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Establishment of Diabetic Foot Ulcer Model in Guangxi Bama Mini-pig

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Abstract **[Objective]** To establish a new simple and steady diabetic foot ulcer model for the clinical and basic study of diabetic skin ulcer. **[Methods]** Five pigs were randomly divided into control group ($n=2$) and experiment group ($n=3$). Pigs in control group were fed with basal diet while those in experimental group were fed with high-fat and high-sucrose diet. When the model was established, the skin ulcer was burned on back, neck and limbs of the pigs after anesthesia. The wounds were examined by histological analysis and the areas of wounds were measured at different time points. **[Results]** Diabetic models were successfully established, there were no significant differences in morphology of ulcers in both groups. Weights of pigs in both groups increased and the food and water intake changed a little. Ulcers in both groups stayed unhealed after 4 weeks. After 4 weeks, the ulcers were examined by histological analysis. The structure of the epidermis and dermis were disappeared along with cell degeneration. The histopathological changes of subcutaneous fatty tissue and underlying muscle tissue were characterized by degeneration. **[Conclusions]** The animal model can simulate clinical diabetic skin ulcer and the method of establishing such model is easy and reliable.

Key words Guangxi Bama mini-pig; Diabetic; Foot ulcer; Model

1 Preface

Diabetic is a kind of chronic metabolic disease. With the growth in the living standard and the change of living environment, the number of diabetic patients increases significantly[1]. Diabetic accompanied by a variety of complications has been a kind of metabolic disorders that threats human health. Diabetic foot ulcer is a complication that has long course of disease, is complicated and difficult to treat and with poor prognosis. It usually lasts a long time and is easy to relapse[2]. With the aging of population, the number of diabetic patients and patients with diabetic ulcers increases. So the number of patients who have to deal with amputation becomes more and more[3]. By statistics, of patients who had gone through non-traumatic amputation, 50% were induced by diabetic ulcers and every year 3% - 6% diabetic patients are accompanied by diabetic foot ulcer[4-5]. So researchers pay high attention to researches about diabetic ulcers[6]. However, there's no good method of prevention and cure for it in clinic currently. In order to promote the researches on diabetic ulcers, Guangxi Bama mini-pig whose organization structure of skin and hematochemistry are similar to those of human was chose as object of study. With the pig, we established a simple and steady diabetic ulcer model and expected that it could provide an ideal and applicable animal model for the clinical and basic study of diabetic skin ulcer.

2 Materials and methods

2.1 Materials

2.1.1 Experimental animals. Five Guangxi Bama mini-pigs (2 - 3 months, male), were purchased from Guangxi Bama mini-pig breeding center of Guangxi University (license key: SCXK Gui 2013 - 0003).

2.1.2 Primary reagents. Domestic analytically pure was commercially available.

2.2 Methods

2.2.1 Preparation of diabetic model. The diabetic model was induced by feeding. Five pigs were randomly divided into control group ($n=2$) and experiment group ($n=3$). The pigs were fed separately, two times a day. Pigs in control group were fed with complete feed while those in experimental group were fed with high-fat and high-sucrose diet (60% complete feed + 30% saccharose + 10% lard oil). The daily food ration was of 2% weight. The pigs were free to drink water. The modeling cycle lasted 6 months.

2.2.2 Preparation of diabetic ulcer model. (1) Animal anesthesia. The pigs were kept off food and water for 12 hours and then measured the weight. 30 min before the operation, they were injected with atropine by tramuscular injection and exposed to anesthesia by tramuscular injection of Sumianxin II and pentobarbital sodium. Sumianxin II (0.1 mL/kg) was injected into buttocks of pigs by tramuscular injection. When the animals could not walk upright, 3% pentobarbital sodium normal saline (0.2 mL/kg) was injected into ear vein. After 15 min, if animals had symptoms of being slow in reacting, muscular flaccidity and having no pain when receiving acupuncture, we could proceed to the establishment of diabetic ulcer model. During the process of diabetic ulcer model, animals are free to breathe in case they were died of suffocation. (2) Choice of ulcer sites. Back, neck and inner sides of limbs were chose as ulcer sites. (3) Preparation of ulcer wound. After the anesthesia becoming effect, the skin ulcer was burned on back, neck and limbs of the pigs with circular soldering iron with

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diameter of 5 – 6 cm that had been burned for 10 min over the stove. Four ulcer wounds were on back, 1 on inner side of every limb and 1 on neck. The necrosis of local skin and subcutaneous fat or even underlying muscle indicated the establishment of model was completed. All animals did not use antibiotics after surgery.

2.2.3 Detecting changes of fasting blood glucose. Observed the changes of activities, food and water consumption of animals in control and experimental group, every day. The changes of fasting blood glucose were detected, every month.

