INFLUENCE OF ORAL ANTI-DIABETIC AGENTS ON WOUND HEALING IN EUGLYCEMIC MALE WISTAR RATS

Ambrish C[1], Torgal SS[1], Patil PA[1], Malur PR[2], Hiremath SV[1]

[1] *Department of Pharmacology, J.N. Medical College, Belgaum, India*

[2]*Department of Pathology, J.N.Medical College, Belgaum, India*

Summary

Oral hypoglycemic drugs like metformin apart from its antihyperglycemic property has been reported to increase angiogenesis & promote wound healing in diabetics. Angiogenesis is required for wound healing hence, any drug which promotes angiogenesis may promote healing. There are controversial reports regarding rosiglitazone on angiogenesis. There is no information about acarbose, another oral antihyperglycemic on wound healing. Paucity of such information, the present study was planned to explore the influence of metformin rosiglitazone and acarbose on excision, resutured incision & dead space wounds in euglycemic male Wistar rats. Metformin and acarbose significantly promoted the healing process in all the three wound models studied. Histopathological studies revealed increased collagen content and granulation tissue in metformin and acarbose treated group compared to control. Metformin and acarbose promoted healing of excision, incision and dead space wounds without significantly altering the blood glucose levels in euglycemic rats. Prohealing activity of these drugs needs to be confirmed clinically.

Key words - wound healing, metformin, acarbose, rosiglitazone

***Corresponding Author**

P.A. Patil
Professor,
Department of Pharmacology,
J.N. Medical College, Belgaum-590010. India
e-mail: drpapatil@yahoo.co.in

Introduction

Wound being one of the common clinical entities, often challenges the clinicians when associated with diabetes mellitus .Insulin, in addition to its hypoglycaemic activity is known to promote wound healing by increasing cellular proliferation, mineralization of the tissue, angiogenesis & decreased apoptosis in diabetic wound.[1]

Glibenclamide, which is known to increase the release the release of insulin, could be expected to promote wound healing. On the contrary, it has been reported to aggravate gastric ulceration.[2] Metformin apart from its antihyperglycemic property has been reported to increase angiogenesis & promote wound healing in diabetics.[3] Angiogenesis is required for wound healing, hence any drug which promotes angiogenesis may promote healing. Metformin by its virtue of its effect on angiogenesis could be expected to promote healing in non- diabetic rats. There are controversial reports regarding rosiglitazone on angiogenesis.[4,5] Guargum, has been reported to possess prohealing effect on gastric ulcers.[6]

There is scanty information about acarbose, another oral antihyperglycemic on wound healing. Paucity of such information, the present study was planned to explore the influence of metformin rosiglitazone and acarbose on excision, resutured incision & dead space wounds in euglycemic male Wistar rats.

Materials and Methods.

Animals and drug treatment

Healthy male euglycemic 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 with free access to water. The study was approved by the Institutional Animal Ethics 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 anaesthesia as described by Erlich and Hunt.[7] Sutures were removed on the 7^{th} day; breaking strength was measured on the 10^{th} post wound day, by the continuous water flow technique as described by Lee.[8] Venous blood was collected from rat tail vein for glucose estimation with the help of glucometer.

Excision wounds were inflicted as described by the method of Morton and Malone[9], 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, 20^{th} 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.[10] 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.[11] 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 animal's venous blood was collected for glucose estimation.

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 Barnes.[12] the dose of metformin (180mg/kg), rosiglitazone (0.72mg/kg) and acarbose (27mg/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

***Resutured incision wounds*:**

The mean breaking strength of wounds in control animals were 288.6 ± 8.449g while metformin ($p<0.001$) and acarbose ($p<0.01$) showed significant increase in breaking strength 410.0 ± 14.71g, 342.1 ± 14.57g respectively, while rosiglitazone group 310.6 ± 6.860g did not show any difference as compared to control (Table 1).

***Dead space wounds*:**

Mean dry weight of granuloma in control animals was 53.65 ± 4.27mg, 85.59 ± 4.96mg, 67.08 ± 5.57mg and 73.92 ± 4.55mg metformin, rosiglitazone and acarbose treated groups respectively. There was significant ($p<0.001$) increase in the granuloma dry weight of metformin treated group while acarbose also showed significant ($p<0.01$) (Table I). Breaking strength of the granuloma in the control group was 210.0 ± 11.55g, while metformin, rosiglitazone and acarbose treated groups it was 310.0 ± 9.309g, 245.0 ± 12.32g, 256.7 ± 13.33g, respectively, indicating significant ($p<0.001$) increase in granuloma breaking of metformin group and ($p<0.05$) in acarbose treated group (Table 1).

