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RESEARCH ARTICLE

Evaluation of dipeptidyl peptidase-4 enzyme inhibition by some commonly used gliptins on cutaneous wound healing in albino rats

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ABSTRACT

Background: Dipeptidyl peptidase-4 enzyme not only inhibits incretins but also many other substrates and has wide distribution. Inhibition of this enzyme may have many implications, one of which is to improve wound healing. **Aims and Objectives:** This aim of this study is to evaluate wound healing property of linagliptin, saxagliptin, sitagliptin, and vildagliptin on excised wounds in non-diabetic Wistar albino rats. **Materials and Methods:** Some commonly used gliptins, linagliptin, saxagliptin, sitagliptin, and vildagliptin were assessed for pro-wound healing property on excised wound in non-diabetic Wistar albino rats. Wound healing was assessed by measuring the scar area at 4,8,12 & 16 days and the time taken for the complete scar formation.On the 16th day, rats were sacrificed, wound tissue biopsy was taken for assessing granulation tissue and serum was subjected for tumor necrosis factor-1α, interleukin-6, inducible nitric oxide synthase, hydroxyproline and glucose estimation. **Results:** Pro-healing property was not uniform among various tested gliptins, only linagliptin and sitagliptin were found to have improved wound healing potential in rat excised wound. **Conclusion:** Gliptins show variable effect on wound healing.

KEY WORDS: Gliptins; Dipeptidyl Peptidase-4 Enzyme; Pro-wound Healing Property

INTRODUCTION

Wound is a common clinical entity encountered in day-to-day practice, most commonly occurring on skin. Some are intentional, and others are accidental. It can be defined as a disruption of anatomical/functional continuity of living tissue produced by various injurious agents. The restoration of this damaged tissue constitutes wound healing, and it includes both tissue repair and regeneration.[1] Wound healing is a specific biological and highly dynamic process, involving complex integrated anatomical, physiological, and biochemical changes, progressing in an orderly manner at an optimal rate, which may differ from species to species and tissues to tissues in the same species.[2] In humans, the healing time is much faster in intestinal wounds and relatively slow in urinary bladder and skin wounds.[3]

With the advancement and in a better understanding of physiology and pathology of wound healing, it is possible to manipulate wound healing favorably by various drugs and factors. The recent studies have identified the numerous changes in wounds that contribute to a delay in healing. They include abnormal pattern of expression and activity of growth factors, cytokines, chemokines, and also protease enzyme.[4] There is also an abnormal decrease in enzyme superoxide dismutase (SOD), leading to increased free radicals and impairing wound healing.[5] The critical stimulus for normal wound healing is relative hypoxia, and an impaired reaction

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to hypoxia could contribute to impaired wound healing. Peak expression of hypoxia inducible factor-1α (HIF) and vascular endothelial growth factor (VEGF) as well as nitric oxide (NO) production promotes angiogenesis and wound healing.[6] Literature survey points out that several drugs and chemicals have been studied to assess their wound healing property, many of them favored,[7-9] some produced no effect,[10] and others retarded wound healing.[11,12] Wound healing is also affected in the presence of other pathologies such as infection, foreign body, malnutrition, diabetes, and many more. Diabetic wound management is a big challenging task for physicians and is associated with complications such as non-healing, chronic ulcer, and sepsis leading to gangrene.

Insulin has been found to promote wound healing, by increasing cellular proliferation, mineralization of tissue, and angiogenesis and by decreasing apoptosis in diabetic wounds.[13,14] Its pro-healing property in non-diabetic individuals is compromised by its hypoglycemic side effects.[15] Gliptins or dipeptidyl peptidase (DPP)-4 inhibitors could be of some usefulness in non-diabetic individuals, as they act by increasing endogenous insulin secretion, with least hypoglycemic risk. They inhibit DPP-4 enzyme, which plays a key role in the degradation of incretins such as glucagon-like peptide-1 and Glucose dependent insulinotropic peptide also known as gastric inhibitory polypeptide, and which inturn increase insulin secretion from β cells of the pancreas. DPP-4 enzyme is widely distributed throughout the body and exists as both membrane bound and as a free plasma circulating form. This enzyme has various other substrates such as high mobility group box-1 protein, HIF-1α, stromal cell-derived factor-1, and many more. These substrates have been involved in angiogenesis, transmigration of endothelial progenitor cells (EPC), and hemopoietic stem cells to the site of damage and initiate tissue repair.[16,17] Saboo et al.[18] have reviewed recently that the DPP-4 inhibitors spare these substrates, thereby promoting angiogenesis and homing of EPC in diabetic foot ulcers, culminating in better wound repair. However, there is sparse information about the pro-healing effect of DPP-4 inhibition on non-diabetic wounds. Hence, the present study was planned to evaluate wound healing property of linagliptin, saxagliptin, sitagliptin, and vildagliptin on excised wounds in non-diabetic Wistar albino rats.

