INFLUENCE OF SOME GROWTH HORMONE MODULATORS ON WOUND HEALING IN WISTAR RATS.

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Summary

Clonidine, Bromocriptine, Levodopa have been reported to release growth hormone (GH), which is essential for cell proliferation and tissue growth. Wound healing essentially involves cell proliferation and is said to be promoted by growth hormone. The drugs like clonidine, bromocriptine and levodopa have been reported to release growth hormones and therefore they would be expected to promote the wound healing. Due to paucity of such information, in the present study the above drugs were investigated in excision, resutured incision and dead space wounds in male Wistar rats. Of all the three drugs tested only clonidine significantly enhanced wound healing in all the wound models, on the contrary bromocriptine retarded the healing while levodopa failed to produce significant effect on healing of all the three wound models.

Key words: Bromocriptine, Clonidine, L-Dihydroxyphenylalanine, Growth hormone, Wound Healing

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Introduction

Growth hormone, a peptide that stimulates growth and cell reproduction in humans and other animals. Secreted from anterior pituitary gland, which is regulated by many factors like Growth hormone releasing hormone (GHRH), somatostatin, glucose, cortisol, etc.

Wound, a common clinical entity as old as mankind, often poses problems in clinical practice. Wound healing is the basic response of living tissue to injury and is influenced by a number of factors including hormones and drugs. Some of the well established factors influencing the wound healing are local factors like surgical technique, blood supply, suture material, suture technique, infection, etc. and systemic diseases like malnutrition, malignancy, metabolic disorders like diabetes mellitus and variety of drugs like cyclophosphamide, 5-flurouracil.[1]

Growth hormone is reported to promote healing of chronic ulcers in diabetic patients[2], as well as that of experimental fractures in rodents[3]. However its use in clinical practice has not been possible because of its non availability and prohibitive cost.

Drugs like clonidine an alpla$_2$ agonist has been used in clinical practice to promote the growth in children with short stature due to growth hormone deficiency.[4] Levodopa, a dopaminergic prodrug used in parkinsonism is also reported to increase growth hormone level[4,5], while another dopaminergic agonist bromocriptine has been reported to possess dual effect on growth hormone levels.[6,7] An attempt has been made in the present study to investigate the possible prohealing effect of these drugs which are known to promote growth hormone release.

Materials and Methods.

Animals and drug treatment

Healthy male Wistar rats weighing 175±25 g, were housed individually acclimatized to laboratory for a week under 12;12 light dark cycle. The animals were fed on standard pellet diet (Amrut brand) and water *ad lib*, where as they were starved over night before the day of experimentation. The study was approved by the institutional animal ethical committee constituted as per CPCSEA guidelines. Depilation at wounding site was done a day before wounding.

Wound models: Resutured incision wounds were inflicted with two 6cm long para vertebral parallel incisions under light ether anesthesia as described earlier.[8] Sutures were removed on the 7th day; breaking strength was measured on the 10th post wound day, by the continuous water flow technique as described by Lee.[9] Venous blood was collected for glucose estimation with the help of glucometer. Adrenals and thymus were removed before sacrificing the animals.

Excision wounds were inflicted as described by the method of Morton and Malone[10], by excising the full thickness circular skin (approximately 500 mm^2) from the nape of neck under

ether anesthesia. Wound closure rate and epithelization time were assessed by tracing the wound on polythene paper from wounding day, followed by 4, 8, 12, 16, 18, 20th day and subsequently on alternate days till complete epithelization (fall of scab without only raw area). Similarly scars were traced on complete epithelization to assess wound contraction by noting scar size and shape.

Dead space wounds were inflicted by implanting sterile cotton pellets (10mg) and cylindrical grass piths (2.5 cm X 0.3 cm) *s.c.* in the groin and axilla alternatively by the technique of D'Arcy *et al* as described by Turner. [11] On the 10th post-wounding day, all the granulation tissues were removed under light ether anesthesia. Cotton pellet granulomas were dried at 60^0 C overnight to record the dry weight which was expressed as mg/ 100g body weight as suggested by Dipasuale and Moli.[12] One of the granulation tissues over the grass piths was opened and trimmed to a rectangular piece for estimation of breaking strength, whereas the other piece was preserved in 10% formalin for histological studies. Before sacrificing the animals venous blood was collected for glucose estimation, while adrenals and thymus were removed to note their weights.

