PHARMACODYNAMICS STUDY OF CHOLECALCIFEROL TO GLUT4 PROTEIN TRANSLOCATION IN MUSCLE FIBER OF HYPERGLYCEMIA MICEWHICH INDUCTED BY STREPTOZOTOCIN

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ABSTRAK

Penyerapan glukosa ke dalam sel-sel otot rangka memerlukan insulin dan jalur sinyal bebas, baik yang mengarah ke translokasi glukosa transporter-4 (GLUT4) ke membran plasma. Resistensi insulin terjadi karena kegagalan signaling insulin untuk mentranslokasi GLUT4 sehingga menyebabkan kegagalan penyerapan glukosa dan mengakibatkan hiperglikemia. Cholecalciferol dikenal memiliki fungsi dalam mengatur homeostasis kalsium terbukti meningkatkan sintesis dan sensitivitas insulin. Tujuan dari penelitian ini adalah untuk menjelaskan peran cholecalciferol dalam meningkatan translokasi GLUT4 ke sel otot rangka. 30 mencit diadaptasi selama satu minggu dan kemudian diinduksi menggunakan 150mg/kgBB streptozotocin (STZ) intraperitoneal. Setelah mengalami hiperglikemia, mencit dibagi menjadi 5 kelompok (n = 6 masing-masing). Kelompok I (kontrol hiperglikemia), kelompok II (25ng cholecalciferol), kelompok III (50 mg cholecalciferol), kelompok IV (100 mg cholecalciferol), dan kelompok V (metformin 300mg/kg BB). Cholecalciferol diberikan secara oral pada hari ke-14. Pada hari ke-15 mencit dikorbankan sehingga dapat diambil jaringan otot gastrocnemiusnya untuk pemeriksaan imunohistokimia menggunakan antibodi GLUT4 poliklonal. Hasil pemeriksaan GLUT4 protein dilakukan dengan mengukur Immuno Reaktif Score (IRS - GLUT4). Berdasarkan analisis statistik menunjukkan bahwa ada perbedaan yang signifikan dalam jumlah sel-sel otot skeletal GLUT4 translokasi antara kelompok (p < 0.001). Berdasarkan analisis regresi univariat ada hubungan yang signifikan antara dosis dan respon (p < 0.001). Cholecalciferol dapat meningkatkan GLUT4 translokasi dalam membran sel otot rangka pada model mencit hiperglikemia. (FMI 2013;49:134-138)

Kata kunci: cholecalciferol, GLUT4, otot rangka, streptozotocin

ABSTRACT

Glucose uptake into skeletal muscle cells require insulin-dependent and insulin independent signaling pathways, both leading to the translocation of glucose transporter-4 (GLUT4) to the plasma membrane. Insulin resistance occurs due to failure of insulin signaling to translocate GLUT4 resulting in the failure of glucose uptake and causing hyperglycemia. Cholecalciferol is known to have a function in regulating calcium homeostasis was shown to increase the synthesis of insulin and increasing insulin sensitivity. The purpose of this study is to explain the role of cholecalciferol to increased GLUT4 translocation in skeletal muscle cells. 30 mice adapted for one week and then induced using 150mg/kg BW streptozotocin (STZ) intraperitoneally, after experiencing hyperglycemia mice were divided into 5 groups (n=6 each), Group I (hyperglycemic control), group II (25ng cholecalciferol), group III (50ng cholecalciferol), group IV (100ng cholecalciferol), and group V (metformin 300mg/kgBBB). Cholecalciferol given orally for 14th days. On day 15th the mice were sacrificed so then the gastrocnemius muscle tissue can be taken for immunohistochemical examination using rat GLUT4 polyclonal antibodies. The assesment of protein GLUT4 was conducted by quantifying Immuno Reactive Score (IRS-GLUT4). Based on statistical analysis showed that there were significant differences in the number of cells of skeletal muscle GLUT4 translocation between groups (p<0,001). Based on univariate regression analyses there was significant doses response relationship (p<0,001). Cholecalciferol may increase GLUT4 translocation in skeletal muscle cell membranes in hyperglycemia mice models.(FMI 2013;49:134-138)

