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IMMUNOMODULATORY ACTIVITY OF DIPEPTIDYLE PEPTIDASE 4 ENZYME INHIBITORS

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ABSTRACT

Objective: To assess the immunomodulatory activity of Dipeptidyl peptidase4 inhibitors(DPP-4) on cellular and humoral immunity. DPP4 inhibitors are a relatively new class of drugs used for the treatment of diabetes.DPP4 inhibitors inhibit DPP4 enzyme. DPP4 enzyme posses immunostimulatory activity .DPP4 enzyme present on T cell surface as CD26 involve in activation of T cell, B cell, natural killer cell, DNA synthesis and release of TGF β. CD26 causes T cell proliferation by binding with adenosine diaminase (ADA) through co-stimulatory mechanism. DPP4 bind to various substrates like RANATES, IL-2, SDF-1β and 1α results in immune stimulation. Several experimental studies suggested potential role of DPP4 in various inflammatory diseases. Sitagliptin is first available DPP4 inhibitor found to be associated with intestinal cancer. On basis of above evidences evaluate immunomodulatory activity of DPP4 inhibitors if significant difference were found.

Method: In this study we evaluated immunomodulatory effect of DPP4 inhibitor by 40/80 compound induced systemic anaphylaxis in mice. In addition Inflammation induced in mice by egg albumin and arthritis induced by using Papain enzyme to evaluate anti-inflammatory activity of Sitagliptin.

Result: Sitagliptin significantly reduce motality in 40/80 compound induced anaphylactic shock in mice. Sitagliptin significantly reduce inflammation in egg induced delayed type hypersensitivity reaction. Sitagliptin significantly ($p<0.01$) reduce inflammation in Papain enzyme induced arthritis inflammatory model.

Conclusion: So findings of our study provide an evidence of antiallergic and anti-inflammatory activity of Sitagliptin. Therefore all observation signifies Sitagliptin posses immnosupressive effect. However further studies needed to clarify mechanism of immune suppression by Sitagliptin.

Key words-Dipeptidyle peptidase 4 inhibitor,Systemic anaphylaxis, Egg albumin induced inflammation, Papain induced arthritis

INTRODUCTION

Dipeptidyle peptidase 4 (DPP4) inhibitors are novel modality for treatment of type 2 diabetes mellitus.It plays crucial role in preventing breakdown of two gut hormones glucagon like peptide (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) known as incretins by Dipeptidyle peptidase enzyme [1]. This result in increase insulin release and reduce glucagon [2].Available DPP4 inhibitors include Sitagliptin, Vildagliptin and Sexagliptin etc. Several DPP4 inhibitors are in clinical development.

Dipeptidyle peptidase 4 enzyme (DPP4) is 766 glycosylated multifunctional and widely distributed serine protease act by cleavage of N terminal dipeptide after proline containing peptide. DPP4 is the enzyme normally present in liver hepatocytes, kidney and intestine[3]. Dipeptidyle peptidase 4 enzyme popularly known for its action on incretins (GLP and GIP) (also known as incretin effect) beside this DPP4 has diverse multifunction in immune system. DPP4 causes T cell proliferation by binding with ADA through co-stimulatory mechanism [4]. It is not only activator of T cell but also activate B cell [5] and natural killer cells. DPP4 involve in DNA synthesis and regulation of TGF-β. TGF β is transforming growth factor which is responsible for regulation and production of immune cells. Therefore release of TGFβ might cause immune suppression [6]. DPP4 causes activation of chemokines. It binds with various substrate e.g. RANATES, IL-2, SDF1α and 1β results in activation of immune system [7]. Several *Invivo* studies shows role of DPP4 in inflammatory disorders e.g. Depression [8], Rheumatoid arthritis[9], Multiple sclerosis[10], Angiodema[11] and Inflammatory bowel syndrome[12]. One of study evident DPP4 inhibitors also plays pivotal role in increasing immune suppression in allograft rejection [13] and decrease expression of DPP4 seen in ovarian cancer. Sitagliptin is first available DPP4 inhibitor use in treatment of type2 Diabetes mellitus. Sitagliptin may contribute to pancreatic cancer. Sitagliptin being DPP4 inhibitor causes cleavage of GLP2. GLP2 has proliferative effect on intestinal cell. Therefore DPP4 inhibition exerted tumor promoting effect on intestinal cancer. Thus, DPP4 shows a variety of functions including regulation of inflammatory, immunological response, signal transduction, and apoptosis. On prolong inhibition of DPP4 enzyme by DPP4 inhibitors may result in immune suppression as shown in figure-1.

