MUSCLE GLUCOSE TRANSPORTER 1 (GLUT-1) EXPRESSION IN DIABETIC RAT MODELS

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ABSTRAK

Glucose transporter-1 (Glut-1) adalah protein transporter glukosa yang ditemukan pada otot rangka, selain Glut-4. Karakteristik Glut-1 berbeda dari Glut-4, Glut-1 tidak bergantung pada stimulasi insulin dalam memfasilitasi difusi glukosa melintasi membran sel. Kami menduga ekspresi Glut-1 tidak berubah pada membran sel otot diabetes mellitus. Penelitian ini bertujuan untuk mengamati perubahan ekspresi Glut-1 secara serial waktu pada model tikus diabetes. Streptozotocin (STZ) dipakai untuk menginduksi model tikus diabetes. Injeksi 150 mg/ kg BB STZ secara intraperitoneal dilakukan pada 18 ekor tikus satu kali. Setiap 6 ekor tikus secara dikorbankan setelah 1,2 dan 3 hari setelah induksi. Setiap tikys diperiksa kadar glukosa darah puasa, histologi pankreas dan otot gastrocnemius. Hiperglikemia (>200 mg/ dl) ditemukan sejak hari pertama dan dipertshankan sampai dengan hari ketiga induksi STZ. Kadar glukosa darah puasa sempat turun bermakna pada hari kedua induksi, meskipun masih dalam rentang hiperglikemia. Pada hari yang sama, ekspresi Glut-1 meningkat bermakna dan dipertahankan tetap terekspresi tinggi sampai dengan keesokan hari. Temuan ini membuktikan Glut-1 justru berperan dalam respon pertahanan fisiologis terhadap stres metabolik pada model diabetes. ***(FMI 2013;49:21-25)***

Kata kunci*: otot, Glut-1, ekspresi, diabetes*

ABSTRAK

Glucose transporter-1 (Glut-1) is one of glucose transporter which found in skeletal muscle. Not like Glut-4, Glut1 is insulin independent in facilitating glucose diffusion across cell membrane. We suggest that Glut-1 expression in skeletal muscle membrane was not affected in diabetes mellitus. This study was aimed to investigate Glut-1 expression by time in skeletal muscle diabetic rat models. Streptozotocin (STZ) was used to induce diabetes in rat models. Intraperitoneal injection of 150 mg per BW STZ was administered to 18 rats in a single load. We sacrificed every 6 rats after the first, second and third day of STZ induction. Each rat models were analyzed for it blood fasting glucose, pancreas and gastrocnemius muscle. Hyperglycemic (more than 200 mg/dl) were found in the first day until the third day of STZ induction. Blood fasting glucose decreases significantly only at the second day of STZ induction, even though it was still in hyperglycemic range. At the same day, Glut-1 expressions rise significantly at muscle membrane and kept in a high expression until the next day. These findings proved that Glut-1 was not only unaffected but also participating in physiologic defense against metabolic stress in diabetic models. ***(FMI 2013;49:21-25)***

Keywords: *Muscle, Glut-1, expression, diabetes*

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized with glucose uptake disturbances. Glucose transporter-4 (Glut-4) fails to facilitate glucose entering to the insulin sensitive cells, such as skeletal muscle, adipose and liver. Recently, there was another glucose transporter expressed at skeletal muscle membrane, namely by glucose transporter-1 (Glut-1). Unlike Glut-4, Glut1 was insulin independent in facilitating glucose diffusion across cell membrane. We suggested that Glut-1 expression in skeletal muscle membrane was not affected in diabetes mellitus. This study was aimed to investigate Glut-1 expression by time in skeletal muscle of diabetic rat models.

MATERIALS AND METHODS

Rattus norvegicus were purchased from Animal Laboratory, Biochemistry Department, Airlangga University, chosen as diabetic model in this study. They were caged and fed individually for about 2 weeks. Rats were 24 healthy male, grouped into 6 control rats and 18 diabetic-induced rats. The diabetic rats were injected with streptozotocin (STZ) and control rats were given only STZ solvent (citric buffer at pH 4.5). Strepto-zotocin was purchased from Bioscience, Malang, East Java, dissolved into citric buffer at pH 4.5. Citric buffer was made by adding 50 mM citric acid in to 0.4 ml NaCl 0.9%, until reached pH 4.5. Monoclonal anti Glut1 antibody (cat# E13180) was purchased from

Springbioscience and monoclonal anti IgG insulin antibody (cat# sc-57046) was purchased from Santa Cruz Biotech. Toluidin O reagent was used to measure blood fasting glucose by spectrofotometric assays.