Hydroxyproline content of 300 mg of granulation tissue was estimated and expressed as µg/300 gm of granulation tissue. It was significantly increased in the metformin ($p < 0.001$) and acarbose ($p < 0.01$) treated group with a mean value of 10.17 ± 0.307 mcg and 7.333 ± 0.494 mcg respectively as compared to the control group with a value of 5.500 ± 0.428 mcg. The mean values of the rosiglitazone treated group which were 5.600 ± 0.328 mcg did not differ significantly from that of control group (Table 1).

Table 1. Effect of various healing agents on resutured incision and dead space wounds

Group (n=6)	Resutured incision wound breaking strength (g)	Granulation tissue		
		Breaking strength (g)	Dry weight (mg % of body weight)	Hydroxyproline (µg/300 mg of wet granulation tissue)
Control (saline)	288.6±8.449	210.0±11.55	53.65±4.277	5.500 ± 0.428
Metformin	410.0±14.71***	310.0±9.309***	85.59±4.963***	10.17±0.307***
Rosiglitazone	310.6±6.860	245.0±12.32	67.08±5.574	5.600±0.328
Acarbose	342.1±14.57 **	256.7±13.33 *	73.92±4.558 **	7.333±0.494*

***Excision wounds*:**

The rate of wound closure in metformin and acarbose treated animals was significantly ($p<0.01$) ($p<0.05$) respectively more on 4th, 12th, 16th, day as compared to that of control. However, there was no significant change in rate of wound closure in rosiglitazone treated animals as compared to control animals. The time for complete epithelization (days) in control group was 20.00 ± 0.447. In comparison to this, metformin17.33 ± 0.210 and acarbose 18.33 ± 0.494 treated group took significantly ($p<0.001$, $p<0.05$) less time for complete epithelization, while rosiglitazone 19.00 ± 0.365 shows no significant changes as compared to control (Table 2).

The mean scar area (mm^2) in the control group was 50.00 ± 4.97. The same in metformin18.50 ± 1.893 and acarbose 26.83 ± 1.797 significantly ($p<0.01$) reduced the scar area (Table 2). Scar were stellate shape in metformin and acarbose group while in control and rosiglitazone treated groups were oval or oblong. Significant reduced scar area in metformin and acarbose group indicates maximum contraction of wound as compared to other groups.

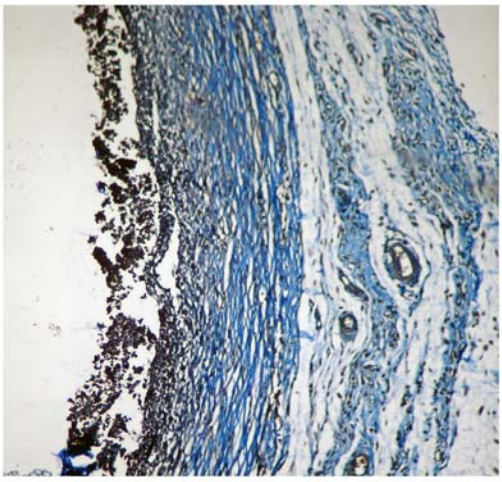
Table 2. Effect of various healing agents on excision wounds

Group (n=6)	Wound closure (% of original area) in mm2 on day (Mean ± SEM)						
	4	8	12	16	18	complete closure (days)	Scar area (mm^2)
Control	19.3±2.74	63.5±3.04	83.9±0.39	95.4±0.69	98.3±0.18	20.0±0.45	50.0±4.97
Metformin (180mg/kg)	32.6±3.85*	77.9±1.76**	89.3±1.22**	98.1±0.36**	100.0±0.0***	17.3±0.21***	18.5±1.89***
Rosiglitazone (0.72mg/kg)	23.3 ±1.78	71.6±2.24	85.1±1.36	96.9±0.43	98.3±0.33	19.0 ±0.36	40.3±1.59
Acarbose (27mg/kg)	29.3±4.21	74.8±1.92 *	87.9±1.48*	97.7±0.51*	99.2± 0.29*	18.3± 0.49*	26.8±1.79**