All the wounding procedures carried out aseptically and none of the animals received local or systemic antimicrobials.

After wounding, the animals were divided into control and treatment groups (n=6, in each) for each wound model to receive treatments. The drugs were administered orally in their therapeutic equivalent doses as calculated with the help of conversion table devised by Pagets and Branes.[13] the dose of clonidine (100µg/kg), bromocriptine (2mg/kg)and levodopa (700 mg/kg) were suspended in 2% gum acacia and were administered once daily in the volume of 5ml/kg, while control groups received equal volume of the vehicle. The duration of the treatment was 10 days for animals inflicted with incision and dead space wounds, whereas it was continued in animals bearing excision wounds till their complete course.

Statistical analysis

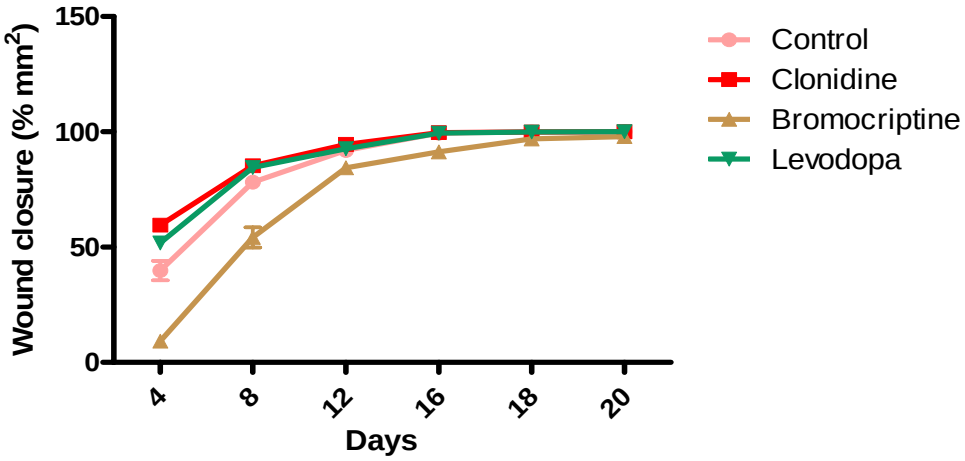
The results were analysed by ANOVA follwed by post hoc Dunnet's test and expressed as mean±SEM. $p<0.05$ was considered as significant.

Results

Excision wound closure rate was significantly ($p<0.05$) increased in clonidine treated group up to 16th day, whereas bromocriptine significantly ($p<0.01$) retarded the wound closure throughout the study as compared to that of control. While levodopa treated group failed to show any significant closure on 4th and 8th day. Epithelisation period was 17 days in clonidine group, which was significantly ($p<0.05$) less as compared to 18 days in control group, whereas bromocriptine prolong time for epithelisation (22.3 days). While it was 18 days in levodopa group which was compared to that of control. (Fig. 1) Scar area was significantly ($p<0.05$) reduced in clonidine treated group and was significantly ($p<0.01$) increased in bromocriptine group as compared to that of control which was compared to that of levodopa. (Table. 1)

The mean breaking strength of resutured incision wound and granulation tissue was significantly ($p<0.01$) increased in clonidine, whereas significantly ($p<0.05$) decreased in bromocriptine and no significance was noted in levodopa treated groups when compared to that of control. The healing of space wounds as assessed by granulation tissue dry weight was significantly ($p<0.05$) promoted by clonidine, whereas bromocriptine and levodopa treated groups did not produce any significant difference when compared to that of control.

