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RESEARCH ARTICLE

Variable anti-edema and anti-granuloma effects of liraglutide and teneligliptin on experimental Wistar albino rat inflammatory models

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ABSTRACT

Background: Diabetes mellitus is not only a chronic metabolic disorder but also an inflammatory one. Antidiabetic drugs are required to address both these entities to reduce the micro- and macro-complications associated with diabetes. Some of the incretin-based drugs have been shown to have decreased inflammatory markers. The studies on inflammatory models are very less. **Aims and Objectives:** The aim of this study is to evaluate anti-inflammatory activities of liraglutide, a glucagon-like peptide-1 analog, and teneligliptin, a dipeptidyl peptidase-4 inhibitor in experimental acute and subacute models of inflammation and also to evaluate their interactions with ibuprofen, a standard non-steroidal anti-inflammatory drugs. **Materials and Methods:** Carrageenan-induced paw edema to assess edema in acute anti-inflammatory action and cotton pellet-induced granuloma method to assess granuloma dry weight of liraglutide and teneligliptin in rats. **Results:** Liraglutide did not show anti-edema and anti-granuloma activity but potentiated the anti-granuloma effect of ibuprofen. Teneligliptin showed only anti-granuloma effect and potentiated both anti-edema and anti-granuloma activities of ibuprofen. **Conclusion:** Liraglutide and teneligliptin individually have variable anti-inflammatory activities, and they also have variable ibuprofen potentiating action. They have potentiated the subacute anti-inflammatory of ibuprofen by their anti-granuloma effect.

KEY WORDS: Liraglutide; Teneligliptin; Anti-inflammatory Action; Drug Potentiation

INTRODUCTION

The incidence of diabetes mellitus (DM) is increasing alarmingly across the globe. The American Diabetic Association is striving hard to reduce the incidence of DM and complications associated with it, by constantly reforming newer guidelines. With the advancement in science and in better understanding the pathogenesis of DM at molecular level, we could come to state an opinion that DM is also a chronic inflammatory disorder rather than a metabolic one, and it is well documented that the inflammation, oxidative stress, and immunity are all interrelated to one another.

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Hyperglycemia is known to activate the various metabolic pathways[1] including diacylglycerol (DAG)-protein Kinase C (PKC) pathway, hexosamine pathway, polyol pathway, advanced glycation endproducts (AGE) pathway, and many more, which leads to both acute and chronic inflammatory cascades, resulting in oxidative stress, depending on the type of stimulus, i.e., intermittent or persistent hyperglycemia and that explains the pathogenesis of complications associated with DM. Various experimental studies have shown that such complications could be significantly reduced by inhibiting these inflammatory signaling mechanisms.[2-4]

Based on this understanding, a novel antidiabetic drugs not only addressing hyperglycemia but also its various associated micro- and macro-vascular complications are the need of the

hour. Anti-inflammation and its associated process such as antioxidation, immunomodulation, anti-atherosclerosis, and many more should be the basis of such novel drugs.

Incretins are the superfamily of polypeptides released from intestinal cells in response to nutritional ingestion. Glucagon-like peptide (GLP)-1 and glucose-dependent insulinotropic polypeptide (GIP) are the important incretins, whose main action is to act on β islet cells of the pancreas to release insulin and responsible for about 50-70% of post-prandial insulin secretion.[5]

GLP-1 and GIP act on their respective receptors which are G protein coupled, linked to AC-cAMP-PKA signaling pathway and result in exocytosis of insulin secretory granules.[6] They are very much short acting as they are rapidly degraded by an enzyme dipeptidyl peptidase (DPP)-4 which exists in various cell types including vascular, intestinal, and renal cells,[7] and its inhibition by DPP-4 inhibitors prolongs the action of them. Wide distribution of DPP-4 receptors explains the possible type wide variety of actions of their receptor blockers. Thus, DPP-4 inhibitors act beyond glycemic control.

