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Evaluation of Incretin Enhancers (Exenatide and Liraglutide) on Intra-Oral Wound Healing in Wistar Albino Rats

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ABSTRACT

Wound healing is an essential survival mechanism in humans. It comprises the following complexly regulated phases: hemostasis, inflammation, proliferation, and remodeling. An effective wound healing occurs if each phase progresses in an organized manner at an optimal rate, which may differ from species to species. To evaluate the wound healing activity 24 Adult Wistar albino rats were selected, divided into 3 groups of 8 each. Group1 served as control and received vehicle (normal saline-10 ml/kg, s.c), Group 2 and group 3 received Exenatide (250 micg/ml s.c) and Liraglutide (0.6mg/mls.c) respectively. The results of the present experimental study would be of some clinical relevance if they could be extrapolated to humans. Among experimented Incretin enhancers (Exentide and Liraglutide) had a wound pro-healing effect. Thus, they become the drug of choice in diabetic wound management, promoting wound healing, yet controlling blood sugar.

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INTRODUCTION:

Wound healing is an essential survival mechanism in humans. It comprises the following complexly regulated phases: hemostasis, inflammation, proliferation, and remodeling. An effective wound healing occurs if each phase progresses in an organized manner at an optimal rate, which may differ from species to species. Many local and systemic factors can impair oral wound healing. Successful oral soft tissue management requires knowledge about intraoral wound healing and its morphologic features. Tissues in the oral cavity follow an essentially identical pattern to complete the healing process without scar formation. In addition, the oral cavity serves as a unique setting thereby wound healing takes place in a saliva-filled environment containing millions of microorganisms.

Daily, dentists perform procedures such as exodontia and implant placement that rely on adequate wound healing. An improved understanding of the local and systemic factors that can impair oral wound healing can help clinicians to control these factors more accurately, resulting in improved patient outcomes.

Due to the pathological and physiological complexity of the healing process, the perfect regeneration of tissues is difficult to achieve. Therefore, the assessment of new treatments are needed and as well as the use of new strategies. The drugs, which decrease inflammation and enhance wound healing and their combinations have been suggested as promising treatments because they accelerate the healing process. Several drugs and chemicals have been studied to assess their wound healing property; many of them are promising for wound healing.

Early in the healing process, initiation of re-epithelialization is a critical step in the closure of the wound. The effect of insulin on wound healing has been confirmed in various animal and wound models, including cutaneous ulcerations, incision wounds, and fracture wounds. Insulin was found to enhance wound healing by faster wound contraction and re-epithelialization. Its pro-healing property in nondiabetic individuals is compromised by its hypoglycaemic side effects. Incretin enhancers could be useful in non-diabetic individuals as they act by increasing endogenous insulin secretion. However, Incretin enhancers are not investigated for their effect on non-diabetic individuals. Some of the literature has information about the pro-healing effect of Incretin enhancers on non-diabetic wounds in the oral cavity. Hence, the present study was planned to evaluate the wound healing property of Exenatide and Liraglutide on intraoral wound healing in non-diabetic Wistar albino rats.

AIM AND OBJECTIVE:

To evaluate wound healing property of Exenatide and Liraglutide on intra-oral wounds in non-diabetic Wistar albino rats.

MATERIALS AND METHODS

Animals

Healthy Wistar albino rats of either sex weighing 150-180 g were procured from the central animal house of the institute. The rats were housed in a clean polypropylene cage kept in experimental condition with 12 h alternate natural light and night cycles at a temperature maintained 23-25°C, with a relative humidity of 50-60%, and allowed free access to standard pellet food and water *ad libitum*. The study was initiated after obtaining approval from the Institutional Animal Ethics Committee (IAEC) (No. IAEC2/Desp.No.58/Dt. 3.01.14).

To evaluate the wound healing activity 24 Adult Wistar albino rats were selected, divided into 3 groups of 8 each. Group1 served as control and received vehicle (normal saline-10 ml/kg, s.c), Group 2 and group 3 received Exenatide (250 micg/ml s.c) and Liraglutide (0.6mg/mls.c) respectively.