AMDCC protocols for diabetic models induction

Diabetic rats were fasted 4 hours before STZ administration. Each rat was given by 150 mg/kg BW streptozotocin via intraperitoneal. Night after STZ injection, 10% sucrose was added in to watery drink bottle to prevent sudden hypoglycaemic. Each day, we sacrificed 6 diabetic rats for the time series observation. Rats were fasted for 6 hours than measured for it body weight before sacrificed at 12 am each days. Blood, pancreas and gasctrocnemius muscles were collected from the rats prior to anesthetic. The protocol was adapted from Animal Models for Diabetic Care Consortioum (AMDCC) with a high dose STZ induction for diabetic animal models. These was certified for ethical clearance from Animal Care and Use Committee, Airlangga University.

Analytical procedure

Blood samples were mixed with tolouidin O reagent than boiled and wait until the color altered. These samples were put in to spectrophotometer one by one for 450 nm absorbency. Fasting glucose level was determined by converting the absorbance using glucose standard curve and equation. Pancreas and gastrocnemius muscles were weighting and placed in to 10% formalin buffer solution before histological processing. These tissues were embedded in to paraffin block than sliced and put on the object glass. Every slide containing slices of tissue was proceded with primary and secondary antibody for immunohisto-chemistry protocol. We determined positive cells percentage in 125 x 125 μm2 visual field using 400 x enlargement scale of light microscope. Positive cells of Glut1 expression were characterized by brownish DAB lining at muscle membrane. Positive cells of insulin expression were characterized by brownish DAB at cytoplasm, marked as beta pancreas.

RESULTS

Loosing weight determined diabetes stage on streptozotocin models

Diabetes was characterized from the alteration of body, muscle and pancreas weight by day. Diabetic rat models showed significant loss (p= 0,0001) of their body, muscle and pancreas weight gradually until observation day 3 . Diabetes stage of streptozotocin models was achieved after 3 x 24 hours administration.

Re-raised fasting glucose levels marked the diabetes stage on streptozotocin models

The fasting glucose levels raised up significantly since 24 hours after streptozotocin administration (p= 0,0001). Glucose levels were above 300 mg/dL, recommended as hyperglycemic on rats. Interestingly, we found a significant decline of glucose levels at 2 x 24 hours after streptozotocin administration but the levels were still in hyperglycemic range (p= 0,019). This only happens a day and the levels were raised again on the next day, higher than before. Re-raised fasting glucose levels marked the diabetes stage at 3 x 24 hours after streptozotocin administration.

Beta pancreas losing its shape, size and population marked the diabetes stage on streptozotocin models

The histological investigations found some complementary evidences to explain the nature of diabetes stage on streptozotocin models. The evidences were collected from beta pancreas and Glut-1 expression in rat muscles. A day after STZ induction, beta pancreas enlarged in scale with edematous tissue around it. At the 2nd day observation, beta pancreases were still in large scale but in irregular shaped, such as cubic, cylindrical and also squamous. At the end of the observation, beta pancreas had reduced size and most of them were squamous in shape. These histological pictures of beta pancreas can be seen in figure 2.

Table 1. Weight characterization of streptozotocin induced rat

	Observation day at			
Weight of	0	1	2	3
Body (g)	188.67±9.33	167 ± 8.65	150.5±11.81	147.5 ± 8.73
Muscle (mg)	1.6 ± 0.13	1.52 ± 0.17	1.6 ± 0.24	1.18 ± 0.22
Pancreas (mg)	1.06 ± 0.15	1.16 ± 0.19	1.12 ± 0.14	0.83 ± 0.24