Incretins-based therapy includes incretin mimetics, i.e., GLP-1 analogs such as exenatide, lixisenatide, liraglutide, and incretin enhancers, i.e., DPP-4 inhibitors such as sitagliptin, vildagliptin, and teneligliptin. They are a novel class of antidiabetic drugs recently gaining importance as they act beyond glycemic control, reducing various inflammatory markers, thus the risk of diabetic complications, but yet to be proved clinically on a larger scale.

Various animal and clinical studies document that GLP-1 analogs and DPP-4 inhibitors have reduced various inflammatory markers such as endothelin (ET)-1, vascular cell adhesion molecule, intracellular adhesion molecule, interleukins (IL)-6, tissue necrosis factor (TNF)-α and NF κβ, and many more.[8] Dipeptidyl peptidase (DDP)-4 inhibitors additionally inhibit the degradation of multiple substrates other than GLP-1, including brain natriuretic peptide (BNP), substance-P, neuropeptide-Y (NPY), stromal-derived factor-1α, and high mobility group proteins B. These substrates play an important role in regulating vascular inflammatory changes,[9] and incretin-based drugs appear to have effects on the occurrence and progression of diabetic complications.

The literature survey reveals that the anti-inflammatory studies of GLP-1 analogs and DDP-4 inhibitors in various acute and subacute inflammatory models are sparse and it is much more harder to find their comparative anti-inflammatory studies. Hence, an experimental study was planned to explore and compare anti-inflammatory activities of some commonly prescribed incretin-based drugs, liraglutide, a GLP-1 analog, and teneligliptin, a DPP-4 inhibitor.

Aims and Objectives

The aim of this study is as follows:

- To evaluate and compare anti-inflammatory activity of liraglutide and teneligliptin in acute and subacute models of inflammation.
- To study the interaction of liraglutide and teneligliptin on inflammation with subtherapeutic equivalent anti-inflammatory dose of ibuprofen.

MATERIALS AND METHODS

Apparently, healthy Wistar albino rats of either sex with normal activity and weighing around 250-300 g were obtained from the central animal house of the institute. They were randomly divided into 5 groups of 6 animals each. They were kept in separate cages under standard laboratory condition with free access to pellet food and water *ad libitum*. They were acclimatized to the laboratory conditions 1 week before starting the experiment.

Drugs ibuprofen, liraglutide, and teneligliptin are obtained from pharmaceutical companies as free samples. Ibuprofen and teneligliptin were obtained in pure powder form of IP grade, whereas liraglutide in injection form.

Clinical doses of these drugs were equivalently converted into rat doses using converting table as described by Paget and Barns,[10] and they were administered to rats using appropriate routes.

Methods

Carrageenan-induced rat paw edema model

This model was used to assess the acute anti-inflammatory activity of treatment drugs.[11] After overnight starvation, rats were administered appropriate doses of drugs. Ibuprofen and teneligliptin in 1% gum acacia suspension given orally using nasogastric tube, whereas liraglutide was injected subcutaneously using tuberculin syringe in treatment groups. Control groups correspondingly received equal volume of 1% oral acacia suspension and normal saline subcutaneous injection.

30 min after drug administration, 0.1 ml of 1 % freshly prepared carrageenan suspension in normal saline was injected into subplantar region of rat left hind paw. The paw edema volume was measured by dipping the hind paw till the level of malleolus in the mercury column of plethysmograph. The readings were taken at 0 min and repeated at regular intervals of 30, 60, 180, and 300 min. The different group readings expressed in volume (ml) were averaged, % inhibition of edema in various treatment groups was calculated, and anti-inflammatory activity was assessed by corresponding decrease in paw edema volume in treatment groups.

