ORIGINAL PAPER

Differential Expression of IL-10 Gene and Protein in Target Tissues of Rattus Norvegicus Strain Wistar Model Type 2 Diabetes Mellitus (T2DM)

Yohanes Bare1,3, Agung Pramana Warih Marhendra1, Tomohiko Sasase2, Fatchiyah Fatchiyah1,3

1Department of Biology, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, East Java, Indonesia
2Biological/Pharmacological Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc, Osaka, Japan
3Research Center of Smart Molecule of Natural Genetics Resources UB, Faculty of Mathematics and Natural Sciences, Brawijaya University, Malang, East Java, Indonesia

Corresponding author: Prof. Fatchiyah Fatchiyah, PhD. Head of Research Center of Smart Molecule of Natural Genetics Resources UB, Biosains Institute Building 1st Floor, Jl. Mayjend Panjaitan, Malang, Indonesia, 65145. Phone: +62341 575841. E-mail: fatchiya@ub.ac.id

ABSTRACT

Introduction: Type 2 diabetes mellitus (T2DM) is a chronic metabolic disease caused by insulin resistance. Insulin resistance leads to hyperglycaemia that causes complication such as microangiopathy and macroangiopathy. The immune system of T2DM will be produce IL-10 as an anti-inflammatory cytokine role immune-stimulator and immunosuppressant in the organ system. This present study investigated of IL-10 gene profile and protein expression in the rat organ (Rattus norvegicus) strain Wistar model T2DM. Material and Methods: This research was used three of male rats group T2DM and three of male of normal rat as a control. The DNA tissues were isolated, amplified and sequenced by using IL-10 gene primer. The IL-10 protein profile and expression of rat tissues was analyzed using Experion-Pro260 gel and dot blotting using IL-10 antibody. Results: This study showed the differential expression of IL-10 gene profile among tissues among normal and T2DM groups. The IL-10 gene sequences, we found eight mutations in brain and twenty-seven mutations on gastric of T2DM group compare with control group, meanwhile there are no mutation in other tissues of both groups. The protein profile of all tissues in both groups was completely diverse as proper. Moreover, the level expression of IL-10 of heart, lung, gastric and kidney of T2DM group was lower than other tissues of both groups. Conclusion: This study concludes that T2DM animal model triggering mutation of IL-10 gene sequences of brain and gastric and induced the increasing level expression of IL-10 of ileum, brain and liver.

Keywords: hyperglycaemia, IL-10 gene, T2DM.

1. INTRODUCTION

The case of Type 2 Diabetes Mellitus (T2DM) in Indonesia has increased, the World Health Organization (WHO) reported the case of T2DM in Indonesia ranked 5th in the South-East Asia Region and continues to increase around 6% of the population by 2030 (1, 2). The T2DM is characterized by insulin resistance, which is causing hyperglycaemia. When the prolong onset of hyperglycaemia caused blood vessels damage and trigger abnormal metabolic activity resulting in diabetic ketoadisis. In addition, the function of the heart as an organ that serves as blood circulation and kidneys as filtration blood up-regulated. Consequently the patient has chronic complications T2DM induces microangiopathy such as retinopathy, nephropathy, neuropathy and also macroangiopathy such as increased risk of cardiovascular disease and peripheral artery disease (PAD). T2DM also affects the damage other organs such as brain and digestive system (3-8).

Hyperglycaemia induces the inflammation by releasing pro-inflammatory (IL-1β, IL-6, TNF-α, etc) and anti-inflammatory cytokine (IL-4, IL-10, IL-11, IL-13, etc). The IL-10 is has important function as immune-stimulator and immunosuppressant to repair the damaged organ (9–11). Yaghini et al (12) reported the in serum IL-10 levels in T2DM patients lower than normal. Recently our study also shown the decreasing expression of IL-10 cause ileum destruction in rheumatoid arthritis animal model (13). We were also found the T2DM rats brain reduced cell proliferation and increased apoptosis in brain cells (14). Though, the cause of decreasing of IL-10 levels on T2DM rats brain still unclear. To examine the abnormality IL-10 gene sequence and IL-10 protein expression of different
tissues, this study prepared T2DM rat animal model and control rat group. This study focused to investigate the differential profile of IL-10 gene sequence and IL-10 protein expression level in target tissues of rat model T2DM.