MATERIALS AND METHODS

The study was initiated after obtaining approval from the Institutional Animal Ethics Committee (IAEC) (No. IAEC2/Desp.No.58/Dt. 3.01.14) and conducted from January to June 2014. Healthy Wistar albino rats of either sex, weighing around 200-250 g of 8-10 months age, were procured from central animal house of the institute. They were divided into 5 groups of 12 each, and among them, 6 rats were to be sacrificed on the 16th day, after starting the experiment. The rats were housed in a clean polypropylene cages kept in experimental condition with 12 h alternate natural light and night cycles at a temperature maintained 23-25°C, with relative humidity of 50-60%, and allowed to a free access of standard pellet food and water *ad libitum*.

The rats were acclimatized to laboratory conditions 1 week before experiments. The drugs such as linagliptin, saxagliptin, sitagliptin, and vildagliptin were obtained from pharmaceutical companies as gifted samples in pure powder form of IP grade. Clinical doses of these drugs were equivalently converted into rat doses using the converting table as described by Paget and Barns,[19] and they were administered to the rats daily per orally using nasogastric tube. Drugs were suspended in 1% gum acacia so as to obtain the required dose in 1 ml and administered every 24 h (10:00 AM) using tuberculin syringe until the complete epithelization of excised wound occurs. Group 1 served as control and received 1% gum acacia, and Groups 2, 3, 4, and 5 received linagliptin, saxagliptin, sitagliptin, and vildagliptin, respectively.

Groups	Treatment
Group 1	Control-gum acacia oral
Group 2	Linagliptin 0.45 mg/kg oral
Group 3	Saxagliptin 0.45 mg/kg oral
Group 4	Sitagliptin 1.8 mg/kg oral
Group 5	Vildagliptin 1.8 mg/kg oral

Methods

Excision wound

The rats were starved overnight but with free access to water, and the backs of the rats were depilated before the day of experimentation without causing any injury. Skin wounds were made as described by Kamper et al.[20] by excising the full-thickness circular skin (approximately 500 mm^2) from the nape of the neck under halothane anesthesia. Measures were taken to control bleeding and infection. Wound closure rate was assessed by tracing the wound on polythene paper and getting its imprints on graph paper, on wounding day (0), followed by 4th, 8th, 12th, and 16th day and subsequently on every alternate day till complete closure has occurred. Falling of the scab without any raw area indicated the time for complete epithelialization and the same was noted. Similarly, scars were traced to assess wound contraction by noting scar size and shape.

Excision wound healing was assessed by:

1) Planimetry, scar shape, scar size, and time of epithelialization
2) Histopathological assessment of wound granulation tissue: Biopsy of cutaneous wound was performed on the 16th day of experiment, after sacrificing rats with

excess dose of ether anesthesia, and biopsied tissue was subjected for histopathological examination, for the assessment of fibroblast population, infiltrating cells, collagen content, and angiogenesis

3) Biochemical analysis: 3 ml of blood was collected from sacrificed animal of each group on 16th day of experiment, and the plasma was separated by centrifugation and stored at −90°C and subjected for the estimation of tumor necrosis factor-1α (TNF-1 α) and interleukin-6 (IL-6) were measured in serum by antibody-captured enzyme-linked immunosorbent assay (ELISA) according to kit manufacturer's instructions (The RayBio ELISA kit). The serum inducible NO synthase (iNOS) and hydroxyproline content were measured by Elisa kit, in accordance with the rat-specific kit protocols (Cusabio, China)
4) Blood glucose estimation using glucometer.

Statistical Analysis

All the results were expressed as mean ± standard error of the mean and significant difference between the mean of different groups was analyzed using One-way ANOVA followed by *post-hoc* (Tukey's) test. The differences in values at level $P < 0.05$ were considered statistically significant.