Table 1: Effects of various drugs on carrageenan paw edema

Drug treatment	Increase in paw volume mean±SEM and % inhibition			
	30 M	60 M	180 M	300 M
Control A oral gum acacia	0.08±0.06	1.81±0.04	2.90±0.08	4.41±0.07
Control B NS injection	0.81±0.04	1.91±0.05	2.87±0.06	4.36±0.06
Ibuprofen 108 mg/kg BW Oral	0.76±0.03 5%	1.36±0.04** 24.79%	2.01±0.04** 30.68%	2.96±0.05** 32.87%
Liraglutide 0.15 mg/kg BW Injection	0.78±0.04 3.70%	1.90±0.03 0.5%	2.77±0.04 3.48%	4.10±0.04 5.96%
Teneligliptin 3.6 mg/kg BW Oral	0.77±0.06 3.75%	1.93±0.06 6.62%	2.82±0.03 2.75%	4.26±0.05 3.40%
ANOVA	1.17	6.36	3.71	11.41

N=6 in each group. ANOVA $F_{4,25}$=2.75 (*P*=0.05), 4.17(*P*=0.01). Dunnett's test *$P \leq 0.05$, **$P \leq 0.01$. SEM: Standard error of the mean, BW: Body weight

Table 2: Interaction of liraglutide and teneligliptin with ibuprofen on carrageenan paw edema

Drug treatment	Increase in paw volume mean±SEM and % inhibition			
	30 M	60 M	180 M	300 M
Control A Oral gum acacia	0.08±0.06	1.81±0.04	2.90±0.08	4.41±0.07
Control B NS injection	0.81±0.04	1.91±0.05	2.87±0.06	4.36±0.06
Ibuprofen 15 mg/kg BW+Liraglutide 0.15 mg/kg BW	0.76±0.05 6.17%	1.76±0.04 7.85%	2.64±0.04 8.01%	4.18±0.07 4.11%
Ibuprofen 15 mg/kg BW+Teneligliptin 3.6 mg/kg BW	0.72±0.3 10.10%	1.56±0.03* 13.81%	2.21±0.07** 23.70%	3.80±0.07* 13.83%
ANOVA	0.26	6.36	1.17	1.34

N=6 in each group. ANOVA $F_{3,20}$=3.09 (*P*=0.05), 4.93 (*P*=0.01). Dunnett's test *$P \leq 0.01$, **$P \leq 0.01$. SEM: Standard error of the mean, BW: Body weight

Table 3: Effect of various drugs on cotton pellet granuloma dry weight

Drug treatment	Granuloma dry weight 9 mg/100 g BW	
	Mean±SEM	% inhibition
Control A Oral gum acacia	29.18±2.01	
Control B NS injection	28.91±1.97	
Ibuprofen 108 mg/kg BW Oral	15.17±0.99**	48.21
Liraglutide 0.15 mg/kg BW Injection	27.11±1.80	6.23
Teneligliptin 3.6 mg/kg BW Oral	22.76±1.7*	24.13
F value	16.61	

N=6 in each group. ANOVA $F_{4,25}$=2.75 (*P*=0.05), 4.71 (*P*=0.01). Dunnett's test **$P \leq 0.01$. SEM: Standard error of the mean, BW: Body weight

Cotton pellet granuloma model

This model[12] was used to assess the sub anti-inflammatory activity of treatment drugs. Under injection thiopentone sodium anesthesia, overnight starved rats were fixed on rat operating table and hair in the axillae were clipped enough to implant 2 sterile cotton pellets, through the small incisions made subcutaneously on both the sides, aseptically. Wounds were sutured after the pellet implantation, and the rats were allowed to recover from anesthesia to administer respective drug and treatment continued daily for 10 days.

On the 11th day, under injection thiopentone sodium anesthesia, the sutures were cut opened to get implanted cotton pellets with granulation tissue around. They were carefully separated from the surrounding tissue and dried overnight at 60°C to find their dry weight, from which initial weight was subtracted to get the net granuloma dry weight, expressed as mg/100 g body weight. The averaged values of treatment group compared with that of control, and the decrease in granuloma dry weight indicates subacute anti-inflammatory activity.

