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Research Article

Effect of Metformin on Wound Healing in Excisional Wound Model

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ABSTRACT

Introduction: Despite many available therapies for wound healing, it's treatment still remains unsatisfactory. Metformin by virtue of it's various actions like anti-inflammatory, anti oxidant and effect on hemostatic mechanisms can prove to be promising for the wound healing especially in diabetics. Materials and methods: Wistar rats were divided into two groups, (n=6 each group) and a wound was created which was treated with petroleum jelly in group A and petroleum jelly based Metformin cream in group B. The wound healing and epithelisation of wound was evaluated at day 4,8,12,16 and 20. Results: Group A showed an average wound size of 348±13.0 on day 4, 278±10.5 on day 8, 119±9.8 on day 12, 86±9.2 on day 16 and 100% wound healing was observed day 20 onwards. Group B showed an average wound measurement of 310±10.0 on day 4, 231±9.0 on day 8, 83±8.5 on day 12 and complete wound healing was observed day 16 onwards in the test group. Conclusion: Metformin proved to be useful in wound healing.

Keywords: Metformin, Wistar Rats, Wound healing, AMPK.

INTRODUCTION

Wound healing is a complex process which passes through the stages of hemostasis, inflammation, proliferation and remodeling or maturation. Hemostasis and inflammation are protective, reactive and remaining two are corrective in nature. Hemostasis minimizes the local blood loss but it also prevents the defence mechanism of body reaching at the site of need locally[1].

Despite many available therapies for wound healing, its treatment still remains unsatisfactory. Metformin by virtue of its various mechanisms like anti-inflammatory[2], anti oxidant[3] and effect on hemostatic mechanisms[4,5] can prove promising for the wound healing especially in diabetics. These effects of metformin are independent of its anti diabetic action. Hence metformin was selected to study it's action on wound healing in excisional wound model in rat.

MATERIAL AND METHODS

This study was carried out in the department of pharmacology and central animal house Bharati vidyapeeth deemed university medical college and hospital Sangli after getting approval from institutional animal ethical committee.[IAEC]

Study was conducted in two groups of wistar rats, selected randomly each consisting of 6 animals and every rat weighing between 200-250gms.Two groups of six rats each were selected, one as control group which received only petroleum jelly and second for test drug metformin cream, to be applied locally. Each animal was housed separately in an individual cage. Light and dark cycle was maintained. They had free access to standard pellet diet and water ad libitum except 12 hours prior to the creation of wound and until rat regained full consciousness after the wound creation. Experiments were carried out between 9.00 to 16.00 hours.

Back of rats were shaved and on the next day surgical intervention was carried out under general anaesthesia with Thiopental sodium in the dose of 25mg/kg body weight. 500mm^2 full thickness circular skin was excised with scalpel blade over the nape of neck. Control animals were treated with local application of petroleum jelly and test animals received local application of 1% metformin cream. 1% metformin cream was prepared by adding 1gm of metformin powder in 99gm of petroleum jelly. Drug and petroleum jelly local application was carried out in the respective group, from the next day of wounding and was continued till the epithelisation with no raw area left behind. Wound area was traced on polythene paper and was measured with the help of planimeter.

Two parameters studied were wound measurement and period of epithelisation. Wound measurement was done from day 4th and then on day 8th, 12th and 16th and when necessary thereafter.

Period of epithelisation was observed in days.

Statistical analysis was carried out by independent t test to compare the wound healing i.e average wound measurements and average time of epithelisation between the control and test group. Probability of $p<0.05$ was considered to be significant. All the analysis was conducted using SPSS ver 13.0.