RESULTS

In the present study, linagliptin, saxagliptin, sitagliptin, and vildagliptin have been investigated for their influence on cutaneous wound healing in normal albino Wistar rats (Table 1).

When compared to control, linagliptin & sitagliptin-treated groups showed significant percentage of wound closure with respective values of 32.56 ± 3.85, 77.88 ± 1.76,89.27 ± 1.22,97.94 ± 0.43 and 34.22 ± 1.07,71.42 ±2.67,94.58 ± 1.15, 99.4 3 ±0.09 at 4th, 8th, 12th 16th days, respectively with the level of significance $P \leq 0.5$ and that showed their pro-wound healing property. Saxagliptin- and vildagliptin-treated groups were insignificant from that of control group values thus found not favoring pro-wound healing effects (Table 2).

The epithelialization was considered to be complete, once the scab falls off without any raw area. Total time for complete epithelialization taken was 16 ± 0.3 and 17 ± 0.4 days with 18.50 ± 5.7 and 26.83 ± 0.95 of scar area (mm^2) with linagliptin- and sitagliptin-treated groups, respectively, when compared with 20 ± 0.2 days and 41.83 ± 5.7 scar area of control group. Saxagliptin and vildagliptin showed insignificant changes. Thus, early and small scar area of linagliptin and sitagliptin groups indicated better collagenesis (Table 3).

As far as biochemical parameters were concerned, on the day 16, in line with earlier results, the serum estimation showed again linagliptin and sitagliptin groups having significant ($P < 0.05$) serum hydroxyproline (780.41 ± 1.57 and 698.75 ± 0.7) and iNOS (96.70 ± 1.57 and 86.42 ± 0.7) concentration, suggesting enhanced collagen synthesis and increased vascularity of the wound tissue, respectively. They also favored wound healing by arresting the ongoing inflammatory process, as suggested by significant $P < 0.05$ decrease in serum TNF-1α and IL-6 (Table 4).

No significant differences in blood glucose levels (mg/dl) were observed after 8 or 16 days of drug treatment in all treatment groups, suggesting their antihyperglycemic action.

Histopathology examination was evaluated on the 16th day after wounding. In linagliptin and sitagliptin groups, wound granulation tissue had comparatively less inflammatory cells and more collagen, fibroblasts, and proliferating capillaries than control, saxagliptin, and vildagliptin groups (pictures not shown).

DISCUSSION

The aim of this study was to investigate the effect of some gliptins/DPP-4 inhibitors such as linagliptin, saxagliptin, sitagliptin, and vildagliptin on non-diabetic cutaneous wound healing. The percentage of wound closure was significantly higher in both linagliptin and sitagliptin groups taking 17 and 16 days for the complete closure by epithelialization with the small scar areas. This was the result of good granulation tissue formed at wound site, as evidenced by biopsy of wound tissue on 16th day, containing relatively less neutrophil infiltration with more collagen, fibroblast, and proliferating capillary vessels. This indirectly points out that both linagliptin and sitagliptin appeared to promote wound healing by halting an ongoing active inflammatory process in the wound. The estimation of biochemical parameters also

Table 1: Percentage closure of excision wound on 4th, 8th, 12th, and 16th day

Days	Control	Linagliptin	Saxagliptin	Sitagliptin	Vildagliptin
4	19.07±1.76	32.56±3.85*	20.27±4.20	34.22±1.07*	21.12±1.78
8	53.57±3.04	77.88±1.76*	54.76±1.92	71.42±2.67*	55.58±2.24
12	81.28±2.96	89.27±1.22*	84.88±1.48	94.58±1.15*	85.07±1.36
16	88.88±1.63	97.94±0.43*	89.69±0.51	99.43±0.90*	90.85±0.43

Values (mean±SEM). *Denotes level of significance ($P<0.05$)

Table 2: Time for complete epithelization and scar area

Parameters	Control	Linagliptin	Saxagliptin	Sitagliptin	Vildagliptin
Time for complete epithelialization (days)	20±0.2	16±03*	18±0.9	17±0.4*	19±0.7
Scar area (mm^2)	41.83±5.7	18.50±15.7*	34.17±0.95	26.83±0.7*	40.33±1.89

Values (mean±SEM). *Denotes level of significance (P<0.05)