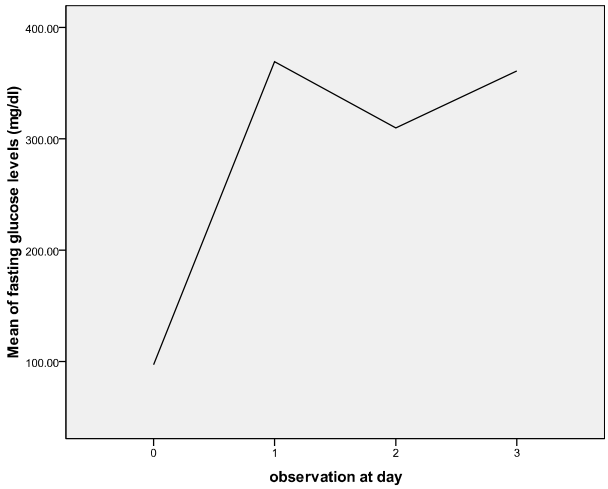

Figure 1. Graph of fasting glucose level (mg/dl) alteration by day

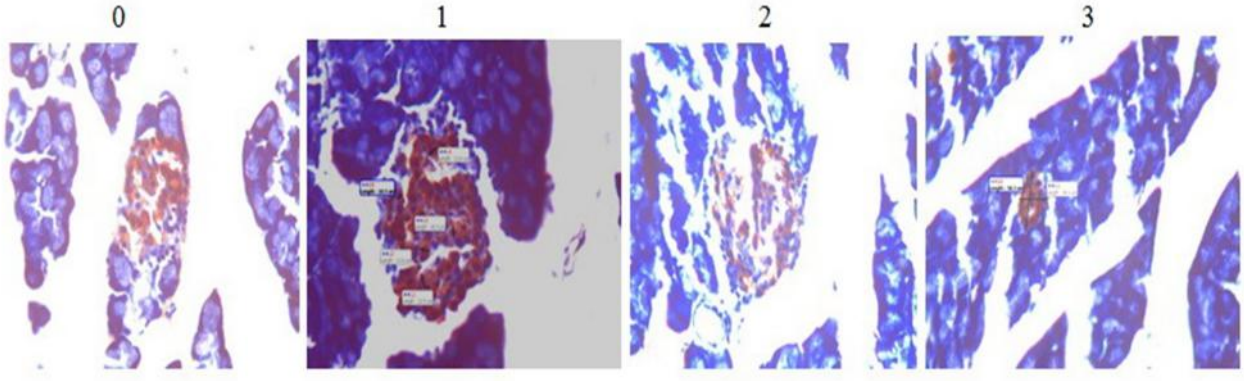

Figure 2 Morphological changes of beta pancreas by day

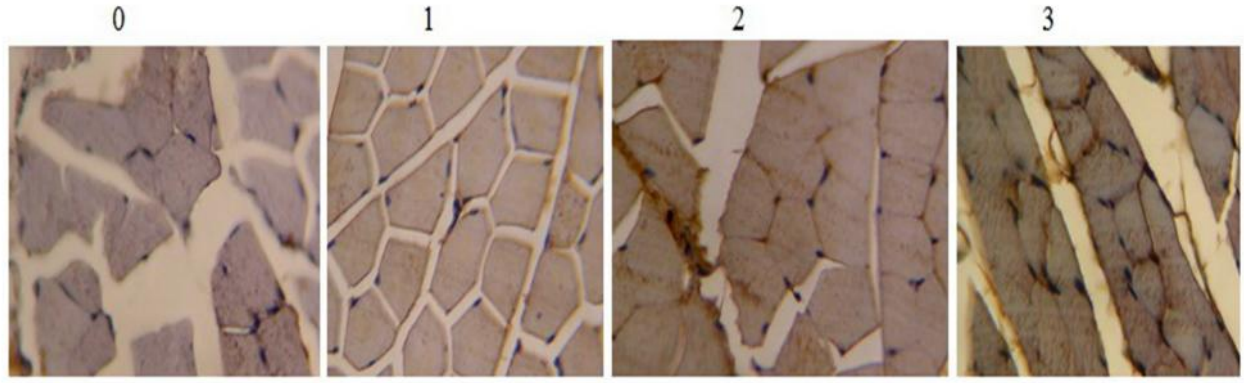

Figure 3. Glut 1 expressed at muscle membrane

Table 2. Fasting glucose levels and Glut-1 expression alteration

Variables	Observation day at			
	0	1	2	3
Glucose (mg/dl)	96.95 ± 19.65	369.23 ± 55.37	309.76 ± 37.55	360.82 ± 42.99
Glut-1 (% positive cells)	0.46 ± 0.29	0.34 ± 0.30	2.10 ± 1.07	2.36 ± 1.91

Raised Glut-1 expression marked the diabetes stage on streptozotocin models

Positive cell of Glut-1 expression was marked as brownish lining at muscle membrane. Positive cells found more in significantly percentage after two days streptozotocin administration (p= 0,013). Muscle Glut-1 in high expression until the end of observation. These histological pictures of muscle Glut-1 expressions can be seen in Figure 3.