2. MATERIAL AND METHOD

Experimental Animal

The experimental animals were using *Rattus norvegicus* strain Wistar obtained from the Laboratory of Experimental Animal, Technical Implementation Units, Integrated Research and Testing Laboratory Gadjah Mada University Yogyakarta, Indonesia. The animals divided into two groups with 3 control rats (C) and 3 T2DM rats (DM). All animal obtained were acclimatized for one week. The T2DM rats group were established from normal rat that fed by high cholesterol food for 2 months and then injected with a single dosage by streptozotocin 25mg/BW a week after the rat positive hypercholesterolemia. Samples were collected from the control rats group (ileum (IC), brain (BC), heart (HC), liver (LiC), lung (LuC), kidney (KC), gastric (GC)) and T2DM rats group (ileum (IDM), brain (BDM), heart (HDM), liver (LiDM), lung (LuDM), kidney (KDM), gastric (GDM)).
Differential Expression of IL-10 Gene and Protein in Target Tissues of Rattus Norvegicus Strain Wistar Model Type 2 Diabetes Mellitus (T2DM) rats group (ileum (IDM), brain (BDM), heart (HDM), liver (LiDM), lung (LuDM), kidney (KDM), gastric (GDM)). This research study has been evaluated and approved by the Ethics Commission of Brawijaya University Malang, East Java (Certificate number, 417-KEP-UB Year 2015).

DNA Isolation, Amplification, and Sequencing

DNA Isolation method according Sambrook et al (15) with some modifications. DNA was amplified using the IL-10 primer from GenBank NC_005112.4. The primer was designed from exon 1 in IL-10 gene sequence, IL-10F 5'-ATA- AAAGGGGACACCGGC-3' and IL-10R 5'-CTCATA- ACCCATGGCTTGGC-3'. Amplification products PCR program hot denaturation 94°C for 5 minutes (1 cycle), denaturation 94°C for 45 seconds, annealing 57°C for 45 seconds, extension 72°C for 45 seconds (35 cycles), and post extension 72°C for 7 min. The PCR products were measured qualitatively using 1.5% agarose gel. DNA sequencing was using ABI 3730xl DNA Sequencer (Koeln, Germany). Alignment

Figure 2. Profile of protein based on molecular weight of protein using analysis experion pro260. The profile of Control rats Group (ileum (IC), brain (BC), heart (HC), liver (LiC), lung (LuC), kidney (KC), gastric (GC)) and T2DM rats group (ileum (IDM), brain (BDM), heart (HDM), liver (LiDM), lung (LuDM), kidney (KDM), gastric (GDM)).
was analyzed by the Bioedit software ver. 7.2.

Profi ling protein and Dot Blotting

Protein isolation was conducted based method on Fatchiyah, et al (16). The protein concentration was measured by using Nanodrop spectrophotometer. Profile protein analysis used Experion Pro260 kit (Catalog Bio-Rad ©, Hercules, CA). Detection of IL-10 protein expression level using dot blotting based on Rohmah et al (17) with some modifi cations. Primer antibody was using mouse anti IL-10 (1:1500 Santa Cruz Biotechnology, Inc) and Anti-mouse IgG labeled with Alkaline Phosphatase conjugated as secondary antibody. Density of IL-10 reaction measured quantitatively by ChemiDoc Gel Imaging (BioRad) and Quantity One program and analyzed by Microsoft Excel.