Table 3: Serum parameters on tissue remodeling on 16th day

Serum parameters	Control	Linagliptin	Saxagliptin	Sitagliptin	Vildagliptin
IL-6 pg/ml	62.56±1.58	37.21±1.57*	59.30±0.95	43.52±0.7*	62.52±1.89
TNF-1α pg/ml	3754.45±57	1392.15±1.57*	3644.02±0.95	1796.82±0.7*	3695.51±1.57
Hydroxy proline ng/ml	554.79±57	780.41±1.57*	578.10±0.95	698.75±0.7*	560.46±1.89
iNOS IU/ml	48.61±57	96.70±1.57*	49.74±0.75	86.42±0.7*	58.66±1.89

Values (mean±SEM). *Denotes level of significance (P<0.05). TNF-1α: Tumor necrosis factor-1α , IL-6: Interleukin-6, iNOS: Inducible nitric oxide synthase

Table 4: Blood glucose level (mg/dl) on the day 0, 8, and 16

Days	0	8	16
Control	117	110	100
Linagliptin	109	95	100
Saxagliptin	101	98	102
Sitagliptin	105	100	95
Vildagliptin	116	108	101

suggests the anti-inflammatory activity of these two drugs, as they significantly decreased TNF-1α and IL-6 levels in the serum, which are the key inflammatory mediators. Further, these two drugs also increased serum iNOS enzyme and hydroxyproline level. Hydroxyproline is a non-essential amino acid synthesized in the liver, required for collagen synthesis which is an integral part of wound healing and repair. Enzyme iNOS is critical for wound healing by producing NO, which ensures enhanced blood flow to the wound, supplying nutrients and eliminating metabolic waste products, indirectly favoring angiogenesis. Thus, linagliptin and sitagliptin appeared to promote wound healing by their property of angiogenesis and collagenation, with some anti-inflammatory action and the stellate shape of the scar, probably suggest that enhanced healing is due to wound contraction rather than enhanced epithelialization.

The results of the present study are in accordance with the earlier studies, where linagliptin accelerated wound epithelialization by increasing myofibroblasts and attenuated the inflammation by decreasing the pro-inflammatory markers COX - 2 and macrophage inflammatory protein-2 in diabetic obese mice.[21] Gupta[22] has reviewed the wound healing effects of linagliptin, which was shown to influence macrophage-mediated inflammatory responses, that may suppress vascular inflammation and improve diabetic wound healing. However, the present study was conducted in non-diabetic wounds, where only linagliptin and sitagliptin groups were found to favor wound healing. Saxagliptin and vildagliptin were found not to have wound pro-healing activity. On the contrary, vildagliptin improved wound healing after 12 weeks of treatment in diabetic patients, by increasing HIF-1α-and VEGF-induced angiogenesis and decreasing oxidative stress.[23] Similarly, it ameliorates oxidative stress and pancreatic beta cells damage by increasing the antioxidative catalase and SOD enzymes and helps in beta cells recovery in Type 1 diabetic rats.[24] The studies on saxagliptin are very much limited.

There is a paucity of information regarding the action of DPP-4 inhibitors on non-diabetic cutaneous wound healing. The present study was attempted to explore their pro-healing activity on non-diabetic wound and also to throw some light on their mechanism of actions for the same. It was angiogenesis (↑iNOS), collagenesis (↑hydroxyproline), and anti-inflammatory actions (↓TNF-α and ↓IL-6), mediated their wound pro-healing action. However, the variable pro-wound healing property across different DPP-4 inhibitors could be explained on the basis of pharmacokinetic and pharmacodynamic properties of the drug. However, the present study would have included more wound models and the direct evidence of tissue remodeling from the wound biopsied tissue rather than from serum. The findings of the present study appear to have some clinical relevance, if they could be extrapolated to humans, in the background of diabetic foot ulcer management. Further studies are required to state that their wound pro-healing action is a drug effect or class effect.

CONCLUSION

The results of the present experimental study would be of some clinical relevance if they could be extrapolated to humans. Among experimented gliptins/DPP-4 inhibitors, only linagliptin and sitagliptin had wound pro-healing effect. Thus, they become the drug of choice in diabetic wound

management, promoting wound healing, yet controlling blood sugar. They could be prescribed even in non-diabetics with wounds or perisurgically, exploiting their pro-healing activity. However, further clinical studies area required for their new role.

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