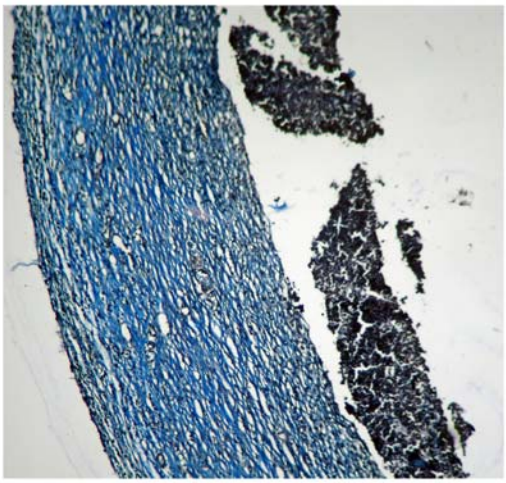
*p<0.05, **p<0.01 and ***p<0.001

The histopathological examination of granulation tissue with Masson's Trichrome Stain (MTS) 20X showed plenty of granulation tissue with increased amount of collagen in metformin treated group as compared to that of control. The amount of granulation tissue and collagen content in rosiglitazone treated group were almost comparable to that of control. The amount of granulation tissue and collagen content in acarbose treated group were increased comparable to that of control group (Fig 1).

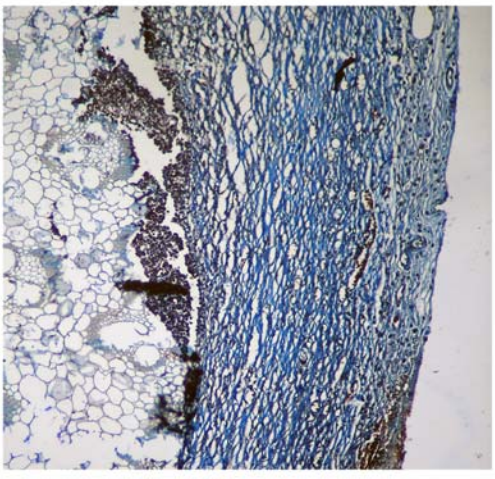
Figure 1. Photomicrographs of Granulation tissue (MTS, 20X)



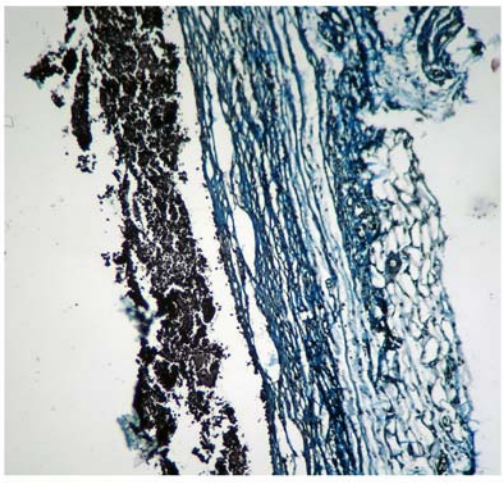
a. Control



b. Metformin



c. Rosiglitazone



d. Acarbose

Discussion

The main objective of this study is to evaluate the influence of anti diabetic drugs like metformin, rosiglitazone and acarbose on healing of excision, resutured incision and dead space wounds in euglycemic male Wistar rats.

The findings of the present study excision wound model clearly indicated that the entire drug treated groups except rosiglitazone, significantly enhanced wound healing as assessed by wound closure rate, time taken for complete epithelization and reduction in scar size. There are scanty reports regarding the influence of metformin, rosiglitazone and acarbose on excision wound.

In resutured incision wound model metformin and acarbose treated groups significantly increased the strength required to break 10 day old resutured incision wound, while rosiglitazone treated group though showed an increase in breaking strength but was not significant as compared to control group. The reports regarding the similar studies could not be traced in literature.

In dead space wound studies metformin and acarbose treated groups significantly increased the granuloma dry weight, granuloma breaking strength and collagen content as indicated by hydroxyproline estimation. The histopathological findings of metformin treated group showed marked increase in granulation tissue and onset of collagen when compared to control group. Rosiglitazone treated group showed a slight increase in collagen content as compared to control. While, collagen content and granulation tissue was more in acarbose treated group when compared to control groups.

