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Analysis of dead space wound granualtion tissue in streptozotocin induced diabetic rats

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ABSTRACT

The aim of the present study was to evaluate the wound healing profiles in streptozotocin induced diabetic rats. The study was carried out in two groups of 6 animals each. Group I animals were served as control, treated with normal saline. Group II animals were diabetes induced rat. Hyperglycemia associated with significant decreasing in granulation tissue hydroxyproline (reflection of collagen content), glycosaminoglycans content (ground substances), proteins and lysyl oxidase were observed in streptozotocin induced diabetic rats. In the study significant decrease in granulation tissue breaking strength, wet and dry granulation tissue weight were observed. The study clearly indicated that streptozotocin interferes in the various phases of wound healing and delays the process.

KEYWORDS

Wound healing;
Diabetic;
Dead space wound;
Granulation tissue;
Streptozotocin.

INTRODUCTION

Diabetes mellitus is not one disease but rather is a heterogeneous group of syndromes and the leading cause of adult blindness and amputation. The metabolic disturbances results in acute and long term diabetes complications, which are responsible for premature death and disability[1]. Diabetic wounds are slow, non-healing wounds that can persist for weeks despite adequate and appropriate care[2]. A series of multiple mechanisms, including decreased cell and growth factor response, diminished peripheral blood flow and decreased local angiogenesis, all of which can contribute to lack of healing in persons with diabetic foot ulcers[3]. The exact pathogenesis of poor wound healing in diabetes is not clearly understood, but evidence from studies involving both human and animal models reveals several abnormalities in the various phases of the wound healing process[4,5]. The present study was designed to evaluate the dead space wound granulation tissue in strepto zotocin induced diabetic rats.

MATERIALS AND METHODS

Drugs and chemicals

Streptozotocin was purchased from Sigma Aldrich Chemicals, Pvt., Ltd., Bangalore and all other chemicals and reagents used were of analytical grade.

Animals

Healthy albino rats of either sex (n=6) and of approximately of the same age, (4-6months) weighing be-

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tween 150-250g were used for the study. They were individually housed, maintained in clean polypropylene cages and fed with commercially pelleted rat chow (M/s Hindustan Lever Ltd. Mumbai) and water *ad libitum*. The experimental protocol was subjected to scrutiny of Institutional Animal Ethical Committee for experimental clearance (No.IAEC/KMC/UA /2000). The animals were divided into 2 groups of 6 animals each.

Group I animals were served as control, treated with normal saline

Group II animals were diabetes induced rats (streptozotocin, 50mg/kg body weight, i.p)[6]

Induction of diabetes mellitus

Diabetes mellitus was induced in Wistar rats by single intraperitonial injection of Streptozotocin (50mg/kg) dissolved in 0.1M citrate buffer (pH 4.5) after overnight fasting for 12hours. The diabetes was determining by assessing blood glucose concentration within 48hours after injection of Streptozotocin. The rats with blood glucose above 250mg/dl were selected for experimental studies.

EXPERIMENTAL

Dead space wound

The wounding procedures were carried out using ketamine (1ml/kg body weight of the rat, i.p) anaesthetized rats. These wounds were created by implanting two polypropylene tubes (0.5cm×2.5cm each), one on either side in the lumbar region on the dorsal surface of each rat. On the 10th post-wounding day, the granulation tissue formed on the implanted tubes was carefully dissected out and weighed[7]. These granulation tissues were collected, weighed and granulation tissue breaking strength was measured. The tissues were dried at 60^{0}C for 24hr and dry weight was noted. The dried granulation tissue acid hydrolysate was prepared and then utilized for the estimation of hydroxyproline[8], hexosamine content[9] and hexuronic acid[10]. A portion of the wet granulation tissue was used for the estimation of lysyl oxidase[11] and tissue protein[12].

Statistical analysis

The results were analyzed using one way analysis of variance (ANOVA) with post hoc Scheffe's test using Graph Pad in Stat (GPIS) software, version 1.13 and were expressed as the mean ±SD. *P* values <0.05 were considered as statistically significant

RESULTS

TABLE 1 shows the level of blood glucose in control and experimental animal group. The level of blood glucose was significantly elevated in streptozotocin treated rats compared to control group. The granulation tissue breaking strength, wet and dry granulation tissue weight was significantly decreased in diabetic rats compared to control (TABLE 1).