Keywords: Cholecalciferol, GLUT4, skeletal muscle, streptozotocin

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INTRODUCTION

Diabetes mellitus (DM) is a problem that faced by world because the increase of incidence, high mortality and morbidity also it needs high cost for treatment and prevention. In 2010, the prevalence of DM in the world is 6,4% (285 million head) and be estimated will increase up to 7,7% (439 million heads). In 2010, Indonesia placed on 9th with 7 million cases and estimated to be 6th with 12 million cases in 2030 (Shaw et al 2010). DM patient has a chronic hyperglycemy caused by relative insulin deficiency and its resistancy in target organ (musculus fiber and adipose cell). Glucose uptake into musculus needs insulin and other

signaling to translocate glucose transporter-4 (GLUT4) from intra cell to the membrane cell. Insulin resistency occur because insulin signaling failed to translocate GLUT4 from citosl to musculus fiber membran, so that glucose uptake failed and cause hyperglycemic (DeFronzo 2004).

The treatment of DM are lifestyle modification, diet, do sport, oral antidiabetic drug (metformin, sulfonilurea, tiazolidindion) and insulin injection. The efficacy of ADG (Antidiabetic Drugs) is acceptable. But, it can't repair the sensitivity of insulin and can't prevent beta cell pranceas degeneration. The development of anthihyperglycemic leads to activate the increase of GLUT4 translocation, one of them is AMPK activation through increase of Ca2+ cytosol (Triplitt 2010, Rotenstein et al 2012). Cholecalciferol is one of D3 vitamin which known has function to regulate the homeostasis of calcium, proven able to increase insulin sensitivity by beta cell of prancreas, increase peripheral insulin sensitivity and decrease D3 vitamin which cause the complication of cardiovascular disease on DM (Chiu et al 2004, Martins et al 2007, Pittas et al 2007).

The treatment by oral cholecalciferol in 14 weeks proven decrease glucose in rat's blood which has DM type 2 (Santos & Vianna 2005). Calcitrion ables to increase insulin sensitivity through the increase of gene expression of insulin receptor and GLUT4 gene. Calcitriol can stimulate GLUT4 translocation on adipose cell culture, 3T3L1 (Maestro et al 2000, Calle et al 2008, Manna & Jain 2012). Based on the research, D3 vitamin could increase the synthesis of insulin and able to increase translocation of GLUT4 in adipose cell. But, the effect of cholacalciferol in translocation from GLUT4 protein to musculus fibers were unclear. Therefore, it needs another research to explain the effect which expected to have explain the function of cholecalcireol to increase the translocation from GLUT4 protein to membrane fibers.

MATERIALS AND METHODS

The 30 male mice (Mus-musculus) strain seiss Webster (Balb/c) which had 25-30 gram body weight were used to this research. The study was conducted in Department of Pharmacology and Patology Anatomy in Medical School, Universitas Airlangga. The mice were study placed on clean and quiet zone, the temperature of 270 C, 12 hours in lighting and 12 hours dark cycles, feeded with pellet and drunk with distilled water. After adaptation, on first day the mice were injected STZ (Sigma Aldrich) single dose 150 mg/kgBB which dissolved in citrate buffer 22,5 mg/ mL intrapertoneally. Mice were fasted in 4 hours to prevent aspiration and empty the stomach by not feeding and sterilization the cge from husk. On first night, after STZ injection, mice given dextrosa liquid 10% to avoid sudden hypoglycemid post injection. 2 days after that, mice were fasted in 6 hours and aspirated mice's blood from the tail to measure blood glucose used On Call Plus Blood Glucose Monitoring System®. Induction be success if blood glucose were 180-500 mg/dL (Etuk 2010).