Fig. 1 Effect of growth hormone modulators on wound closure in excision wounds



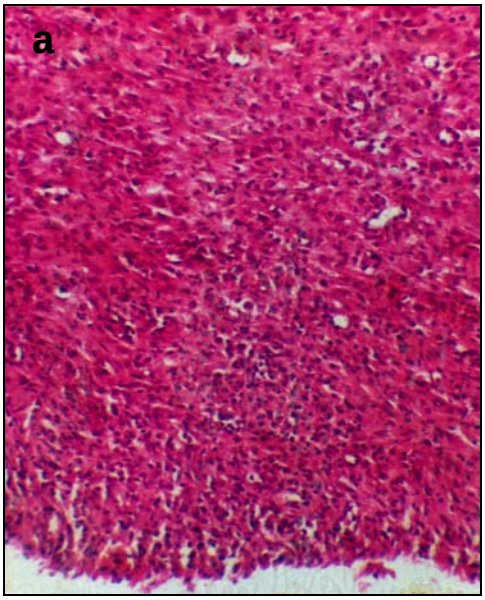
Histological studies

Increased number of fibroblasts and collagen tissue in clonidine (Fig 2c) treated group, whereas levodopa (Fig 2b) treated group did not show any significant improvement when compared to that of control (Fig 2a). In contrast to both bromocriptine (Fig 2d) treated group showed sever fat necrosis and calcification.

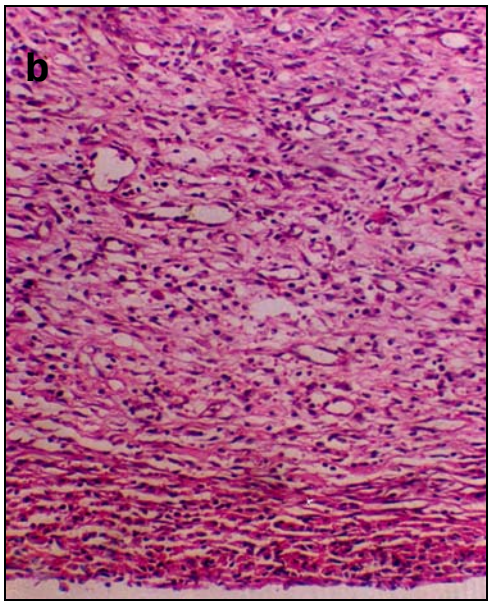
In Bromocriptine treated group the histopathological studies of some vital organs (kidney, liver, lung) were carried out. Where in kidney showed areas of haemorrhages and necrosis of tubules (Fig 3a); liver showed foci of necrosis with intense inflammation (Fig 3b); lung showed acute congestion with perivascular deposition of blue amorphous material, surrounded by dense inflammatory cells. (Fig 3c)

The blood glucose levels were significantly ($p<0.01$) increased in clonidine (109.0±8.6) as well as in bromocriptine (102.8±7.8) groups, whereas levodopa (82.0±5.8) treated group did not show any significant variation as compared to that of control(73.0±3.7).(Fig. 4) The thymus weight was significantly ($p<0.01$) increased in clonidine (112.7±5.7) whereas there was significant ($p<0.001$) decreased in bromocriptine (40.8±2.8) groups when compared to the corresponding control values (76.4±4.9). The adrenal weight were significantly ($p<0.001$) decreased in clonidine (22.6±1.4) treated group, whereas significantly ($p<0.01$) increased in bromocriptine (36.0±0.7) treated group as compared to that of control (31.9±0.8). The mean adrenal and thymus weights in levodopa treated groups 27.3±2.0 and 79.2±5.4 were comparable to that of respective controls. (Fig. 5)

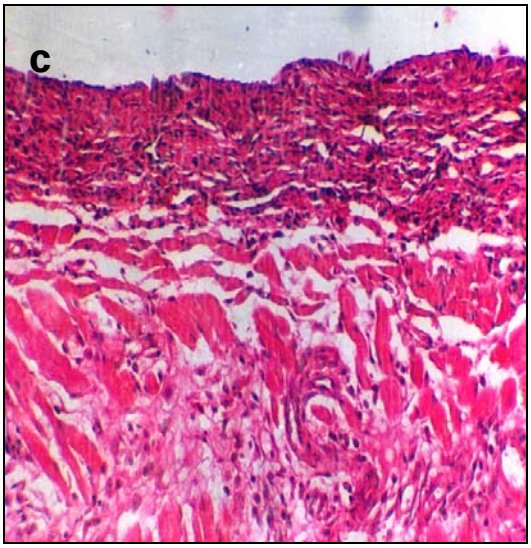
Figure 2. photomicrographs of granulation tissue of different drug treated groups. H & E stain (100X)



