

Epidermal Modification in Skin of Streptozotocin-induced Diabetic Rats

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Abstract

Delayed wound healing is one of complications in the diabetic dermopathy, and potentially causes chronic ulceration and limb amputation. To investigate the effects of diabetes on the skin, male Sprague-Dawley rats were used. After 24 weeks of a single dose of streptozotocin administration, the skin was removed as part of the histological process to produce microscopic slides and was viewed under a light microscope. It was shown that the thickness of the epidermis in long-termed diabetic rats decreased. Additionally, in the long-termed diabetic group, hypertrophic keratinocytes and pyknotic melanocytes were also observed in the stratum basale. The size of keratinocyte and its nucleus in the stratum spinosum was smaller. In addition, there was a dense-stained layer with shreds in the stratum corneum. It was concluded that long-termed diabetes causes these pathological aspects of the skin, and might further contribute to chronic wound formation. Therefore, skin problems should be managed with early diagnosis and treatment, in order to improve quality of life in diabetic patients.

Keywords: Diabetic dermopathy, diabetes mellitus, epidermis

Introduction

Diabetes mellitus (DM) is the most common chronic disease, and tends to increase in all countries around the world [1-3]. DM affects various organ systems in the body, such as the kidneys (nephropathy), nervous system (neuropathy), vascular system (angiopathy), and integumentary system (dermopathy) [4-7]. Indeed, skin is the first line of defense from external factors. Up to 60 % of diabetic patients show skin disorders [8]. The effects of diabetes on skin include complications associated with angiopathy and neuropathy [9,10]. Diabetic dermopathy appears as a pink to red skin rash, dry flaky skin, and carotenoderma (yellow skin) or acanthosis nigricans (brown to black skin) [11-15]. Moreover, chronic diabetic skin disease delays the wound healing process, which raises the risk of infection and tissue necrosis, later leading to amputation.

In previous studies of both human and animal models, diabetes can induce skin alterations. Light microscopic investigation of epidermis revealed decreased epidermal thickness, increased melanin pigments, active Langerhans cells [10,16-20], keratinocyte degeneration, defective collagen fibers, and elevated inflammatory cell infiltration [10,21-23]. Evaluation of skin contents has shown that lipids, in terms of fatty acid and cholesterol, decrease, but triglyceride increases in the stratum corneum of the epidermis. However, there is a significant decrease in the water content of diabetic skin [24,25]. The alterations of skin components relate to their morphological changes; therefore, this study was proposed to investigate the histological alterations of the epidermis in streptozotocin (STZ)-induced diabetic rats, compared to the control. The results might provide a useful basic knowledge for understanding and clarifying structural impairments in diabetic skin.

Materials and methods

Animal and diabetic induction

Male Sprague-Dawley rats, 200 - 270 g, were obtained from the National Laboratory Animal Center, Mahidol University, Salaya. This experiment was performed and approved by the National Research Council's Guide for the Care and Use of Laboratory Animals. Following a week of acclimatization, each animal was made to fast for 10 to 12 h. After that, the rats were measured for body weights and for urine and blood glucose levels. Then, the animals were randomly assigned into 2 main groups; STZ-induced diabetic (N = 4) and control (N = 3) groups.

Diabetes was induced with a single dose of STZ (60 mg/kg body weight in citrate buffer at pH 4.5) via intraperitoneal injection. The animals in the control group received the same volume of citrate buffer alone. Thereafter, the urine glucose levels and body weights were measured daily. Blood glucose levels were measured as a standard for diagnosing diabetes at 48 and 72 h after induction and before sacrifice. The animals were sacrificed at 24 weeks.

Tissue preparation for histological study

At the end of experiment, each animal was anesthetized by halothane inhalation before the rib cage was opened and the aorta perfused with 500 ml of 0.9 % NaCl solution. Then, 300 ml of Bouin's solution was injected to preserve the tissues. The skin at the foot was immersed in the same fixative overnight and rinsed several times in 70 % ethanol before the histological process was done. Next, the specimens were dehydrated in a graded series of ethanol, cleared in xylene, infiltrated, and embedded in paraffin. Then, the embedded specimens were serially sectioned at 10 μ m thickness and stained with hematoxylin and eosin (H&E), respectively.

Statistical analysis

Body weights and blood sugar levels were analyzed using the Mann-Whitney U test (SPSS 18.0 software). The data were expressed as mean \pm standard deviation (SD) from control vs STZ-treated groups. Differences at $p < 0.05$ were considered significant.

Results and discussion

STZ-diabetic rats showed characteristic signs of diabetes, such as glucosuria, increased water and food intake with hyperglycemia, whereas body weight decreased, as shown in **Table 1**.

Table 1 Comparisons of urine and whole blood glucose levels and body weights of STZ-induced diabetic and control groups.