Hydroxyproline concentration of granulation tissue was significantly decreased in the streptozotocin induced diabetic rats. Glycosaminoglycan contents like hexuronic acid and hexosamine concentration was significantly decreased in the experimental group. Tissue protein concentration was very low in the case of diabetic rats when compared to control. Lysyl oxidase level was significantly decreased in the experimental group (group II) (TABLE 2).

DISCUSSION

The results of the present study clearly demonstrated

TABLE 1: Physical and biochemical analysis of granulation tissue in streptozotocin induced diabetic rats

Group	Blood glucose (mg/dl)	Tissue breaking strength (g)	Wet tissue weight (mg/100g rat)	Dry tissue weight (mg/100g rat)
Wounded control	80.1±7.2	285.49±14.37	240.5±13.09	31.58±5.80
Diabetic induced	276.38±14.1[a]	178.5±11.20[a]	169.5±10.32[a]	22.5±4.50[a]

Values are mean ± SD of 6 replications. P values: [a]:<0.01vs control

TABLE 2: Biochemical analysis of granulation tissue in streptozotocin induced diabetic rats

Group	Hydroxyproline (mg/g tissue)	Hexosamines (mg/g tissue)	Hexuronic acid (mg/g tissue)	Tissue protein (mg/g tissue)	Lysyl oxidase (SFU)
Wounded control	14.72±4.02	10.49±2.37	12.11±3.09	41.58±3.80	1711±69
Diabetic induced	10.38±2.10[a]	7.1±1.20[a]	9.5±1.32[a]	27.5±2.50[a]	1128±47[a]

Values are mean ± SD of 6 replications. (SFU- Spctroflourimetric units), P values: [a]:<0.01 vs control

that streptozotocin showed a definite antihealing action along with its hyperglycemic effect. Hyperglycemia, is a primary clinical manifestations of diabetes mellitus, is associated with micro and macro vascular diabetic complications[13]. Diabetes leads to modification of collagen such as advanced glycation and cross-linking which play an important role in the pathogenesis of diabetes mellitus[14]. Wound healing deficits in diabetes are diverse, multifactorial, complex and interrelated and are believed to be caused by impaired blood flow and oxygen release from increased blood sugar[15]. Collagen, fibrin and keratin accumulate advanced glycation Amadori end products which affects binding of regulatory molecules, susceptibility to proteolysis and finally decrease the ability of protein cross-linkage[16]. Di Girolamo et al postulated that defects in wound healing are caused by the hyperglycosylation of the locally synthesized cellular fibronectin[17].

A decrease in granulation tissue breaking strength and hydroxyproline content of diabetic wounds may be due to decrease in collagen concentration and stabilization of fibers. The decrease in wet and dry granulation tissue weight indicated low protein concentration and collagen bundle formation. The glycosaminoglycans are a major component of the extracellular matrix of skin, joints, eyes and many other tissues and organs. In spite of its simple structure it demonstrates remarkable viscoelastic and hygroscopic properties which are relevant for dermal tissue function. The glycosaminoglycans are known to stabilize the collagen fibers by enhancing electrostatic and ionic interactions with it and possibly control their ultimate alignment and characteristic size. In our study, the levels of hexuronic acid and hexosamine levels were decreased considerably, it is likely that the observed decrease in tissue breaking strength is not only due to collagen concentration but also due to its improper deposition and alignment. The process of maturation of collagen fibrils is catalysed by lysyl oxidase. This enzyme is involved in the formation of cross-links, therefore play a very important role in the maturation process and in wound healing. The decreased enzyme activity in our study may result in the decreased cross linking and poor breaking strength of the granulation tissue.

Overproduction of reactive oxygen species results in oxidative stress thereby causing cytotoxicity and delayed wound healing. Streptozotocin damages pancreatic b-cells possibly by generating free radicals and thus widely used for the induction of experimental diabetes mellitus. Streptozotocin generated lipidperoxidation and DNA breaks in pancreatic cells have been demonstrated[18] Prakasan et al have reported an elevated lipid peroxidation and lowered antioxidants in streptozotocin induced diabetic mellitus[19]. This could be the other reasons for poor wound healing profiles in sterptozotocin induced diabetic rats.

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