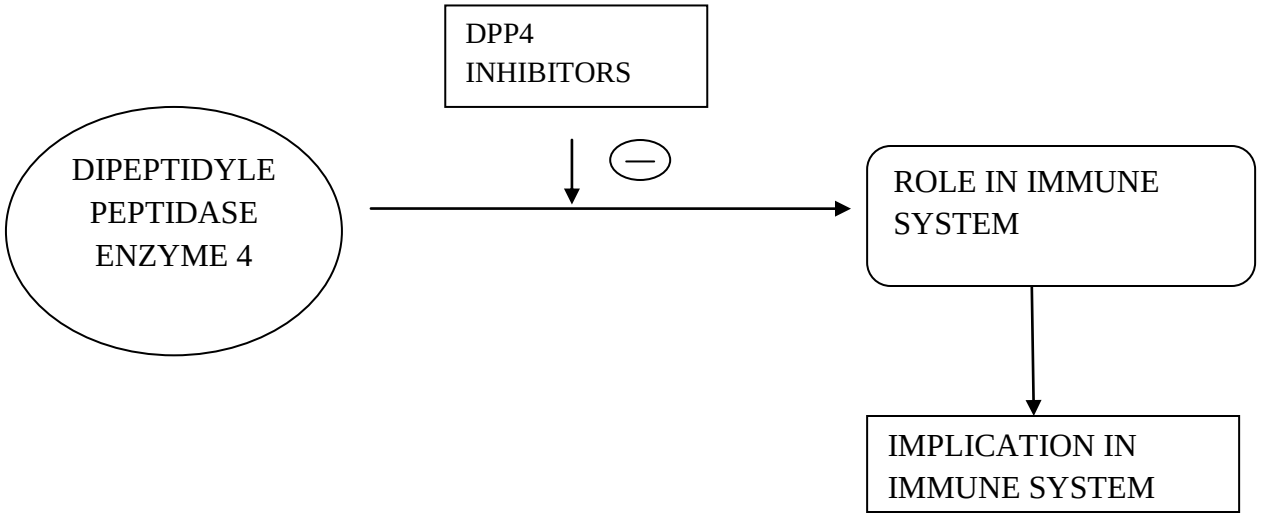

Fig-1 immune suppression by DPP4 inhibitors

Experiment with DPP4 inhibitors Sitagliptin indicate immune suppressive activity of DPP4 inhibitors

2. Meterials and Method

2.1. Drug and Reagents

Papain enzyme, Compound 40/80,egg albumin were purchased from Sigma etc. Sitagliptin were purchased from M.S.D Pharmaceuticals Pvt. Limited and Mycophenolate mofetil were purchased from Cipla Pharmaceuticals, Roorkee.

2.2. Animals

The original stock of male Swiss-albino mice (4 weeks in age and 25–35 g in weight) were taken from animal house of Shri Guru Ram Rai of Technology and Sciences. The animals were housed 4 per cage in a laminar air flow room maintained under a temperature of 22±2 °C throughout the study. Care and treatment of the mice were approved by the Committee for Animal Experimentation, Department of Pharmacy, Shri Guru Ram Rai Institute of Technology and Sciences, Uttarakhand Technical University. The animals were divided into four groups of four animals each.