After hyperglycemic, mice were divided into 6 groups, they were negative-controlled groups (propylene glycol), 1st dosage group (cholecalciferol (Sigma Aldrich) 25 mg), 2nd dosage group (cholecalciferol (Sigma Aldrich) 50 mg), 3rd dosage group (cholecalciferol (Sigma Aldrich) 100 mg) and positive-controlled group (metformin 300mg/kgWB). Cholecalciferol (Sigma Aldrich) were given in 14 days in peroral. In 15th day the mice were sacrifised to take the gastrocnemius muscle tissue and observed its imunohistokimia. This observation used GLUT4 antibody policlonal (bioworld). GLUT4 protein expression on every sample was scored semi-quantitatively based on modifying Remmele metode (Kaemmerer et al 2012). Immunoreactive score (IRS) or Remmele scale index was the result of IRS multiplied by color intensity score of imunoreactive cell (table 1).

Table 1. Immunoreactive score (IRS) were result of positive-cell percentage score (A) multiplied by color-reaction intensity score (B)

Positive-cell percentage score (A)	Color-reaction intensity score (B)
Score 0: No postive cell	Score 0: No color reaction
Score 1: The positive cell less than 10%	Score 1: The colour intensity were weak
Score 2: The positive cell less 11%-50%	Score 2: The colour intensity were average
Score 3: The positive cell 51%-80%	Score 3: The colour intensity were strong
Score 4: The positive cell more than 80%	

All normality of the data were tested by Shapiro-Wilk. Data-analysis technique used one-direction Anova with error rate were 5%. To knew the relation of response dosage, it used simple linier-regretion test with SPSS program.
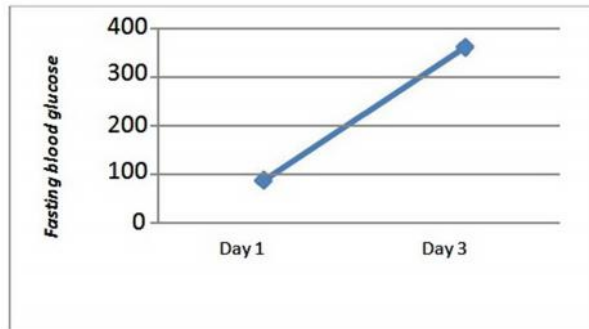
RESULTS

The result of STZ induction

Figure 1.Changes of Fasting Blood Glucose Levels after administration of STZ

From figure 1 above was obtained that mice which given with 150 mg/kgBW injection intraperitoneal single dose had significant hyperglycemia on third day after STZ induction. This was consistent with previous studies, which used doses of STZ to induce hyperglycemia was a single dose of 150 mg / kg and showed the result of hyperglycemia on day two and the seventh and last up to 8 weeks, so time up to 8 weeks could be used to test a material suspected to have hypoglycemic effects (Ventura-Sobrevilla et al 2011, Tian et al 2010). The used of these dosage was alleged to have occurred Non Insulin Requirement (NIR) phases on the stage of the pathophysiology of diabetes in mice with reason that hyperglycemia control group can stay alive until day 14, and the treatment group with metformin may respond well, even without insulin (Yamazaki et al 2009).

GLUT4 protein translocation after cholecalciferol administration

The positive result of GLUT4 protein translocation in muscle fiber was it had brown spot in the cytoplasm.In skeletal muscle cells of normal mice, the translocation of GLUT-4 protein which expressed on the cell membrane was strong enough. While the skeletal muscle cells of mice that experienced hyperglycemia, GLUT-4 translocation proteins expressed weakly. GLUT4 protein translocation assessment done by counting immunoreactive score (IRS-GLUT4) with a