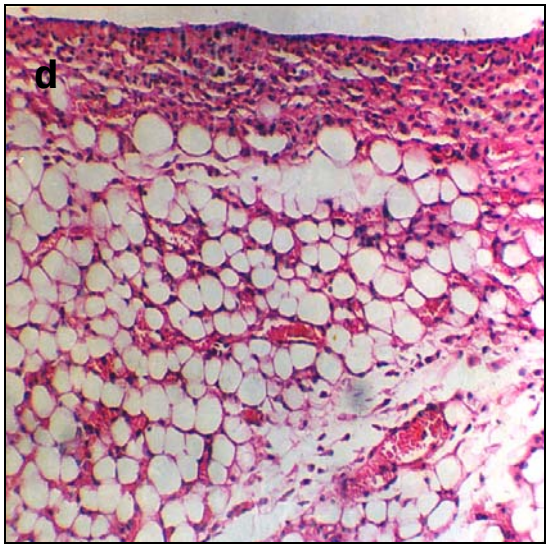
a. Control



b. Levodopa



c. Clonidine



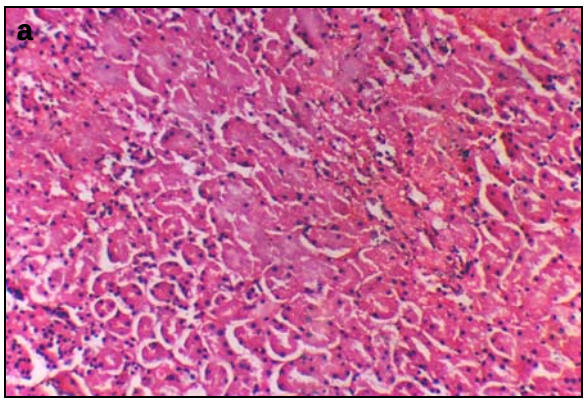
d. Bromocriptine

Table 1. effect of various treatments on excision wound, resutured incision and dead space wounds.

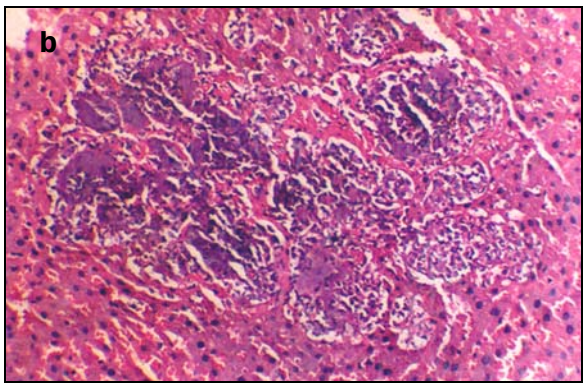
Groups	Wound closure (%/ mm^2) on day						Complete epithelisation (days)	Scar area (mm^2)	Resutures wound breaking strength (g)	Granulation tissue	
	4	8	12	16	18	20				Breaking strength (g)	Dry weight (mg%)
Control	39.8 ±4.3	78.2 ±2.2	91.9 ±0.9	99.4 ±0.1	99.9 ±0.1	100 ±0.0	18.5 ±0.6	45.0 ±3.8	227.8 ±12.4	322.8 ±37.5	24.6 ±2.3
Clonidine (100µg/kg)	59.5 ±2.4**	85.3 ±1.1*	94.5 ±0.5**	99.7 ±0.1*	99.9 ±0.1	100 ±0.0	17.0 ±0.4*	32.3 ±3.1*	318.0 ±15.3**	467.1 ±28.0**	33.7 ±2.9*
Bromocriptine (2mg/kg)	9.2 ±2.2***	54.2 ±4.4***	84.4 ±2.3*	91.3 ±1.9*	96.9 ±0.8**	97.9 ±0.7**	22.3 ±1.4	61.5 ±2.8**	183.9 ±12.9*	233.0 ±9.0*	26.9 ±0.6
Levodopa (700mg/kg)	51.8 ±2.5*	84.5 ±1.5*	92.7 ±0.9	99.4 ±0.1	99.9 ±0.1	100 ±0.0	18.2 ±0.5	49.3 ±6.3	253.5 ±6.5	333.8 ±12.2	28.8 ±0.8