2.3. Inflammation induced by egg albumin

The study was carried out using mice adopting the method of (Winter *et al.,* 1962)[14]. Increase in the mice hind paw linear circumference induced by sub-plantar injection of a phlogistic agent (egg albumin) was used as the measure of acute inflammation. Adult swiss albino mice of either sex were used for this study. They were fasted for 24h before use and were only deprived of water during experiment. Animals were divided into 4 groups. Each consists of 4 animals. Group1 served as control group and treated with only egg albumin. Animals in Group2, Group3 and Group4 received standard treatment (Mycophenolate mofetil 100mg/kg), vehicle (Distill water) and test drug (Sitagliptin 200mg/kg) intraperitonally (i.p.) for two weeks respectively. After two weeks of post treatment standard, test and vehicle in mice, each animal received 0.1ml of undiluted fresh egg albumin i.p in subplanter region of hind paw. The linear paw circumference was then measured using the cotton thread method of Bamgbose and Noamesi (1981). Linear paw circumferences of rats were determined just before injection of the phlogistic agent and at 30 min interval for 4 h.

Fig-2 edema induced by egg albumin

Inhibition (%) = [(Increase in paw oedema, control - Increase in paw oedema, treated) ÷ Increase in paw oedema, control] × 100

2.4. Papain induced arthritis

Protocol was taken from method[15] .A new model employing latex of papaya as an inflammagen has been develop for inducing rheumatoid arthritis. Group1 served as control group and treated with only papain enzyme. Animals of Group 2, 3 and 4 received standard (Mycophenolate mofetil 200mg/kg), vehicle (distill water) and test (Sitagliptin 200mg/kg) respectively for two weeks. Animals were fasted overnight. After post treatment of standard, test and vehicle, arthritis induced by using papain enzyme in right hind paw of mice. Papain enzyme (1µM) prepared with 0.05M of sodium acetate buffer .0.1ml of this suspension was injected in rat hind paw produce concentration dependent inflammation. Edema was assessed for difference in paw oedema between control and 1,2,3,4 and 5hr after administration of papain enzyme by measuring paw circumference.

2.5. Compound 48/80-Induced Systemic Anaphylaxis

Mice were given an intraperitoneal injection of 8 mg/kg body weight (BW) of the mast cell degranulator, compound 48/80.Mycophenolate mofetil administered by I.P. route at a dose of 100 mg/kg and Sitagliptin was administered I.P at doses of 200mg/kg for two weeks before the compound 48/80 injection (n=10/ group). Mortality was monitored for 1 h after induction of anaphylactic shock.

2.6 .Statistical analysis

The data are presented as the mean±S.E.M. Statistical significance was tested by a two-way analysis of variance (ANOVA) followed by Dunnett's test in delayed type hypersensitivity and papain induced arthritis. Statistical significance was tested by a one-way analysis of variance (ANOVA) followed by Dunnett's test in systemic anaphylaxis induced by compound 48/80. A probability value of less than 0.05 is considered to be significant.

3. Result

3.1. Effect of Sitagliptin on Egg Albumin Induced Delayed Type Hypersensitivity

Sitagliptin showed significant ($P< 0.05$) anti-inflammatory activity against acute inflammation.
(Table 2) (Graph 4). It suppressed increase in the mice paw edema caused by egg albumin. Sitagliptin 200mg/kg i.p and standard Mycophenolate mofetil 100mg/kg i.p was administered for two weeks before inducing arthritis. The inhibition by the Sitagliptin was maximal after 4hours of administration of phlogistic agent. The effect which was significant when compared to control was comparable to that of the standard drug,Mycophenolate mofetil