score of 0-12, with classification 0-1 = negative expressions, 2-3 = weak expression, 4-8 = moderate expression, 9-12 = strong expression . IRS-GLUT4 from each treatment group as in Figure 3.
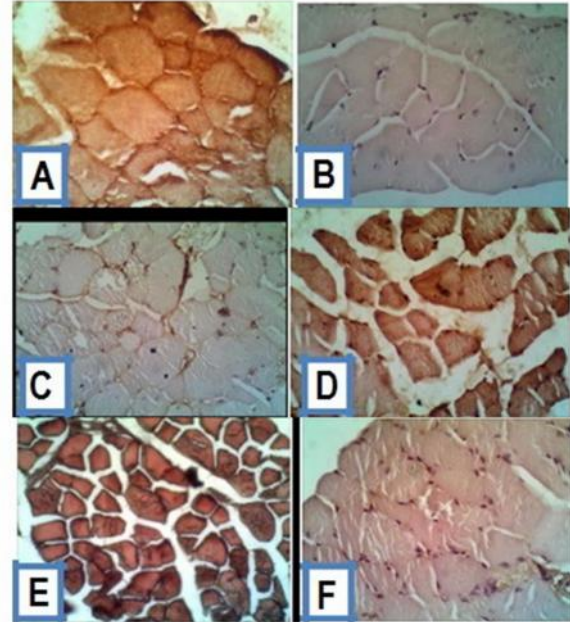

Figure 2. Imunohistokimia coloring of GLUT4 protein translocation in muscle fibers with maglinancy of 400x. (A) normal muscle fiber of mice. (B) negative control (hyperglycemia). (C) dose I. (D) Dose II. (E) Dose III. (F) Moethormin.
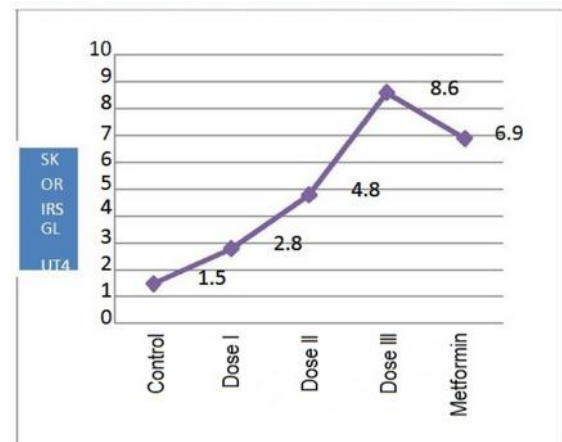

Figure 3.The average graphic of IRS score on every treatment group

Based on this research could be seen that there were significant differences in IRS score for GLUT4 translocation of skeletal muscle cells between treatment

groups (p <0.001). So that this research proved that the administration of oral cholecalciferol can increase GLUT4 protein translocation of skeletal muscle cells. To determine the relation between the increase of cholecalcifereol with effect of the increase of GLUT4 protein translocation in muscle fiber was calculate and the result was on Figure 4.
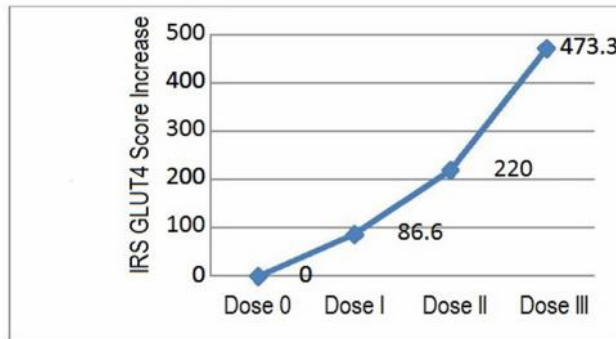

Figure 4. The relation of the percentage increase in dose of cholecalciferol rise with the increase of effect GLUT4 translocation proteins of skeletal muscle cells

The result of regression analysis showed that there was a relation between the dose of cholecalciferol with a percentage increase of GLUT4 translocation (p <0.001), with a correlation value of 0.916 and a positive correlation direction so that it could be concluded that the greater doses of cholecalciferol so GLUT4 protein translocation will be greater too.