2.2.4 Intravenous glucose tolerance test (IVGTT). An IVGTT was conducted every 60 days. Animals were kept off feed for 16 hours before test and injected 50% glucose solution (1.3 mL/kg) into ear vein in 3 min. Blood samples of caudal veins were collected at 0, 3, 10, 30, 60, 90 and 120 min after intravenous injection of glucose solution. The serum was separated after centrifugal separation. Concentrations of blood glucose and insulin were determined.

2.2.5 Observation and evaluation of diabetes ulcer wound. (1) Observation of ulcer wound. After operation, the animals were fed separately. The size, color, infection and intertion status of animals in various groups were observed and recorded 1 time every 3 days. (2) Evaluation of ulcer wound. The wounds areas of groups were measured along the edges with vernier caliper on 1, 7, 12, 21, 28 days after preparation of ulcer wound. The intertion status of wounds was photoed. The healing rates of wounds were calculated according to the formula: the healing rate = (the original wound area – the actual measured wound area) / the original wound area × 100%. Healing of 100% ulcer surface was complete healing. Healing of 50% ulcer surface was partial healing. Healing of less than 50% ulcer surface was not healing. (3) Histopathological observation. Skin tissues of ulcer wounds were obtained at 1, 7, 12, 21, 28 days after preparation of ulceration. They were fixed with 10% formaldehyde, dehydrated with ethyl alcohol and embedded with paraffin. Then they were cut into slices and stained with HE and then observed by microscopically.

2.2.6 Statistic analysis. The data was analyzed by SPSS16.0 statistical software. Mean ± standard deviation ($\bar{x} \pm s$) was regarded as measurement data. Differences between the two groups were analyzed by *t*-test. $P < 0.05$ indicated that differences were of statistical significant. $P < 0.01$ was as very significant.

3 Results and analysis

3.1 Determining blood index

3.1.1 Changes of fasting blood-glucose. The food and water consumption and weight of animals increased significantly after feeding with high-fat and high-sucrose diet. All animals´ activities and metal status were good. There´s no dead animal (Table 1). After feeding with high-fat and high-sucrose diet for 5 months, blood glucose level of animals in experimental group reached (10.23 ± 1.94) mmol/L and was of significant differences when compared with that of control group ($P < 0.05$).

3.1.2 TVGTT. At the end of the 6th month, IVGTT was conducted. After intravenous injection of glucose, blood glucose level of Guangxi Bama mini-pig in the ulcer group reached 4.6 mmmol/L at 0 min, 17.8 mmol/L at 3 min, 18.0 mmol/L at 10 min, 13.0 mmol/L at 30 min, 11.0 mmol/L at 60 min and 11.0 mmol/L at 90 min. At 10 min, the blood glucose level reached the peak, 18 mmol/L. From 30 min, the blood glucose level decreased slowly and at 90 min it´s still higher than the initial blood glucose level and the sugar tolerance lowered. At 120 min, blood glucose level could not be detected. The result indicated that the model was successfully established and could be used for preparation of diabetes ulcer model.

3.2 Observation of ulcer wound On 7 days after operation, the shape of ulcer wound was circle and it was clear and kermesinus. There was a little of secreta which decreased little by little. On 12 days, edges of wound started the centripetal contraction. The contractions of control and ulcer group were of significant difference ($P < 0.05$). On 21 days, the healing rate of ulcer wound was less than 50% and its shape was similar to oval and its edges were irregular. The control group and ulcer group differed significantly ($P < 0.01$). On 28 days, the healing rate of ulcer wound was less than 50% and its shape was similar to oval and its edges were irregular. The control group and ulcer group differed significantly ($P < 0.01$). Some wounds were infected during the process of experiment, since the pigs were not treated with antibiotics (Table 2).

Table 1 Changes of blood glucose levels of control and experimental group mmol/L

Group	Measure time//d					
	30	60	90	120	150	180
Control group	4.20 ± 2.26[a]	4.20 ± 0.28[a]	5.65 ± 1.63[a]	4.55 ± 0.07[a]	4.20 ± 0.99[a]	3.35 ± 0.64[a]
Experimental group	8.40 ± 2.89[a]	7.30 ± 1.36[a]	7.17 ± 1.95[a]	6.30 ± 0.26[b]	10.23 ± 1.94[b]	9.07 ± 3.07[a]