TREATMENT	DOSE	After 00minute	After 30minute	After 60minute	After 90minute	After 120minute	After 150minute	After 180minute	After 210minute	After 240minute
Inflammatory control	-	1.768±0.02	1.818±0.01	2.11±0.06	2.42±0.19	2.90±0.23	3.33±0.23	2.42±0.19	2.90±0.23	3.33±0.23
Distill water	10ml/kg	1.76±0.02 **(NI)**	1.812±0.02 **(NI)**	2.10±0.02 **(NI)**	2.40±0.02 (0.82%)	2.89±0.02 (0.33%)	3.30±0.01 (0.90%)	2.40±0.06 (0.82%)	2.87±0.06a (1.03%)	3.00±00 (9.90%)
Mycophenolate mofetil	100mg/kg	1.75±0.02 **(NI)**	1.78±0.02 (1.65%)	1.72±0.01 (18.48%)	1.62±0.03 (33.05%)	1.56±0.04^{a*,b*} (46.20%)	1.37±0.16^{a****b***} (58.85%)	0.91±0.18^{a****b***} (62.39%)	0.62±0.1^{a****b****} (78.62%)	0.45±0.05^{a****b***} (86.48%)
Sitagliptin treated	200mg/kg	1.76±0.02 **(NI)**	1.79±0.01 (1.1%)	1.82±0.02 (13.74%)	1.66±0.06 (31.40%)	1.49±0.04^{a**b**} (48.62%)	1.25±0.13^{a****b***} (62.46%)	1.09±0.14^{a****b***} (54.95%)	0.75±0.08^{a****b***} (74.13%)	0.50±0.02^{a****b***} (84.98%)

Table-1- Effect of Sitagliptin on Egg Albumin Induced Inflammation in mice.

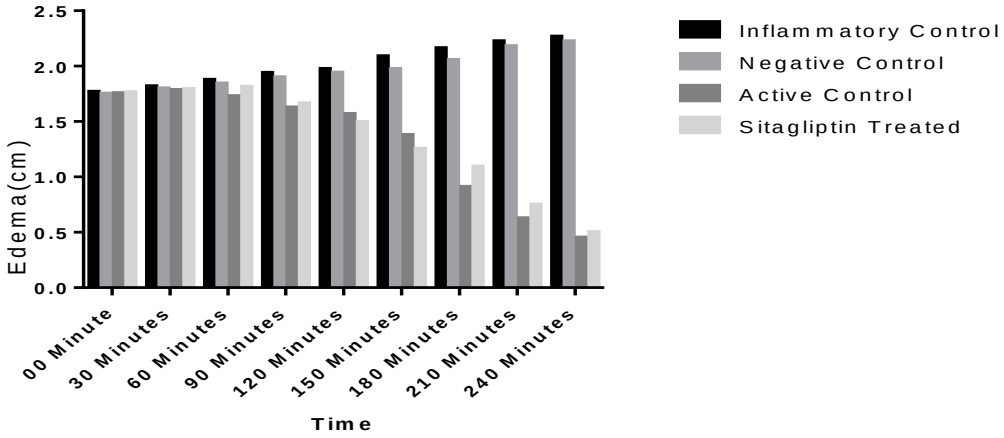

Fig-4 Effect of Sitagliptin on egg albumin induced inflammation

3.2.Effect of Papain induced arthritis

Papain induced arthritis is another way to test the immunosuppressive effect of Sitagliptin. Arthritis induced in mice by papain enzyme increases inflammation (marker of increase in immunity) which is significantly ($P< 0.05$) suppressed by Sitagliptin (Table-1) (Graph-3). Sitagliptin at 200mg/kg i. and standard Mycophenolate mofetil 100mg/kg i.p for two weeks before inducing arthritis.