RESULTS

The values observed in all groups were averaged, tabulated, and subjected to statistical analysis using ANOVA followed by Dunnett's test. The level of significance was calculated at value $P \leq 0.05$.

Table 4: Interaction of liraglutide and teneligliptin with ibuprofen on cotton pellet granuloma dry weight

Drug treatment	Granuloma dry weight mg/100 g BW	
	Mean±SEM	% inhibition
Control A Oral 1% gum acacia	29.18±2.01	
Ibuprofen 15 mg/kg+Teneligliptin 3.6 mg/kg	23.16±1.18*	20.63
Control: B NS injection	28.91±1.97	
Ibuprofen 15 mg/kg+Liraglutide 0.15 mg/kg	23.76±1.05*	17.85
F value	1.32	

N=6 in each group. ANOVA $F_{3,20}$=3.09 (*P*=0.05), 4.93 (*P*=0.01). Dunnett's test *$P \leq 0.05$. SEM: Standard error of the mean, BW: Body weight

In this acute inflammatory model, the increase in paw edema volume was recorded at 30, 60, 180, and 300 min. Ibuprofen, a standard non-steroidal anti-inflammatory drugs (NSAID) significantly (*P* 0.01) decreased paw edema with % inhibition of 24.79, 30.68, and 32.87, respectively, at 60, 180, and 300 min, when compared with control A. Liraglutide and teneligliptin both decreased paw edema insignificantly, when compared with Control B and Control A, respectively Table 1.

Here, the interaction of liraglutide and teneligliptin with subtherapeutic anti-inflammatory dose of ibuprofen was evaluated Table 2. It was found that only teneligliptin significantly (*P* 0.05) decreased paw edema volume at 60, 180, and 300 min with respective % inhibition of 13.81, 23.7, and 13.83, respectively. Thus, potentiation of anti-edema effect of ibuprofen by teneligliptin in this acute inflammatory model.

In this subacute inflammatory model, ibuprofen decreased granuloma dry weight with mean value of 15.17 ± 0.99 (48.21% inhibition), when compared to Control A. Teneligliptin also decreased granuloma dry weight significantly with mean value of 22.76 ± 1.71 (24.13% inhibition) but not to that extent of ibuprofen Table 3.

While evaluating the interaction of liraglutide and teneligliptin with subtherapeutic anti-inflammatory dose of ibuprofen, both of them significantly reduced granuloma dry weight with $P \leq 0.05$ and respective mean values are 23.16 ± 1.05 (17.85% inhibition) and 23.16 ± 1.18 (20.63% inhibition) and thus potentiation of sub anti-inflammatory activity of ibuprofen by both drugs Table 4.