	Groups	
	Control	STZ-induced DM
Urine glucose levels (mg/dL)	0	> 500
Whole blood glucose levels (mg/dL)	122.00 \pm 11.19	451.62 \pm 22.72 (> 300 mg/dL indicated as DM)
Body weights (g)	405.20 \pm 38.56	239.50 \pm 13.86*

* $p < 0.05$ compared to control group

The normal epidermis, the outermost layer of skin, was divided into 5 layers: the stratum basale, stratum spinosum, stratum granulosum, stratum lucidum, and stratum corneum. The innermost layer, stratum basale, was a layer of proliferating columnar keratinocytes and melanocytes. The stratum spinosum included many polyhedral shaped keratinocytes with oval nuclei. The stratum granulosum was

composed of keratinocytes with keratohyalin granules. The stratum lucidum, found only in the thick skin, also contained dead keratinocytes. The outermost layer, stratum corneum, was made of dead keratinocytes (**Figure 1A**). In the diabetic group, all layers of epidermis were thin compared to the control. The cells in the stratum basale were large and pyknotic nuclei of melanocytes were observed. Keratinocytes, especially in the stratum spinosum, relatively decreased their nuclear size (**Figures 2B** and **3B**), compared to the control (**Figure 3A**). The thickness of the stratum corneum was obviously thin with more shredded keratin.

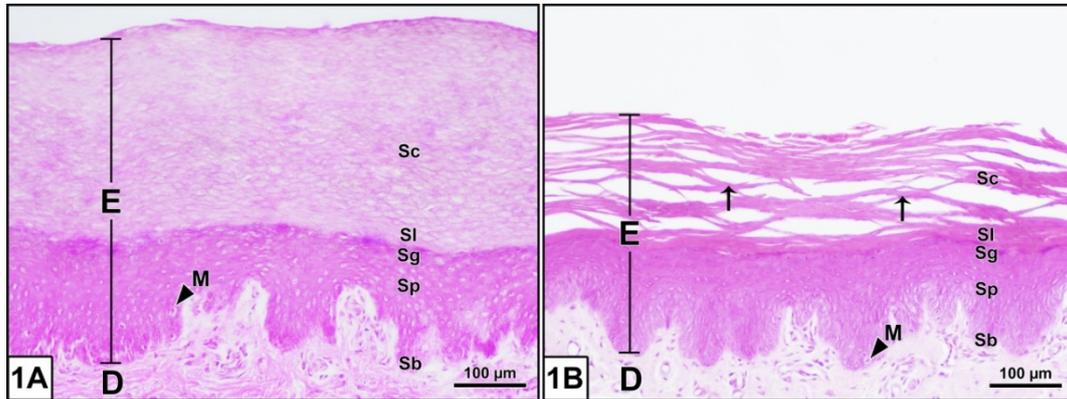


Figure 1 Optical micrographs of skin from the feet of the control (A) and STZ-induced diabetic groups (B). The skin was composed of epidermis (E) and dermis (D). Epidermis included 5 main layers: stratum corneum (Sc), stratum lucidum (Sl), stratum granulosum (Sg), stratum spinosum (Sp), and stratum basale (Sb), respectively. Melanocyte (M) was inserted in the stratum basale. Diabetic skin revealed the decreased epidermal thickness and obvious shredded keratin at the stratum corneum (black arrows). 10× magnification.

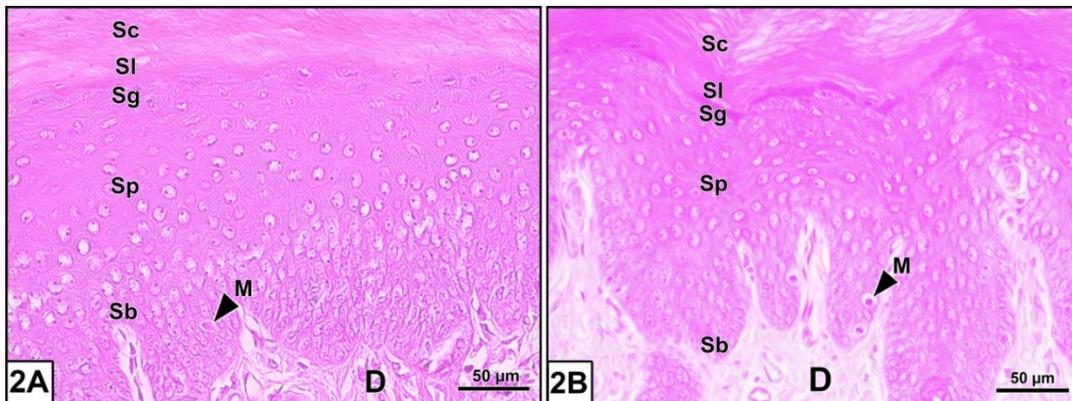


Figure 2 Optical micrographs of skin from the feet of the control (A) and STZ-induced diabetic groups (B). Five layers: stratum corneum (Sc), stratum lucidum (Sl), stratum granulosum (Sg), stratum spinosum (Sp), and stratum basale (Sb) in diabetic rat decreased in their thickness. Melanocyte (M) in the stratum basale had pyknotic nuclei. Dermis (D). 20× magnification.

