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Sphingosine kinase 1 improves cutaneous wound healing in diabetic rats

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ABSTRACT

Background: Diabetes is one of the most prevalent human metabolic diseases. Wound healing in diabetes is frequently impaired and treatment remains challenging. Sphingolipid metabolites play important roles in the regulation of glucose metabolism. SPK1 is the key enzyme in the sphingolipid metabolic pathway. S1P/SPK plays a pivotal role in the signalling pathways of diverse cellular processes including proliferation, differentiation, migration, apoptosis in diverse cell types.
Methods: To investigate the role of sphingosine kinase 1 (SPK1) in skin injury, plasmids containing the *SPK1* gene (pcDNA3-FLAG-SPK1) were applied to cutaneous wounds on a streptozotocin-induced diabetic rat model over a 21-day period. The wound area and rate of wound healing were determined. The histopathological features of the healed wounds were also observed, and SPK1 expression in the skin was detected by immunohistochemistry.
Results: There was a significant decrease in wound area in diabetic rats treated with 125 and 60 μg/wound pcDNA3-FLAG-SPK1 ($P < 0.001$–0.01). The mean sizes of the wounds were 0.67 ± 0.15 cm^2, 0.83 ± 0.18 cm^2, and 1.09 ± 0.23 cm^2 in both treated and diabetic control group at the 7th day post-treatment respectively. In addition, wound healing in diabetic rats of test group was accelerated. At the 7th day, the mean rates of healing were $73.2 \pm 5.7\%$ and $66 \pm 7.3\%$ in test group of 125 and 60 μg/wound respectively, and $55.4 \pm 9.9\%$ in diabetic control group ($P < 0.001$–0.01). Histology revealed that tissue sections from the treated diabetic rats contained more granulation tissue and capillaries than that of the control rats. There was high SPK1 expression in the skin of the treated diabetic rats.
Conclusions: *SPK1* gene therapy may represent a novel approach to cutaneous wound healing.

Introduction

Diabetes is one of the most prevalent human metabolic diseases, reaching pandemic proportions [1]. The morbidity of diabetes has increased dramatically over the past few years, and afflicts more than 100 million people worldwide. Delayed wound healing is considered one of the most common, disabling, and costly complications of diabetes. People with diabetes have poor circulation, poor resistance to infection, and poor local nutrition, thus their wounds are highly susceptible to infection [2]. This impairment in diabetic wound healing represents a significant clinical problem, leading to chronic non-healing ulcers ultimately resulting in infection, gangrene, or even amputation [3]. Thus, accelerating wound healing must be the primary goal of wound care.

Sphingosine kinase (SPK), a highly conserved lipid kinase present in organisms as diverse as humans, mice, flies, yeast, and plants, catalyses the phosphorylation of sphingosine to generate sphingosine-1-phosphate (S1P), a potent lipid mediator that plays important roles in a wide variety of mammalian cellular processes. SPK1 and SPK2 are the only two cloned isoforms of mammalian SPK. SPK1 is located predominantly in the cytosol; small amounts are associated with cellular membranes [4]. Sphingolipid metabolites play important roles in the regulation of glucose metabolism. SPK1 is the key enzyme in the sphingolipid metabolic pathway, forming an essential checkpoint to regulate the relative levels of bioactive sphingolipid metabolites, ceramide, sphingosine, and S1P. Previously [5], we demonstrated that adenovirus-mediated *SPK1* gene transfer (Ad-SPK1) improved blood glucose and lipid profiles, reduced body weight and adiposity, reversed hepatic steatosis, increased energy expenditure, and improved insulin sensitivity in diabetic mice. In this study, we investigated the effect of SPK1 on wound healing in diabetes mellitus.

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SPK1 catalyses the phosphorylation of sphingosine to form S1P [6]. An increasing amount of evidence indicates that S1P/SPK plays a pivotal role in the signalling pathways of diverse cellular processes in various cell types, including proliferation, differentiation, migration, and apoptosis [7–10]. SPK1 also promotes cell growth in soft agar [11]. After demonstrating markedly improved blood glucose levels in type 2 diabetic KK/Ay mice by Ad-SPK1, we designed the present study to assess the effect of SPK1 on wound healing in diabetic rats.

Materials and methods

Animals and plasmid

Healthy adult male Wistar rats (approximately 10 weeks of age; average body weight of 250–300 g) were used in this study. All rats were housed at a constant temperature and humidity in a room with an artificial 12-h light/dark cycle and had free access to food and water. Constructs of wild-type SPK1 (pcDNA3-FLAG-SPK1) were kindly provided by Dr. Stuart M. Pitson (Hanson Institute, Adelaide, Australia) [12]. All animal experiments were conducted following the institutional guidelines and approved by the Ethical Committee for Animal Care and Use, The Military Medical Science Academy of the PLA.

