P-0602 – Establishment Bama minipigs models of diabetes mellitus and the skin changes of diabetic minipigs

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Fig1 Changes of blood glucose and insulin in minipigs before and after STZ injection

Objective

To establish the Bama minipig models of diabetes mellitus and to observe the diabetic effects on skin in Bama minipigs.

Methods

8 male Bama minipigs aged 2.5 months were fed with high-fat and high-sugar diet to 24-months old, then a small dose of streptozotocin (STZ, 60mg/Kg) was injected by intravenous. The diagnostic criteria for diabetes are consistent with those for adults. The body weight, abdominal circumference, blood pressure, fasting blood glucose were monitored regularly during the modeling period, and we also performed oral glucose tolerance test (OGTT) and insulin release test (IRT). The skin tissues were collected from minipigs usually on both sides of the back to the buttock, and the time points were Prediabetes (Pre-DM), diabetes for 1 month (DM1), 3.5 months (DM3.5) and 5 months (DM5). After HE staining, the changes of skin tissues were observed under light microscopes, and the thickness of epidermal and dermal were measured. Picrosirius red staining was used to observe the distribution of collagen fibers and calculate the content of type I/III collagen fibers. The distribution of microvessels density (MVD) and advanced glycation end products (AGEs) were observed and quantified by immunohistochemistry (IHC). Ultrastructure of vascular endothelial cells and nerve cells were observed under electron microscopy. Statistical analysis was performed by one-way repeated measure ANOVA and Dunnett's test, $P < 0.05$ was significant statistical difference.

Results

1. The fasting blood glucose of minipigs in both groups were normal at the age of 24 months, but the OGTT-IRT results showed that two minipigs had impaired glucose tolerance and hyperinsulinemia (Fig1A, B). The blood glucose of all minipigs had reached the diagnostic standards of diabetes at 30-months old after STZ injection in different times and doses (Fig1C). 2. Observing skin tissues of minipigs at different time

points, we found the epidermal and dermal thickness of minipigs were significantly lower after diabetes ($P=0.034$; $P=0.006$). The contents of type I and type III collagen were not significantly different compared with Pre-DM ($P=0.511$; $P=0.526$), but the ratio of collagen I/III changed significantly ($P=0.007$). The MVD was significantly lower after diabetes ($P=0.008$). While the AGEs content of skin tissues was not significantly different compared with Pre-DM ($P=0.057$) (table 1).

Table 1 Skin thickness, collagen ratio, MVD and AGEs in experimental group

Items	Sphericity test	Time	Compared to Pre-DM	Adjusted P value
Epidermal Thickness	$P=0.92$, Yes	$F=3.759$	Pre-DM vs. DM1	0.373
		$F=0.034$	Pre-DM vs. DM3.5	0.616
		$F=0.006$	Pre-DM vs. DM5	0.073
Dermal Thickness	$P=0.018$, No	$F=11.178$	Pre-DM vs. DM1	0.008
		$F=0.006$	Pre-DM vs. DM3.5	0.437
			Pre-DM vs. DM5	0.024
Collagen type I/III	$p=0.281$, Yes	$F=6.791$	Pre-DM vs. DM1	0.023
		$F=0.016$	Pre-DM vs. DM3.5	0.893
			Pre-DM vs. DM5	0.981
MVD	$p=0.673$, Yes	$F=3.687$	Pre-DM vs. DM1	0.571
		$F=0.008$	Pre-DM vs. DM3.5	0.023
			Pre-DM vs. DM5	0.007
AGEs	$p=0.095$, Yes	$F=3.869$	Pre-DM vs. DM1	0.067
		$F=0.071$	Pre-DM vs. DM3.5	0.633
			Pre-DM vs. DM5	0.191

Conclusions

High-fat and high-sugar diet combined with low-dose STZ can induce stable diabetes in Bama minipigs successfully. Chronic hyperglycemia had certain effects on skin tissues. In brief, the epidermal and dermal structures were abnormal and became thinner; the MVD was reduced with different degrees of expansion; edema and inflammatory cell infiltration were observed in the local dermis. And there were disordered collagen arrangement with abnormal collagen structure and imbalanced ratio of collagen I/III. This may be one of the reasons why diabetic skin is more vulnerable to damage and difficult to heal after injury.