TREATMENT	DOSE	Edema (Mean ± SEM)								
		After 00minute	After 30minute	After 60minute	After 90minute	After 120minute	After 150minute	After 180minute	After 210minute	After 240minute
Control	-	1.83±0.23	1.92±0.02	2.12±0.07	2.31±0.11	2.60±0.41	2.83±0.11	3.02±0.05	3.17±0.04	3.27±0.04
Distill water	10ml/kg	1.83±0.02 (NI)	1.92±0.02 (NI)	2.14±0.06 (NI)	2.37±0.07 (NI)	2.72±0.08 (NI)	2.93±0.02 (NI)	3.02±0.03 (NI)	3.11±0.03 (NI)	3.24±0.02 (NI)
Mycophenolate mofetil	100mg/kg	1.82±0.02 (0.54%)	1.89±0.04 (1.56%)	2.15±0.08 (1.41%)	2.28±0.11 (1.29%)	2.19±0.05[a*] ***b**** (15.76%)	2.07±0.03[a*] ***b**** (26.85%)	2.00±0.04[a*] ***b**** (33.77%)	1.87±0.03[a*] ***b**** (41%)	1.72±0.01[a] ****b**** (47.40%)
Sitagliptin treated	200mg/kg	1.83±0.02 (NI)	1.92±0.02 (NI)	2.11±0.08 (0.47%)	2.29±0.11 (0.86%)	2.23±0.06[a*] **b**** (14.23%)	2.12±0.05[a*] ***b**** (25.08%)	1.96±0.02[a*] ***b**** (35.09%)	1.88±0.01[a*] ***b**** (40.69%)	1.75±0.02[a] ****b**** (46.48%)

Values are mean ± SEM (n = 4) [a]p< 0.05 vs Inflammatory control

[b]p< 0.05 vs Negative control

Values in parenthesis represent percent inhibition of edema. (Two way ANOVA, Non Repeatative measure)

NI = No inhibition

Table-1- Effect of Sitagliptin on Papain induced arthritis in mice.

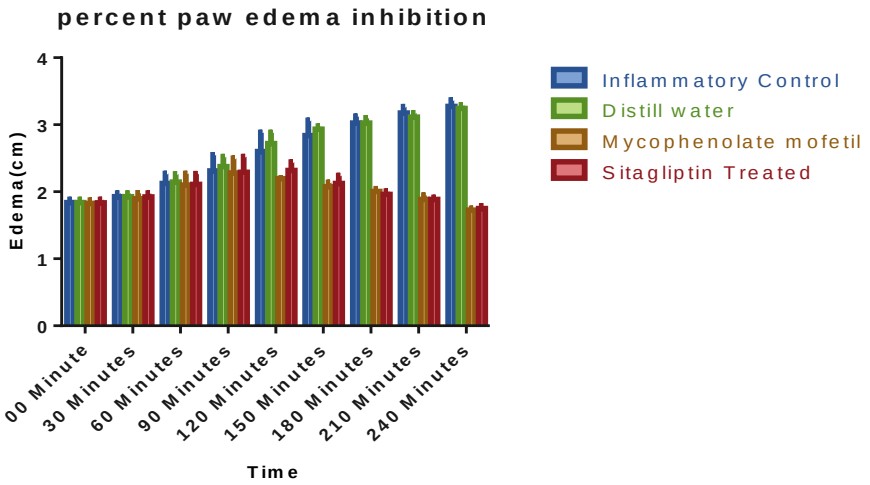

Fig-3 Effect of Sitagliptin on Papain Induced Arthritis

3.3. Effect on systemic anaphylactic shock induced by compound 48/80 in mice

As shown in Table 1, injection of compound 48/80 (8 mg/kg, I.P.) resulted in a fatal shock in 100% of the mice. Mycophenolate mofetil produced 80% reduction in mortality of the rat. Sitagliptin pretreatment at doses 100 mg/kg, i.p reduced the mortality rate.