RESULTS

Group A showed an average wound size of 348±13.0 on day 4, 278±10.5 on day 8, 119±9.8 on day 12, 86±9.2 on day 16 and 100% wound healing was observed day 20 onwards. Group B showed an average wound measurement of 310±10.0 on day 4, 231±9.0 on day 8,

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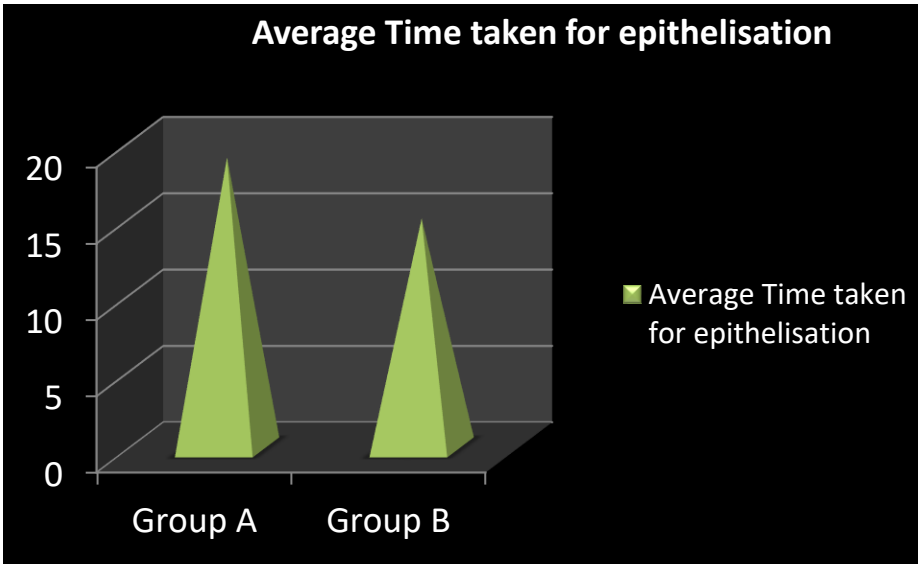
Table 1: Comparison of average wound measurement between the control and test group.

Groups	Wound area mm^2			
	Day 4	Day 8	Day 12	Day 16
Group A (control group)	348±13.0	278±10.5	119±9.8	86±9.2
Group B (test group)	310±10.0	231±9.0	83±8.5	Complete wound healing (0.0±0.0)
P value	< 0.001	<0.002	<0.002	< 001

*p value <0.05 is considered significant
Independent t test



Graph 1: Average time taken for epithelisation of wound.

83±8.5 on day 12 and complete wound healing was observed day 16 onwards in the test group. Independent t test results showed a statistically significant difference in the average wound measurements between both the groups at day 4, 8, 12, 16. (table 1)

The average time taken for epithelisation of the wound was found to be on an average of 19 ± 1.0 for group A and 15± 1.0 for group B. Independent t test shows a significant difference between the average time taken for epithelisation of the wound between group A and Group B. (Graph 1)

DISCUSSION

Our study reveals that metformin has the potential of wound healing as it was seen that, this drug significantly enhanced wound healing. There are not many studies regarding the effect of metformin on wound healing and that too on cutaneous wound healing.

When metformin was applied locally in sustained release form, using biodegradable metformin drug eluting membrane, had shown improvement in wound repair[6]. Metformin was tried for peri implant wounds in rat model of type 2 diabetes mellitus which showed improved wound healing along with control in blood sugar[7]. But study done by Fatima et al had an observation that metformin treated animal and diabetic foot ulcer patient had shown increase in wound area, but also showed protective effect on the need for amputation which was correlated with the anti-inflammatory effect of metformin[8].

The possible mechanisms by which metformin can promote the wound healing would be considered on the basis of its various actions like anti inflammatory, anti oxidant and action on hemostasis.