Reagent and antibody

Streptozotocin (STZ) was purchased from Sigma (St. Louis, MO, USA). Rabbit anti-FLAG antibody was obtained from OriGene (Rockville, MD, USA).

Induction of diabetes and wounding

Forty male rats were used; five were randomly selected as normal controls. Diabetes was induced in the remaining rats by tail intravenous injection of the pancreatic β-cell toxin STZ (freshly dissolved in sterile saline, 2%) at a dose of 50 mg/kg body weight. Blood glucose levels were measured using an acute glucometer (Roche, USA) 1 week after the STZ injections. Animals that developed average blood glucose concentrations exceeding 18 mmol/L were selected and assigned randomly to four groups.

Wounding was performed as described previously [13]. Briefly, rats were anaesthetised with intraperitoneal Nembutal (50 mg/kg). The dorsal skin was shaved and cleaned with povidone-iodine solution. Two full-thickness skin wounds (approximately 1.8-cm diameter) were created on the back of each rat: the area was marked with a stamp before the outlined skin was cut.

Therapeutic intervention and wound area

Different concentrations of plasmid-SPK1 constructs (pcDNA3-FLAG-SPK1; 15, 30, 60, 120 μg/wound) were applied to the wounds every 3 days. The construct was not applied to rats in the diabetic control group. Wound size was recorded with a transparent film after anaesthesia at 0, 3, 7, 10, 14, and 21 days after wounding. The wound size was then calculated by weighing the film. Animals were sacrificed 7, 14, and 21 days after wounding and skin specimens were obtained and fixed in 10% paraformaldehyde for histological study.

Rate of wound healing

The percentage of wound closure was calculated as follows, using the initial and final wound area drawn on glass slides during the experiments: % of wound healing = (original area − unhealed area)/original area × 100%.

Histopathological assay

A skin specimen was obtained from each group on day 7, 14, 21 after wounding for histopathological examination. Specimens were immediately fixed in 10% (v/v) neutral buffered formalin, and the solution was replaced every 2 days until the tissues had hardened. Each specimen was embedded in a paraffin block and thin sections (3 μm) were prepared and stained with haematoxylin and eosin (H&E) for general morphological observation.

Immunohistochemistry

Tissue sections that had been dewaxed and rehydrated routinely were incubated with 3% H_2O_2 for 30 min. The slides were washed with phosphate-buffered saline (PBS, pH 7.4) twice. The sections were blocked with 5% bovine serum albumin in Tris-buffered saline for 20 min. The spent solution was discarded and the sections were incubated with anti-FLAG antibody (1:2000) at 4 °C overnight. The slides were washed with PBS, incubated with rabbit secondary antibody (1:5000) for 1 h, and followed by incubation with streptavidin–horseradish peroxidase for 20 min. The antibody binding sites were visualised by incubation with diaminobenzidine–H_2O_2 solution.

Statistical analysis

All values are expressed as mean ± standard error of the mean (SEM). One-way analysis of variance followed by Student's unpaired *t*-test was used to compare parametric data. $P < 0.05$ was recognised to indicate statistical significance.

Results

Effect of SPK1 on blood glucose levels of diabetic rats

There was a significant elevation in blood glucose level (>18 mmol/L) after 1 week in the rats that had received STZ. Blood glucose levels were determined when the plasmid treatment was administered, as shown in Table 1; all diabetic rats had average blood glucose levels that exceeded 18 mmol/L, although there was no statistically significant difference among the six groups. Our data indicate that SPK1 did not directly affect the blood glucose levels of diabetic rats.

Table 1
Effect of SPK1 on blood glucose levels of diabetic rats.

Group	Dose μg/wound	Times	Blood glucose (mmol/L)			
			0 d	7 d	14 d	21 d
Control	Physiological saline	3	5.6 ± 0.3	5.8 ± 0.5	5.1 ± 0.2	6.4 ± 0.5
Diabetic control	Physiological saline	3	20.3 ± 2.7	26.2 ± 4.0	30.7 ± 5.4	31.0 ± 1.3
SPK1	120	3	20.6 ± 4.5	24.9 ± 54.2	25.7 ± 2.4	27.4 ± 2.5
SPK1	60	3	19.9 ± 0.07	27.8 ± 5.8	31.3 ± 3.0	26.9 ± 0.8
SPK1	30	3	20.2 ± 2.4	28.9 ± 4.2	28.8 ± 5.3	28.2 ± 2.9
SPK1	15	3	19.9 ± 2.9	26.2 ± 6.1	27.0 ± 6.4	25.4 ± 7.2