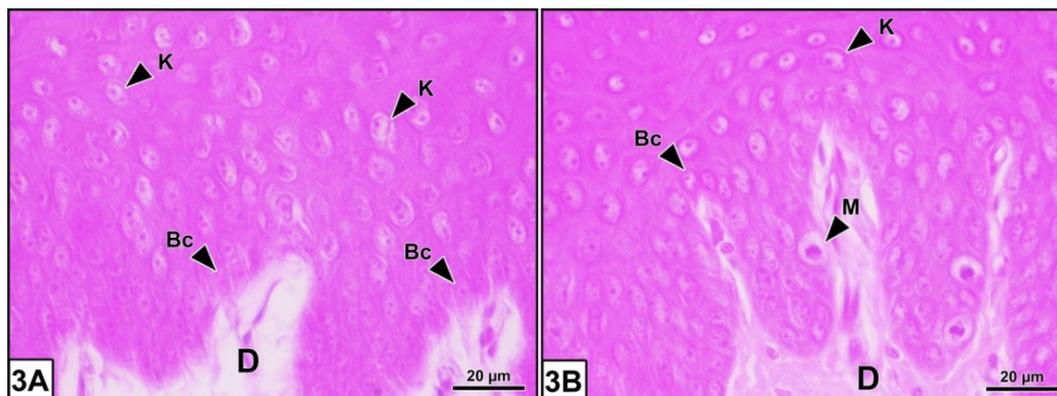


Figure 3 Optical micrographs of skin from the feet of the control (A) and STZ-induced diabetic groups (B). Diabetic skin showed a slight decrease in cell size of keratinocyte (K) in stratum spinosum. Basal cell (Bc), melanocyte (M), dermis (D). 40× magnification.

In the long-termed DM, it was found that all layers of epidermis were thinner than those of the control. In the stratum basale, cells were enlarged, whereas smaller keratinocytes with nuclear shrinkage in the stratum spinosum and pyknotic nuclei of melanocytes were found. Additionally, the corneocytes in the stratum corneum showed dense staining with a shredded appearance. In high glucose levels, reactive oxygen species (ROS) is generated via polyol, advanced glycation end product, oxidative stress, and glycolysis pathways [26]. In addition, elevated ROS increases Ca^{2+} level in keratinocytes, which activates calpain, a protease for cytoskeletal destruction, such as cytokeratin intermediate filaments. The size of keratinocytes in the stratum spinosum became smaller in the diabetic group. Moreover, ROS stimulates tumor necrosis factor- α to active caspase 3 to generate phospholipase, nuclease, and protease. The phospholipase destroys cell and nuclear membranes, so both of the membranes shrink in the keratinocytes of stratum spinosum. Additionally, nuclease damages DNA, changing them to fragments as chromatin clumping in the pyknotic nuclei, which are discovered in melanocytes. Furthermore, protease destroys cell organelles. Interestingly, destroyed Golgi complexes are associated with decreased numbers of lamella bodies, which contain phospholipid, glycosceramides, and cholesterol. Then, water in the skin evaporates; after that, the skin becomes dry in the DM. As a result, the stratum corneum shreds, as shown in the diabetic patients. Furthermore, high glucose levels inhibit insulin-like growth factor signaling [27,28]; thus, cellular proliferation in the stratum basale decreases. Moreover, it has been demonstrated that some, but not all, basal cells in stratum basale in the DM can proceed with DNA duplication [29]. Therefore, some basal cells were hyperfunctional to produce cells in other layers. In addition, increased ROS, as mentioned above, induces mitochondrial dysfunction [30]. In fact, mitochondria play a central role in the source of energy biosynthesis and activities of the cell by generating adenosine triphosphate (ATP). Therefore, ATP is reduced in mitochondrial dysfunction, causing the blocking of the Na^+/K^+ pump, leading to Na^+ accumulation in cytoplasm. High intracellular Na^+ concentrations induce Cl^- , together with H_2O influx, for ionic balance of basal cells, to maintain cell viability. Consequently, hyperfunction and water influx in the cells of stratum basale lead to cell swelling.

Conclusions

Histological changes of all layers in the diabetic epidermis were investigated, which relate to impaired wound healing and infection of diabetic patients. Furthermore, transmission electron microscopic investigation will be performed to evaluate the ultrastructure of cell death, including the membrane, nucleus, and its organelle, as well as to investigate cell surface alterations of cells such as desmosome. These data will provide essential information for studies on skin lesions caused by diabetes.

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