*p<0.05, **p<0.01 and ***p<0.001

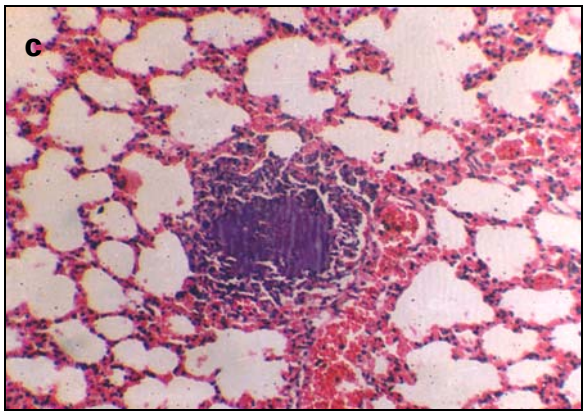
Figure 3. Microphotographs of histopathological study of some vital organs in bromocriptine treated group. H & E stain (100X)



a. Kidney



b. Liver



c. Lung

Fig. 4 Blood glucose levels in different drug treated groups

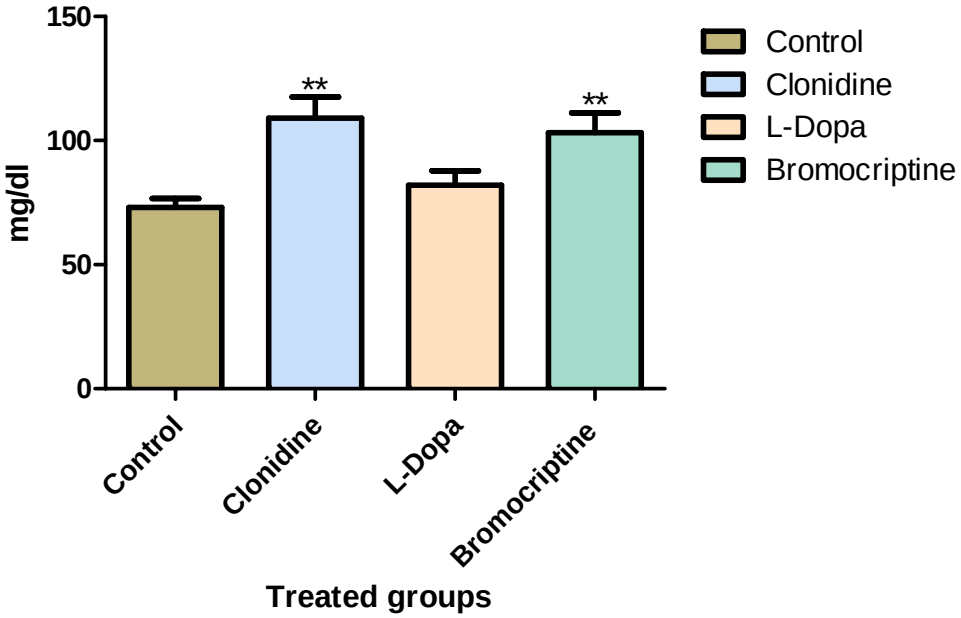
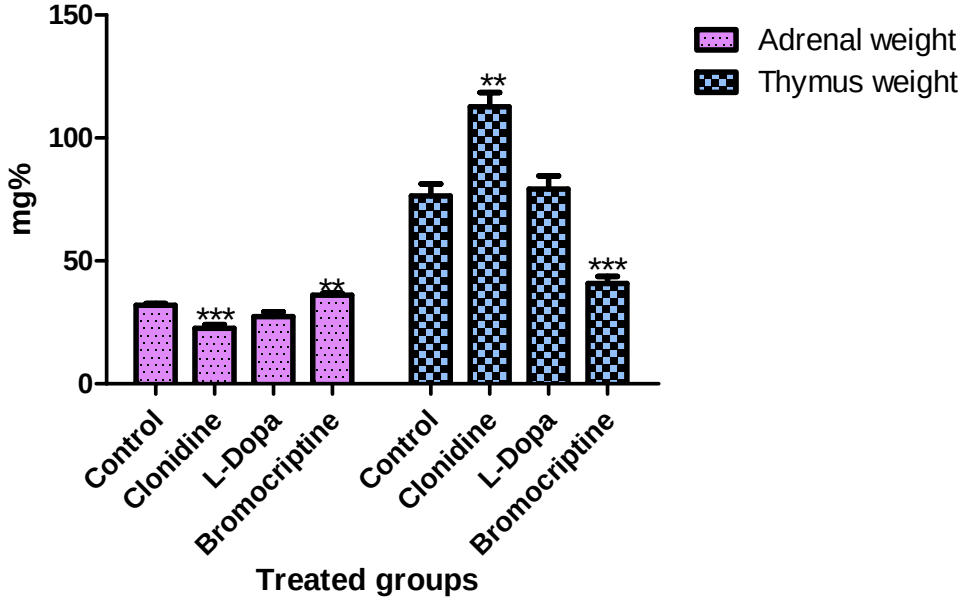