DISCUSSION

As mentioned earlier, the main objective of the study was to assess the anti-inflammatory activity of some GLP-1 analogs and DPP-4 inhibitors in various inflammatory models. Liraglutide, a GLP-1 analog, and teneligliptin, a DPP-4 inhibitor, were selected by choice. Acute carrageenan-induced rat paw edema and cotton pellet-induced granuloma models were chosen, as they are simple, basic, and time-tested anti-inflammatory screening methods. In carrageenan-induced rat paw edema model, neither liraglutide nor teneligliptin showed significant anti-edema effect, whereas ibuprofen showed by inhibiting cyclooxygenase-derived prostaglandins. Edema occurs in 2 phases, an early phase involving mainly histamine, serotonin, and prostaglandins and a late phase involving prostaglandins, lysosomes, and proteases, starts from 2 h, and continues up to 5 h.[13] Liraglutide and teneligliptin appeared not to inhibit these inflammatory mediators and thus failed to show anti-edema effect. This is in very much contrast with various other studies, where incretin-based drugs are showed to be anti-inflammatory on the basis of reduction in the inflammatory markers in different tissues and plasma,[8] and anti-edema effect was not assessed in those studies as in our present study. In cotton pellet granuloma model, assessing subacute anti-inflammatory action of drugs, teneligliptin, showed significant anti-granuloma effect by decreasing granuloma dry weight, thus appeared to have an action on inflammatory mediators of subacute inflammation. Teneligliptin, a DPP-4 inhibitor also inhibits degradation of many other substrates of DPP-4 enzyme such as BNP, NPY, substance-P, and many more which have regulatory action on inflammation and probably that explains its anti-granuloma effect. Interestingly, when interactions of liraglutide and teneligliptin with subtherapeutic anti-inflammatory dose of ibuprofen were evaluated, its only teneligliptin significantly potentiated the anti-edema and anti-granuloma actions of ibuprofen. Liraglutide potentiated only anti-granuloma effect, and it neither has anti-edema action of its own nor potentiated that of ibuprofen. These variable effects on inflammation could be explained on the basis of various pharmacokinetic factors such as drug bioavailability, metabolism, concentration at site, and pharmacodynamic factors such as drug dose, duration, and inflammatory mediators involved.

Many earlier clinical and experimental studies document that many GLP-1 analogs and DPP-4 inhibitors have decreased various inflammatory markers locally and systemically, giving an indirect evidence to their anti-inflammatory activities. Sitagliptin[14] and liraglutide inhibited expression of pro-inflammatory genes[15,16] in mouse islet cells and cytokines expression in cultured human cells.[17] Vildagliptin showed a decrease in plasma TNF-α.[18] Studies exhibiting direct evidences of cellular events and vascular changes are very much lacking in abovementioned studies. In this present study, an attempt was made to visualize the direct evidence of the anti-edema and anti-granuloma effects in acute and subacute inflammatory models.

The present study findings are also different from other recent studies, where many DPP-4 inhibitors[19] and GLP analogs[20]

have shown a significant anti-edema effect, but teneligliptin and liraglutide were not studied there.

These variable effects on inflammation could be explained on the basis of various pharmacokinetic factors such as drug bioavailability, metabolism, concentration at site, and pharmacodynamic factors such as drug dose, duration, and inflammatory mediators involved. Our study used drugs liraglutide and teneligliptin whose anti-inflammatory studies are very much limited and so also their comparison and interaction with ibuprofen. Our study was attempted to assess the anti-inflammatory activities with respect to edema and granuloma only, and other events of inflammation[21] were not assessed.

CONCLUSION

Hence, in DM, incretin-based therapy not only decreases blood glucose level but may also the progression of diabetes and its complications associated, by virtue of their anti-inflammatory effects. However, further, long-term clinical studies are required to strengthen this view to claim clinical benefits. These drugs could have potential uses in various chronic inflammatory conditions. On the other hand, these drugs may increase the side effects of NSAIDS, when co-prescribed with, in diabetic patients and much more studies are required to look into this aspect.

