals is significantly lower than normal control (p<0.05) as well as treatment groups (p<0.01). The number of entries in open arms in the animals treated with a Sitagliptin or Linagliptin was observed to be significantly higher as compared to disease control group (p<0.01).
C. Time spent in open arms (Figure 6)

The extent of time spent in open arms is also an indicator of exploratory drive in rats. Results show that the time spent in open arms is significantly lesser in disease control group as compared to treatment groups (p<0.01). The time spent in open arms in animals treated with Sitagliptin or Linagliptin was found to be significantly high as compared to disease control group (p<0.05). The time spent in open arms in animals treated with Sitagliptin or Linagliptin along with Alprazolam was found to be higher as compared to animals treated with Sitagliptin, Linagliptin or Alprazolam alone (p<0.05).

b. No of entries in open arms

![Graph of No of entries in open arms vs Test Interval]

**Fig 5: No of entries in open arms vs Test Interval.**

a. Time spent in open arms

![Graph of Time spent in open arms (sec) vs Test Interval]

D. Number of entries in closed arms (Figure 7)

The number of entries in closed arms indicates the extent of anxiety induced in animals. Results show that the number of entries in closed arms in disease control animals is significantly higher than normal control (p<0.05) as well as treatment groups (p<0.01). The number of entries in closed arms in the animals treated with Sitagliptin or Linagliptin were observed to be significantly lower as compared to disease control group (p<0.01).

E. Time spent in closed arms (Figure 8)

The extent of time spent in closed arms is also an indicator of anxiety or fear in rats. Higher the time spent in closed arms, more is the anxiety in animals. Results show that the time spent in closed arms is significantly higher in disease control group as compared to normal control group (p<0.01) and treatment groups (P<0.05). The time spent in closed arms in animals treated with Sitagliptin or Linagliptin was found to be significantly lesser as compared to disease control animals (p<0.05). Additionally, the time spent in closed arms in animals treated with Sitagliptin or Linagliptin along with Alprazolam was found to be significantly lesser as compared to animals treated with Sitagliptin, Linagliptin or Alprazolam alone (p<0.05).

a. Time spent in closed arms
Fig 8: Time spent in closed arms vs Test Interval.

3. Porsolt’s Forced Swim Test

A. Time of Immobility (Figure 9)

The forced swim or Despair swim test is a potential indicator of evaluating the onset and intensity of depression in rats. The time of immobility depicts the onset of depression in animals. Results show that the time of immobility is significantly higher in disease control group as compared to normal control group (p<0.01) as well as treatment groups (P<0.01). The time of immobility in animals treated with Sitagliptin or Linagliptin was found to be significantly lesser as compared to disease control animals (p<0.05). Additionally, the time of immobility in animals treated with Sitagliptin or Linagliptin along with Alprazolam was found to be significantly lesser as compared to animals treated with Sitagliptin, Linagliptin or Alprazolam alone (p<0.05).

b. Antidepressant Activity (Despair swim test)

a. Time of Immobility
B. Time Of Mobility (Figure 10)

In accordance with the time of immobility, the time of mobility also indicates the onset and extent of depression in animals. The time of immobility depicts the onset of depression in animals. Results show that the time of mobility is significantly less in disease control group as compared to normal control group (p<0.01) as well as treatment groups (P<0.01). The time of mobility in animals treated with Sitagliptin or Linagliptin was found to be significantly high as compared to disease control animals (p<0.05). Additionally, the time of mobility in animals treated with Sitagliptin or Linagliptin along with Alprazolam was found to be significantly higher as compared to animals treated with Sitagliptin, Linagliptin or Alprazolam alone (p<0.05).

a. Time of mobility
Diabetes mellitus is emerging as one of the leading metabolic disorders in both developed and developing countries. Diabetes and its complications are known to affect all organs including the CNS in the course of time. Amongst many factors like heredity, lifestyle, eating habits etc, stress is one of the common factors, responsible for the pathogenesis of diabetes. It is also now evident from studies that stress plays a pivotal role in the development of diabetic complications [38-39].

Anxiety and depression are CNS disorders which are emerging as a major cause of concern in the health care sector. DPP-IV inhibitors are a relatively recent addition in the treatment options for Type 2 diabetes. Sitagliptin and Linagliptin, DPP- IV inhibitors are known to act by stimulation of Incretin release and hence, indirect stimulation of insulin release.

Apart from their role in glycemic control, recent reports have emerged stating their possible roles in GIT, Inflammation, CNS, kidneys etc, owing to the pleotropic nature of DPP-IV receptors [7-8]. There are various reports of the role of GLP-1 and DPP-IV inhibitors in neuro-protection and neuro-inflammation [41-42]. Also, there have been recent reports of these drugs in ethanol tolerance and withdrawal studies [43-44]. There are reports of studies of these drugs in cognition and brain atrophy [45] and in neurodegenerative conditions [46]. Stress and inflammation are significantly linked with diabetes and its complications. Results of the study show that Sitagliptin and Linagliptin have a significant anxiolytic and antidepressant activity.

The locomoter and exploratory drive was significantly increased in animals treated with Sitagliptin or Linagliptin as compared to disease control animals as evident from the number of transitions. Furthermore, the time spent in light
chamber was also seen to be significantly more in animals treated with Sitagliptin and Linagliptin as compared to disease control animals.

It is worth noting that Sitagliptin, and Linagliptin, although not anxiolytic drugs, significantly alleviated conditions of anxiety in animals. Studies show that stress is characterised by an increase in brain cortisol levels [40]. These elevated levels of cortisol are reported to suppress insulin levels in the brain [13]. In accordance with the obtained results, it may be hypothetised that drugs like Sitagliptin and Linagliptin may have a pleotropic effect on the modulation of Cortisol levels.

This is possible as depleted insulin levels have a direct bearing on Cortisol levels in brain. Furthermore, results of the antidepressant study show that Sitagliptin and Linagliptin have a significant effect in reduction of depression in rats. This was evident from the decrease in time of immobility in animals treated with Sitagliptin or Linagliptin. The results further substantiate the possible role on these drugs in the modulation of HPA-axis and mediators of depression.

Further studies on chronic models of depression like Chronic Mild Stress model can provide more information into the possible mechanisms of anxiolytic and antidepressant activities of these drugs. Whether, they have any role in direct or indirect mediation of anxiety and depression has to be investigated further.

**Conclusion**

Results of the present study show that Sitagliptin and Linagliptin have a significant anxiolytic and antidepressant activity. This suggests that Sitagliptin and Linagliptin may have indigenous anxiolytic and antidepressant activities, through molecular mechanisms which are yet to be investigated.

The possible area of involvement includes the modulation of cortisol and the role of GLP-1 in the HPA-axis. Further studies need to be done to investigate the exact mechanisms of the observed activities of these drugs.

**Acknowledgements**

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