Groups	**Treatment**
Control	Normal saline10 ml/kg, s.c
Exenatide	250 micg/ml s.c
Liraglutide	0.6 mg/ml s.c

Method:

Excision wound

The rats were kept starved overnight with free access to water. The animals were anesthetized as described by Kamper *et al.* under halothane anesthesia. The skin of the impressed area was excised to full thickness to obtain a wound area of about 500 mm^2. Measures were taken to control the 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 the 4^{th}, 8^{th}, 12^{th}, and 16^{th} day and subsequently on every alternate day till complete closure has occurred. Falling off 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) Biochemical analysis: 3 ml of blood was collected from a sacrificed animal of each group on the 8th and 16th day of the experiment, and the plasma was separated by centrifugation and stored at −90°C and subjected for the estimation of hydroxyproline content was measured by Elisa kit, following the rat-specific kit protocols (Cusabio, China).

3) Blood glucose estimation using a glucometer.

Statistical Analysis

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

RESULTS AND DISCUSSION:

Table No. 1: Percentage closure of excision wound on 4th, 8th, 12^{th,} and 16th day

Day	Control	Liragluitide	Exentide
4th	20.07±1.76	37.12±0.68*	38.27±0.86*
8th	63.57±3.04	84.53±1.71*	82.12±1.49*
12th	82.28±2.96	96.23±1.83*	93.20±1.80*
16th	86.87±1.63	98.81±1.0*	96.22±2.46*

Values Mean ±SEM* denotes significance **(p<0.05)** in of data Exenatide and Liraglutide, compared to control various time points studied.

Table No. 2: Time for complete epithelization and scar area

Days	Control	Liragluitide	Exentide
Time for complete epithelization (days)	21±0.4	16±0.2	15±0.2
Scar area (mm^2)	42.84±4.5	24.33±1.75*	25.00±1.58*

Values Mean ±SEM*denotes Significant ($p<0.05$) decrease in time (days) for epithelization in Exentide and Liraglutide compared to control.

Table No. 3: Effect of Drug on the normal wound healing rats Hydroxyproline levels plasma ng/ml

Day	Control	Liragluitide	Exentide
8th	491.36 ± 57	498.14 ± 1.75	480.86 ± 1.58
16th	504.79 ± 57	651.39± 1.75*	670.01± 1.58*

Values Mean ±SEM* denotes a Significant ($p<0.05$) Increase in hydroxyproline (HYP) content in Exentide and Liraglutide compared to control.

Table No. 4: Blood glucose level (mg/dl) on day 0, 8 and 16

Day	Control	Liragluitide	Exentide
0	110	106	108
8th	105	101	99
16th	101	98	97

In this study, Exenatide and Liraglutide have been investigated for their influence on intra-oral wounds in non-diabetic Wistar albino rats.

When compared to the control, Exentide and Liraglutide treated groups showed a significant percentage of wound closure at the 4th, 8th, 12th, and 16th days respectively with the level of significance $P \leq 0.5$ and that showed their pro-wound healing property (Table 1).

The epithelialization was considered to be complete, once the scab falls off without any raw area. Total time for complete epithelialization taken was 16 ± 0.2 and 15 ± 0.2 days with 24.33 ± 1.75and 25.00 ±1.58 of scar area (mm2) with Exentide and Liraglutide -treated groups respectively when compared with 21 ± 0.4 days and 42.84 ± 4.5 scar area of the

control group. Thus, early and small scar areas of Exentide and Liraglutide groups indicated better collagenases (Table 2).

As far as biochemical parameters were concerned, on day 16, in line with earlier results, the serum estimation showed again Exentide and Liraglutide groups having significant ($P < 0.05$) serum hydroxyproline concentration, suggesting enhanced collagen synthesis and increased vascularity of the wound tissue, respectively (Table 3).

No significant differences in blood glucose levels (mg/dl) were observed after 8 or 16 days of drug treatment in all treatment groups (Table 4).

The present study attempted to explore their pro-healing activity on the non-diabetic wound and also to throw some light on their mechanism of action for the same. It was angiogenesis (↑iNOS) and collagenases (↑hydroxyproline), that mediated their wound pro-healing action. However, the variable pro-wound healing property of Incretin enhancers could be explained based on the pharmacokinetic and pharmacodynamic properties of the drug. However, the present study would have included more wound models and 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, to treat non-healing wounds in non-diabetic patients in the oral cavity. Further studies are required to state the wound pro-healing activity of these drugs further clinical trials to evaluate intraoral wound healing is in need.

CONCLUSION

The results of the present experimental study would be of some clinical relevance if they could be extrapolated to humans. Among experimented Incretin enhancers (Exentide and Liraglutide) had a 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 intraoral wounds, exploiting their pro-healing activity. However, further clinical studies are required for their new role.

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