Fig. 5 Adrenal and Thymus weights in different treatment groups.



Discussion

Wound healing is the basic response of living tissues to injury. Growth hormone has been reported to have a beneficial influence on healing of poorly healing wounds. GH enhances chondroitin sulphate helps conversion of proline to hydroxyproline thereby enhancing collagen synthesis.[14] It has been reported to increase overall protein synthesis involving its action on DNA and the production of specific mRNA. Enhanced DNA synthesis with subsequent mitosis is also stimulated resulting in proliferation of various tissues.

Results of the present study clearly indicated that clonidine promoted healing of all the wound models employed. Eventhough clonidine is reported to have no influence on resutured incision wounds when given in the daily dose of 90µg/kg body weight,[15] in the present study it has shown significant prohealing activity.

The discrepancy could be explained by difference in the dose and the route of administration. Clonidine increases the growth hormone secretion by stimulating the release of stomatomedins.[16] The mechanism of clonidine in release of GH is reported to be central alpha adrenoceptor mediated.[17] It is reported to suppress plasma cortisol level [18,19] and increases blood glucose level[20], in the present study clonidine has promoted wound healing probably by releasing GH as indicated by hyperglycemia as well as thymotropic effect, typical of GH action and by decreased cortisol activity as indicated by decreased adrenal weights.

Previous reports suggest that levodopa augments the release of GH probably by increasing the hypothalamic secretion of GHRH[4,5,21], however it may suppress release in acromegaly. It is also reported to have decreased the plasma cortisol concentration[22] and hypoglycemic effect due to its peripheral effects via dopamine or other metabolites.[23] But in the present study levodopa has not produced any significant influence on different parameters of wound healing nor on blood glucose, thymus and adrenal weights.

Bromocriptine is reported to have a dual effect on the release of GH, normally it increases the release but in acromegaly it decreases the release. It is reported to reduce growth of GH3 (growth hormone releasing tumor) cells. It is also reported to act by inhibiting the effect of somatostatin and allowing the hypothalamic GHRH to have great stimulatory action[24] on anterior pituitary. The histopathological changes observed in kidney, liver and lung are in agreement with the earlier reports, where in it has been reported that bromocriptine produces toxic effects.[25,26,27,28,29] In the preent study bromocriptine has significantly retarded thymus weight, indicating decreased GH activity, while hyperglycemia and increased adrenal weights indicate the increased cortisol activity by bromocriptine.

Mortality rate in control as well as clonidine and levodopa treated animals was nil, while bromocriptine group it was 13% by 10^{th} day and 35% by 20^{th} day. The histopathology of some vital organs like kidney, liver and lung showed toxic effects as well as the animals showed sever piloerection, haemorrhagic conjunctivitis and depressed motor activity prior to death.

Conclusion

The results of the present study if extrapolated to humans suggest clonidine to be an useful drug to promote wound healing. Clonidine if used to heal the chronic ulcers, more often in diabetics the hyperglycemia effect can be overcome by increasing the dose of hypoglycemic, which needs to be confirmed clinically.

Acknowledgement

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