Anti inflammatory action of metformin- Beyond glucose lowering action, metformin has many pleiotropic benefits like vascular protection and reduced inflammation of endothelium and smooth muscle cells[2,9,10]. As response to injury or inflammatory stimuli there is accelerated migration of monocytes to the site of inflammation which eventually differentiate in to macrophages and play an important role in the immune response and inflammation. Metformin was proved to inhibit this differentiation due to increased AMPK activation and by reduced STAT 3 activity. Phenotypically polarized macrophages are either pro inflammatory M 1 or anti-inflammatory M2. AMPK signaling pathway promote polarization to anti-inflammatory M 2 macrophages and suppress pro inflammatory M 1[11].

Enhanced activation of AMPK by metformin in the endothelial cells inhibit pro inflammatory cytokines and nuclear factor kappa B [Nf-kB][9,10]. This factor mediates cytokines, cell adhesion molecule, receptor signaling proteins, growth factors and other proteins of immune cells in endothelium, smooth muscles and macrophages[12-14]. Activation and transcription of Nf-kB stimulates several pro inflammatory genes encoding cytokines IL-6 and IL-8. Metformin was found to inhibit the release of these cytokines from the vascular smooth muscle cells, endothelial cells and macrophages[10]. IL-8 play an important role in the monocyte migration and adhesion to

endothelial cells. IL-6 triggers the acute phase response[15]. In the smooth muscle cells metformin suppressed Nf-kB activated pro inflammatory responses. Metformin was found to suppress Akt, Erk ½ and Nf-kB translocation and block the pro inflammatory signal transduction via Nf-kB downstream of PI3 kinase[10].

TNF alfa promotes growth, activation and migration of leucocytes to the vascular wall[16] resulting into increased accumulation of mononuclear phagocytes and enhanced inflammation[17]. Metformin was demonstrated to inhibit the production of TNF alfa[18].

Metformin reduces inflammation as observed by the decrease in circulatory markers of inflammation like ICAM-1, VCAM-1, highly sensitive C reactive proteins, macrophage migration inhibitory factor and soluble intercellular adhesion molecule[19-21]. Increased AMPK activation by metformin reduces inflammatory response in vascular smooth muscle cells through AMPK phosphatase and tensin homologue pathway[22]. Activation of AMPK reduces STAT 3 phosphorylation and inhibit the monocyte to macrophage diiferentiation[11].

Metformin targets lysosomes and reduces inflammation.[23] It also reduces inflammatory marker like YKL-40[chitinase-3-like protein-1] the effect which is independent of glycemic control[24].

Metformin also inhibits the extracellular matrix accumulation, basement membrane thickening, decreases endothelial permeability which is increased in inflammation and improves vasodilator dysfunction[25].

Antioxidant action of metformin-Oxidative stress is known to contribute for the inflammation and inflammation itself enhances oxidative stress[26]. Metformin has antioxidant property which is exerted through inhibition of mitochondrial respiration and reduction in generation of reactive oxygen species.[27] In diabetes mellitus it reduces advanced glycation end products by controlling the hyperglycemia[28]. Antioxidant system gets benefited by metformin through the increase in reduced glutathione[27].

Effect of metformin on hemostasis- Metformin is known to reduce the levels of the plasminogen activator inhibitor-1 and von Willebrand factor, fibrinogen and factor VII[4,5]. It also affects the fibrin structure and function by decreasing factor XII activity[29]. This all results in to inhibition of intravascular coagulation which allows the defence machinery of the body to reach the site of inflammation and wound including the antibiotics and supportive treatment.

Animal studies done in rats have shown that metformin accumulated several folds in tissues[30].

All these actions of metformin like anti-inflammatory, antioxidant and effect on hemostasis help in the wound repair and accelerate the wound healing. This action of metformin will prove to be helpful in the diabetic wound repair. Studies need to be done meticulously to quantify this action of metformin in the clinical set up so that use of this drug can be encouraged for wound healing and repair. It appears that once there is diabetic atherosclerosis it might hinder the wound healing. But metformin can be tried in the initial stages of the disease before the advancement of diabetic atherosclerosis for the treatment of wounds or even otherwise also, as metformin is known to reduce atherosclerosis.

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