DISCUSSION

The administration of STZ caused DNA changes in pancreatic beta cells and DNA fragmentation via DNA alkylation. STZ was a NO donor in large doses, which it caused destruction of pancreatic beta cells and caused DNA damage. STZ also produced ROS that causes DNA fragmentation and cell damage. The barriers to synthesis and secrete insulin due to administration of STZ caused hyperglycemia in mice (Szkudelski 2001), (Lenzen 2008). The mechanism that alleged increase of GLUT4 protein translocation in muscle fibers cells was through the binding of cholecalciferol and its receptor (VDR) in muscle fibers cells. Cholecalciferol will be activated by the enzyme 1- -hydroxylase to its active form is 1,25(OH)2D3. The bond between 1,25(OH)2D3 to the VDR receptor in muscle fibers cells would lead the genomic and non-genomic effects. Genomic effects caused by binding of 1,25(OH)2D3 to VDR in muscle fibers nuclei. This bond will activate gene transcription of mRNA and synthesis of Ca2+ binding protein (calbindin D-9k and calmodulin), thereby it able to maintaince the intracellular calcium concentration in muscle fibers cell (Ceglia & Harris 2013).

Cholecalciferol also have non-genomic effects due to binding of 1,25 (OH) 2D3 to the VDR receptor in the membrane. This bond will provide a faster effect than genomic effects. Cholecalciferol will increase cytosolic Ca2+ through Ca2+ release from the sarcoplasmic reticulum and Ca2+ influx from the extracellular to the cytosol. Cholecalciferol activates phosphoinositol-3-kinase (PI3K) and adenilil cyclase (AC) with the final result is an increase of cytosolic Ca2+. First, PI3K activates phospholipase C (PLC) subsequently forming diacyl glycerol (DAG) and inositol-triphosphate (IP3). IP3 will release Ca2+ from the sarcoplasmic reticulum. Ca2+ activates AMPK through CAMK kinase (CaMKK). AMPK activation is a signal for GLUT4 translocation to the plasma membrane and it uptakes glucose into muscle cells so it can decrease fasting blood sugar levels (Santos et al 2008, Seshadri et al 2011).

Pharmacological activity of a drug is determined by the presence of the drug binding to the receptor. The intensity magnitude of pharmacological effects that appear depend on the concentration or amount of drug that reaches the receptor and the type of drug-receptor binding. The increase of drug concentration in serum followed by a rise in the probability of a pharmacological response and will be followed by a simultaneous increase in toxic effects. The increase in dose of cholecalciferol cause the increase of concentration of 1,25 (OH) 2D3 in the serum, so the probability of bond between 1,25 (OH) 2D3 and receptor of VDR will increase and activates signal transduction so that increasing GLUT4 translocation in muscle fibers cells (Katzung et al 2006). The examination to show insulin activity was not counducted in this research so it needed other examination used HOMA-IR and a research to know the level of 25(OH)D3 serum in subject of the study which has given cholecalciferol. The future research of chalciferol to the clinical test is important to be done so it can be useful.

CONCLUSION

The administration of chalciferol able to increase GLUT4 protein translocation in muscle fiber cells in hyperglycemic mice which has inducted by streptozotocin. And there are obtained a relation betreen cholecalciferol dose with the effect of GLUT4 protein translocation increase in muscle fiber cell which the

greater cholecalciferol dose will produce the greater increase of GLUT4 protein translocation too.

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REFERENCES

Calle C, Maestro B, Arencibia MG (2008). Genomic actions of 1,25-dihydroxyvitamin D3 on insulin receptor gene expression, insulin receptor number and insulin activity in the kidney, liver and adipose tissue of streptozotosin-induced diabetic rats. BMC Molecular Biology9, 65

Ceglia L and Harris S (2013). Vitamin D and its role in skeletal muscle. Calcif Tissue Int 92, 151-162

Chiu KC, Chu A, Go VL, Saad MF (2004). Hypo-vitaminosis D is associated with insulin resistance and cell dysfunction. Am J Clin Nutr 79, 820-825

DeFronzo RA (2004). Pathogenesis of type 2 diabetes mellitus. Med Clin N Am 88, 787-835