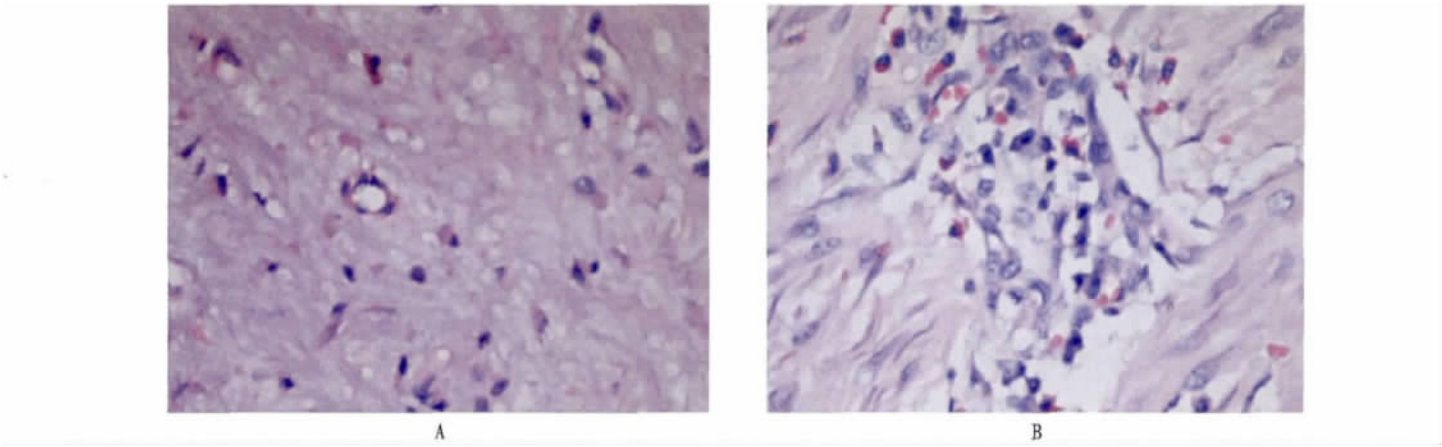
Note: The differences between the same letters were not significant and the differences between different letters were significant in the same column. (Similarly hereinafter).

Table 2 Comparison of areas of ulcer wounds in different periods

Group	*n*	Measure time//d				
		1	7	12	21	28
Control group	2	22.65 ± 0.50[a]	22.28 ± 0.89[a]	22.66 ± 1.28[a]	20.95 ± 0.71[a]	19.19 ± 1.04[a]
Experimental group	3	21.60 ± 0.59[a]	21.19 ± 0.33[a]	29.57 ± 3.04[a]	29.61 ± 2.63[b]	27.94 ± 2.16[b]

3.3 Histopathological observation According to Fig. 1, compared with the control group, the layer of epidermis cells was not clear and the multilayer was lack. The dermis structure disappeared. Cell necrosis was observed and cellular structure was

missing. We could only observe a little part of cells. Some collagens became atrophic and degenerated. Some basal nuclei became consolidated or fragmented. Arrangement of collagen fibers was in a mess. Angiotelectasis and even embolism were observed. Characters of subcutaneous adipose tissue and muscle tissue like degeneration, shrink or disappearance were observed. They were also calcified. The phenomenon indicated that ulcer had already formed.



Note: A. Control group; B. Diabetic ulcer group; ×400.

Fig. 1 Pathology detection of animal ulcer tissues in groups

4 Conclusions and discussions

At present, many kinds of animals have been used for establishing diabetic and diabetic ulcer model. So choosing animals that have similar body structure, function, metabolism and diseases of human is the key to the success of experiment. Since Guangxi Bama mini-pig whose organization structure of skin and hematochemistry are similar to those of human, so it was an ideal choice for simulating diabetic and diabetic ulcer model [7]. Diabetic ulcer model was obtained by vulnerating pigs of diabetic model [8-9]. The model could simulate pathogenesis of diabetic skin ulcer in clinic which was very complicated. The pathogenesis was as followed: local ischemia and hypoxia induced by vascular disease; foot protective sensation became weaken or defected induced by peripheral nerve lesions [10-11]; local was not sensitive to the pressure and other mechanical stimulation; repeated stimulation and complicated with infection and so on [12]. So in order to prepare diabetic ulcer model we need to consider many factors. Guangxi Bama mini-pig was as object of study. Diabetic ulcer model was established by using animal hyperglycemia combined with superposition of local acute trauma. It was different with ischemia and hypoxia, local mechanical stimulation, chronic infection induced by clinical chronic nerve vascular lesions in diabetic skin ulcer. And the sample size was small. So the model has to be further improved. The model was initially used to simulate the pathogenesis of diabetic skin ulcer and provided an ideal and applicable animal model for the clinical and basic study of diabetic skin ulcer. We still need to further study on how to improve the model and make it better for simulating the pathogenesis.

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