DISCUSSION

We successfully induced rats as models of diabetes mellitus by STZ administered protocol. A day after STZ induction, our rats showed hyperglycemic and significantly body weight reduction. These signs were similar with those found in STZ diabetes model protocol (AMDCC). Diabetic state was reached earlier in our study compared with AMDCC protocol. It might be caused by difference of the strains used in both studies. Wistar strain in our study differs from Sprague Dawley strain in the protocol. Wistar strain rats are more easily to become stress than Sprague Dawley rats. We also found the Wistar rats could survive only in 4 days induction before they died.

Diabetic model of STZ rat provided short diabetic nature with complete stages similar to those in early classification. At the first day, hyperglycemic was suddenly found in rat models, even though beta pancreases were still in a good shape and function. We suggested that our rat models were in the stage of non-insulin requirement for treatment (NIR). Interestingly, a day thereafter, fasting glucose levels decreased significantly, even though they remained hyperglycemic. On the other hand, beta pancreas enlarged and became irregular in shaped. It must be the stage of insulin requirement for controlling glucose level (IRC) according to the classification. On the 3rd day, fasting glucose level re-raised and beta pancreas lost it size and original shape. It quite matched with the stage of insulin requirement for survival (IRS), because 60% rat diabetic models died on the 4th day.

We also tried to compare the classification of diabetes with muscle Glut-1 expression in diabetic rat models. On the 1st day observation we had not found any significant changes at the diabetic rat muscles. Surprisingly, we found that muscle Glut-1 expression were significantly higher on day 2 observation, the same day with the falling glucose level phenomena or at the same time with IRC stage of diabetes classification. Glut-1 stayed in high expression at muscle membranes until the end of observation (American Diabetes Association 2004).

Our findings contradicted those of Ciaraldi's study (Ciaraldi et al 2005) who found a significant loss of Glut-1 expression in most biopsy muscle specimens from diabetes samples. Samples of Ciaraldi's study were human and mostly severe diabetes for at least a year in uncertain stage of diabetic classification. The nature of diabetes in human developed slowly; quite different from those in our rat model.

Glut-1 is expressed at sarcolemma in basal condition, e.g. fasting. Stress induces Glut-1 expression in many membrane cells as part of response to defense against molecular disturbances. We suggested that STZ succeeded to create environmental stress which activated some stress protein pathways. Hyperglycemic generates a lot of mitochondrial reactive oxygen species (ROS) which induces oxidative stress in many cells, including muscles. Cells, including muscles, respond to fight against radical oxidant by activating mitogen activated protein kinases (MAPKs). Protein 38 MAPK is one of these kinases which activate Glut-1 as an alternative glucose transporter in diabetic muscles.

Our findings showed that muscle Glut-1 expression marked a border between reversible and irreversible stage of diabetes. We suggested that reversible stage was periods before muscle Glut-1 expression raised significantly. If it is true, any treatment to repair beta pancreas will prevent diabetes develop to the irreversible one. Further, we need to prove whether insulin is an effective treatment to prevent irreversible stage of diabetes when given based on Glut-1 expression.

Table 3. Resume of variable alteration observed by day on stretptozotocin models

Variables	Observation day		
	0→1	1→2	2→3
Body weight (g)	↓	↓	↓
Pancreas weght (mg)	-	-	↓
Muscle weight (mg)	-	-	↓
Fasting glucose level (mg/dl)	↑	↓	↑
Glut1 expression (%)	-	↑	↑
Population & size of beta pancreas	↑	↑	↓
Survival response	Reversible		Irreversible
Windows periods			

Glut-1 expression was still observed from muscles e.g. biopsy and surgery specimens, which was not easy to collect. Further, we need to explore other variables which have strong correlation with Glut-1 expression. These variables are used to arrange a discriminator function which may distinguish whether someone is still in reversible stage or already in irreversible stage. Then, everyone can easily determine the stage of diabetes and decide what must be done.

CONCLUSION

Muscle Glut-1 expression changes dynamically in STZ diabetic rat models. GLUT-1 expression marks a border between reversible and irreversible stage of diabetes.

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