Estimation of blood glucose levels at the end of the study in all the drug treated groups did not show any significant variation as compared to control groups.

On the basis of above findings it could be inferred that metformin and acarbose but not rosiglitazone have significantly promoted healing process in all the three wound models employed in the present study.

The present study was not aimed to probe into the detailed mechanism of action of the drugs. However, based on the pharmacological action as reported in earlier studies the following mechanisms could be proposed for their effect on wound healing.

The prohealing activity of metformin in diabetic individuals may be due to its antihyperglycemic activity as diabetic patients are more prone for infection,[13] apart from this metformin is also known to increase angiogenesis through activation of AMPK in hepatocytes.[3]Studies have also shown that metformin possesses antioxidant property which may favour for its prohealing activity.[14]

Rosiglitazone has not shown any improvement in the present as compared to control study, this may be due to its anti-inflammatory activity.[15]The other drug used in the present study acarbose though has promoted wound healing the exact mechanism behind it could not be traced.

Conclusion

Prohealing activity of metformin and acarbose could be exploited to treat non healing wounds in non diabetic patients which accounts for more than 70% of chronic non healing ulcers, since they are devoid of severe hypoglycemic adverse effects. To exploit the prohealing activity of these drugs further clinical trials are needed.

Acknowledgement

The authors are grateful to the Principal, J. N. Medical College, Belgaum for providing facilities. Thanks to Mr. A. V. Karvekar and Mr. M. D. Kankanwadi for their skilful assistance.

References

1. Gandhi A, Beam HA, Parsons JP, Lin SS. The effect of local insulin delivery on diabetic fracture healing. Bone. 2005;37(4):482-490.
2. Toroudi HP, Rahgozar M, Bakhtiarian A, Djahanguiri B. Potassium channel modulators and indomethacin-induced gastric ulceration in rats. Scand J Gastroenterol. 1999;34(10):962-966.
3. Zhou G, Myers R, Ying Li, Chen Y. Role of AMPK in mechanism of metformin action. J Clin Invest.2001; 108(8):1167-1174.
4. Sheu WH, Ou HC, Chou FP, Lin TM, Yang CH. Rosiglitazone inhibits endothelial proliferation and angiogenesis. Life Sci. 2006;78(13):1520-1528.
5. Chu k, Lee ST, Koo JS, Jung KH, Kim M, Roh JK, et al. PPR-gamma-agonist. Rosiglitazone promotes angiogenesis after focal cerebral ischemia. Brain Res. 2006; 1093(1):208-218.
6. Harju E, Sanjanti J. Protective effect of nutrients against stress induced gastric ulcers in the rats. Surg Gynecol Obstet.1987; 165:530-534.
7. Ehrlich HP, Hunt TK. Effect of Vit A and glucocorticoids on inflammation and collagen synthesis. Ann Surg. 1973; 222:119-126.
8. Lee KH. Studies on mechanism of action of salisylates. J Phar Sci. 1968; 57:1042-1043.
9. Mortan JJP, Malone MH. Evaluation of vulnary activity by an open wound procedure in rats. Arch Int Pharmacodyn. 1972; 196:117-126.
10. D'Arcy PF, Howard EM, Muggleton PW, Townsend SB. The anti-inflammatory action of griseofulvin in experimental animals. *J Pharm Pharmacol* 1960; *12* : 659-65.
11. Dipasquale G, Meli A. Effect of body weight changes on the formation of cotton pellet induced granuloma. *J Pharm Pharmacol* 1965; *17* : 379-82.
12. Pagets GE, Barnes JM. Toxicity tests. In : Laurence DR Bacharach AL, editors. *Evaluation of drug activities: pharmacometrics*. London and New York: Academic Press; 1964 p. 134-66.
13. Ridray S. Hyperinsulinemia and smooth muscle cell proliferation. Int J Obes Relat Metab Diord. 1995; 1:39-51.
14. Ramazan, Mehmet, Fatih. Effect of metformin or gliclazide on lipid peroxidation and antioxidant levels in diabetes millets. Turk J Med Sci. 2008; 38:6.
15. Franz Meisner, Daniel Walcher, Florence Gizard, Xaver Kapfer. Effect of Rosiglitazone Treatment on Plaque Inflammation and Collagen Content in Nondiabetic Patients. Arteriosclerosis, Thrombosis, and Vascular Biology. 2006; 26:845.