REFERENCES

1. Kawanami D, Matoba K, Utsunomiya K. Signaling pathways in diabetic nephropathy. Histol Histopathol. 2016;31(10):1059-67.
2. Inoue T, Inoguchi T, Sonoda N, Hendarto H, Makimura H, Sasaki S, et al. GLP-1 analogue liraglutide protects against cardiac steatosis, oxidative stress and apoptosis in Streptozotocin-induced diabetes in rats. Atherosclerosis. 2015;240(1):250-9.
3. Ishibashi Y, Matsui T, Takeuchi M, Yamagishi S. Glucagon-like peptide-1 (GLP-1) inhibits advanced glycation end product (AGE)-induced up-regulation of VCAM-1 mRNA levels in endothelial cells by suppressing AGE receptor (RAGE) expression. Biochem Biophys Res Commun. 2010;391(3):1405-8.
4. Ishibashi Y, Nishino Y, Matsui T, Takeuchi M, Yamagishi S. Glucagon-like peptide-1 suppresses advanced glycation end product-induced monocyte chemoattractant protein-1 expression in mesangial cells by reducing advanced glycation end product receptor level. Metabolism. 2011;60(9):1271-7.
5. Nauck MA. Incretin-based therapies for Type 2 diabetes mellitus: Properties, functions, and clinical implications. Am J Med. 2011;124 1 Suppl: S3-18.
6. Seino Y, Fukushima M, Yabe D. GIP and GLP-1, the two incretin hormones: Similarities and differences. J Diabetes Investig. 2010;1:8-23.
7. Mulvhill EE, Drucker DJ. Pharmacology, Physiology and mechanisms of actions of dipeptidyl peptidase-4 inhibitors. Endocr Rev. 2014;35(6):992-1019.
8. Tang ST, Zhang Q, Tang HQ, Wang CJ, Su H, Zhou Q, et al. Effects of glucagon-like peptide-1 on advanced glycation endproduct-induced aortic endothelial dysfunction in Streptozotocin-induced diabetic rats: Possible roles of rho kinase-and AMP kinase-mediated nuclear factor ?B signaling pathways. Endocrine. 2016;53(1):107-6.
9. Kawanami D, Matoba K, Sango K, Utsunomiya K. Incretin-based therapies for diabetic complications: Basic mechanisms and clinical evidence. Int J Mol Sci. 2016;17(8). pii: E1223.
10. Paget GE, Barns JM. In Evaluation of Drug Activities: Pharmacometrics. In: Laurence DR, Bacharach AL, editors. Vol. 1. New York and London: Academic Press; 1964.
11. Winter CA, Risley EA, Nuss GW. Carrageenin-induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. Proc Soc Exp Biol Med. 1962;111:544-7.
12. Vogel HG, Vogel WK, Editors. Drug Discovery and Evaluation: Pharmacological Assays. Germany: Springer Publications; 1997. p. 759.
13. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenin edema in rats. J Pharmacol Exp Ther. 1969;166(1):96-103.
14. Makdissi A, Ghanim H, Vora M, Green K, Abuaysheh S, Chaudhuri A, et al. Sitagliptin exerts an ant inflammatory action. J Clin Endocrinol Metab. 2012;97(9):3333-41.
15. Huang C, Yuan L, Cao S. Endogenous GLP-1 as a key self-defense molecule against lipotoxicity in pancreatic islets. Int J Mol Med. 2015;36(1):173-85.
16. Nauck M. Incretin therapies: Highlighting common features and differences in the modes of action of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. Diabetes Obes Metab. 2016;18(3):203-16.
17. Velmurugan K, Balamurugan AN, Loganathan G, Ahmad A, Hering BJ, Pugazhenthi S. Antiapoptotic actions of exendin-4 against hypoxia and cytokines are augmented by CREB. Endocrinology. 2012;153(3):1116-28.
18. Akarte AS, Srinivasan BP, Gandhi S, Sole S. Chronic DPP-IV inhibition with PKF-275-055 attenuates inflammation and improves gene expressions responsible for insulin secretion in Streptozotocin induced diabetic rats. Eur J Pharm Sci. 2012;47(2):456-3.
19. Urmila AK, Netravathi BA, Somnath MM. Effect of dipeptidyl peptidase 4 inhibitors on acute and chronic models of inflammation in male wistar rats: An experimental study. Int J Appl Basic Med Res. 2017;7(1):26-31.
20. Chaudhuri A, Ghanim H, Vora M, Sia CL, Korzeniewski K, Dhindsa S, et al. Exenatide exerts a potent anti-inflammatory effect. J Clin Endocrinol Metab. 2012;97(1):198-207.
21. Pise HN, Padwal SL. Evaluation of anti-inflammatory activity of *Nigella sativa*: An experimental study. Natl J Pharm Pharmacol. 2017;7(7):701-11.

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