	Dose mg/kg i.p	Mortality (%)
Control		100
Mycophnolate mofetil	100	80
Sitagliptin	200	20
vechile	100	5

Mice were given Sitagliptin for two week prior to intraperitoneal injection of compound 48/80 (8 mg/kg).Mortality rate (%) within 1 h after the injection of this compound was represented as:

$$\frac{\text{Number of dead mice}}{\text{Total number of mice}} \times 100$$

4. DISCUSSION

This is first report on immunosuppressive effect of Sitagliptin on humoral and cell mediated immunity by indirect method. Our results reported immunosuppressant activity of sitagliptin demonstrated by using model of anaphylactic shock induced by 40/80 compound,delayed hypersensitivity induced by egg albumin and papain induced arthritis. Anaphylaxis is defined as a serious allergic reaction that is rapid in onset and may cause death [16,17]. The prevalence of anaphylaxis is estimated to be as high as 2%, and appears to be rising, particularly in the younger age group. Sitagliptin posses anti-allergic activity evaluated by anaphylactic shock induced by compound 48/80 in mice .Mechanism of anaphylactic shock due to massive release of histamine from mast cell and basophil [18]. Compound 40/80 directly activate G protein. Compound 40/80 increases permeability of lipid bilayer membrane results in perturbation of membrane indicating that in membrane permeability may be essential trigger for release of mediator from mast cell. Major finding showed that Sitagliptin significantly reduced mortality in mice. Sitagliptin might stabilize lipid bilayer membrane thus preventing perturbation induced by compound 40/80. Sitagliptin is also posses' anti-inflammatory activity evaluated by delayed hypersensitivity induced by egg albumin and papain induced arthritis .Acute inflammation by egg albumin due to release of histamine and serotonin results in edema in right hind paw of mice. New model of papain enzyme used to induce arthritis in right hind paw of mice which is characterized by pain, edema and redness of paw. Papain is obtained by cutting the skin of the unripe papaya and then collecting and drying the latex which flows from the cut [19]. Papain induces inflammation by increasing prostaglandin synthesis. Papain involves in destruction of osteoclast and causes arthritis [20].Data from studies table-2 and graph showed that Sitagliptin significantly reduce inflammation. Sitagliptin might reduce release of mediators. Our finding signifies Sitagliptin posses' immune suppressive effect. Sitagliptin is selective DPP4 inhibitor inhibit DPP4 enzyme. DPP4 is the enzyme popularly known for its incretin effect. Beside this DPP4 posses immunomodulatory activity as mentioned above.DPP4 involve in T cell, B cell and natural killer cell activation. It also causes release of IL 10, 12 and interferon gamma which involve in T cell proliferation. As several studies evident DPP4 involve in immune regulation which is inhibited by DPP4 inhibitor might result in immune suppression. Sitagliptin found to be associated with intestinal cancer [20]. Sitagliptin inhibit enzyme DPP4 which results in increase in GLP-2.GLP-2 posses' proliferative effect on prolong inhibition of DPP4 enzyme by using DPP4 inhibitors. Sitaglipin is DPP4 inhibitor intestine may contribute to intestinal cancer. Sitagliptin also found to be associated with pancreatic cancer and ovarian cancer. Our finding mainly demonstrated role of Sitagliptin in immune system. However further studies needed to clarify exact mechanism of immune suppression by Sitagliptin.

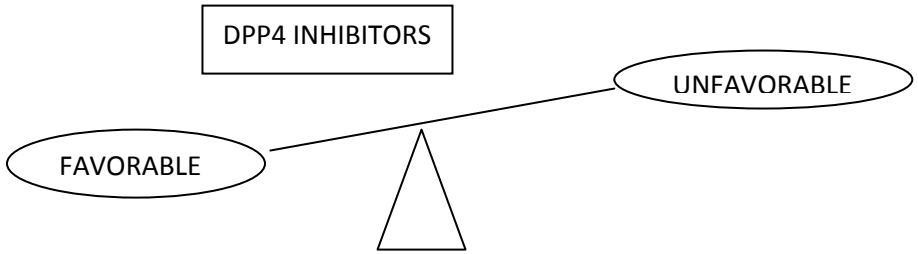

Fig-3-Diverse Role of DPP4 Inhibitors

5. CONCLUSION

The present study establishes the anti-allergic and anti-inflammatory activities of DPP4 inhibitors Sitagliptin.This signify immunosuppressant activity of Sitagliptin. Further research research need to be done for more clarification about immunosuppressive role of DPP4 inhibitor.

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