Etuk EU (2010). Animals models for studying diabetes mellitus. Agric Biol J N Am 1, 130-134

Kaemmerer D, Peter L, Lupp A, Schulz S, Sänger J, Baum RP, Prasad V, Hommann M (2012). Comparing of IRS and Her2 as immunohistochemical scoring schemes in gastroenteropancreatic neuroendocrine tumors. Int J Clin Exp Pathol5, 187-194

Katzung BG, Masters SB, Trevor AJ (2006). Basic and Clinical Pharmacology. 11th ed., Singapore, McGraw Hill Companies

Lenzen S (2008). The mechanisms of alloxan-and streptozotocin-induced diabetes. Diabetologia 51, 216-226

Maestro B, Campión J, Dávila N, Calle C (2000). Stimulation by 1,25-dihydroxyvitamin D3 of insulin receptor expression and insulin responsiveness for glucose transport in U-937 human promonocytic cells. Endocrine Journal 47, 383-391

Manna P and Jain SK (2012). Vitamin D upregulates glucose transporter 4 (GLUT4) translocation and glucose utilization mediated by Cystathionine-γ-Lyase (CSE) activation and H2S formation in 3T3L1 adipocytes. Available from http://www.jbc.org/cgi/doi/10.1074/jbc. M112.407833. Accessed October 16, 2012

Martins D, Wolf M, Pan D, Zadshir A, Tareen N, Thadhani R, Felsenfeld A, Levine B, Mehrotra R, Norris K (2007). Prevalence of cardiovascular risk factors and the serum levels of 25-hydroxyvitamin D in the United States. Data from the Third National Health and Nutrition Examination Survey. Arch Intern Med 167, 1159-1165

Pittas AG, Lau J, Hu FB, Dawson-Hughes B (2007). The role of vitamin D and calcium in type 2 diabetes. A Systematic Review and Meta-Analysis. J Clin Endocrinol Metab92, 2017-2029

Rotenstein LS, Kozak BM, Shivers JP, Yarchoan M, Close J, Close KL (2012). The ideal diabetes therapy: What will it look like? How close are we? Clinical Diabetes 30, 2

Santos JM, Ribeiro SB, Gaya AR, Appell HJ, Duarte JA (2008). Skeletal Muscle pathways of contraction enhanced glucose uptake. Int J Sports Med 29, 785-794

Santos RS and Vianna LM (2005). Effect of cholecalciferol supplementation on blood glucose in an experimental model of type 2 diabetes mellitus in spontaneously hypertensive rats and Wistar rats. Clinica Chimica Acta 358, 146-215

Seshadri KG, Tamiselvan G, Rajendran A (2011). Role of vitamin D in diabetes. J Endocrinol Metab 1, 47-56

Shaw JE, Sicree RA, Zimmet PZ (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Research and Clinical Practice 87, 4-14

Szkudelski T (2001). The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. PhysiolRes 50, 536-546

Tian HL, Wei LS, Xu ZX, Zhao RT, Jin DL, Gao JS (2010). Correlation between glucose level and diabetes signs in streptozotocin induced diabetic mice. Global Journal of Pharmacology 4, 111-116

Triplitt C (2010). Improving treatment success rates for type 2 Diabetes: Recommendations for a Changing Environment. Available from http://www.ajmc.com/publications/supplement/2010/a292_10aug/a292_10aug_triplitt_s195to200/1#sthash.G8uD162puf. Accessed October 16, 2012

Ventura-Sobrevilla J, Boone-Villa VD, Aguilar CN, Román-Ramos R, Vega-Avila E, Campos-Sepúlveda E, Alarcón-Aguilar F (2011). Effect of varying dose and administration of streptozotocin on blood sugar in male CD1 mice. Proc West PharmacolSoc 54, 5-9

Yamazaki M, Kato A, Kato C, Fujii E, Adachi K, Miyoshi A, Suzuki M (2009). Segmentation of the patophysiological stage of diabetic changes in the db/db mouse. J Toxicol Pathol 22, 133-137