3. RESULTS

IL-10 Gene Profi le

The IL-10 gene amplifi cation (Figure 1A) was successfully demonstrated with 1.5% gel agarose electrophoresis of 470bp. The sequence target DNA exon 1 of IL-10 gene size 166bp. The similar type of mutation that occurs are fi ve mutations that change G to A, G→C, T→G, C→G, A→C, this mutation induced the amino acid (Ser→Arg→Asp) also changed into Asn-Thr-His. Besides that, the other mutation in brain (BDM) are G→A, T→A, A→G, G→C, C→G, induced amino acid Ser into Arg and in gastric (GDM) are A→C, C→G, G→T, T→A, A→G, G→C, C→G, C→A, A→G, G→C, C→G, induced amino acid (His→Ala→Met→Glut→Glu→Pro→Gln→His→Pro) to be Pro→Gly→Arg→Thr→lys→Ser→Arg→Cys→Phe (Figure 1D). In GDM we found new amino acid in number twenty-eight is Glut as absent in other tissues both of group.

Protein Profi le

The protein profiles found in the control rats group (C) and T2DM rats group showed different results (Figure 2). In organ ileum (IC) found 37 bands, brain (BC) found 42 bands, heart (HC) found 36 bands, liver (LiC) found 18 bands, lung (LuC) found 40 bands, kidney (KC) found 63 bands, gastric (GC) found 39 bands, ileum (IDM) found 19 bands, brain (BDM) found 26 bands, heart (HDM) found 17 bands, liver (LiDM) found 25 bands, lung (LuDM) found 19 bands, kidney (KDM) found 13 bands, gastric (GDM) found 28 bands. Protein profi le in the control rats group (C) and T2DM rats group showed diff erent amounts of protein level, the number of protein bands in the normal group was higher than T2DM.

Identification of IL-10 protein expression using specifi c antibody showed that blue-purple visualization on spots of positive control and proteins from protein sample. Binding of proteins and antibody specifi c showed that control positive was higher of mean density than T2DM (Figure 3A). In this study we found diff erent amount of density (Figure 3B). The density of IL-10 in IDM, BDM, HC, LiDM, LuC, KC and GC higher than among of tissues.

4. DISCUSSION

The IL-10 gene mutations that occur in BDM and GDM cause diff erent eff ect on organ function. Mutations in BDM are a type of substitution mutation, in which there is a change of base in some parts replaced with another base (Figure 1C). This mutation accounts for about 5% of the total DNA sequence of IL-10 and the mutation leads to increased pro-infl ammatory cytokines as mediators of damaged organ. The IL-10 gene mutations occurring in the brain will cause the increased performance of the IL-10 as anti-infl ammatory. The infl ammation in T2DM microglial cells brain increasing immunocompetent cells has potent and diverse eff ects on essentially all hematopoietic cells that infl ate the brain following injury (14, 18)

Figure 3. Level of IL-10 expression in different tissues of T2DM and Normal Rats. (A) Level IL-10 protein was identified by dot blot analyzed using IL-10 antibody. (B) Statistic Analysis of dot blot. Positive control (C+) from LiC, negative control (C−) using PBS, Control Group (ileum (IC), brain (BC), heart (HC), liver (LiC), lung (LuC), kidney (KC), gastric (GC)) and T2DM rats group (ileum (IDM), brain (BDM), heart (HDM), liver (LiDM), lung (LuDM), kidney (KDM), gastric (GDM)).
Mutations occurring in GDM are a type of substitution mutation with a mutation count of about 16% of the total DNA sequence. Mutations occurring in the GDM will cause decreased effectiveness of IL-10 performance in gastriacs an anti-inflammatory cytokine resulting in increased pro-inflammatory mediation TNF-α exacerbates damaged organ in the T2DM. Mutation of IL-10 in gastricdoes not regulate the homeostasis of the gastric mucosa and induce the development of mucosal metaplasia. Therefore, further investigation on the role of epithelial IL-10 in gastric tissue is needed (19, 20). Kryukov, et al (21) concluded that 20% of new mutations in humans result in loss of function, while 53% had adverse effects and 27% were neutral effectively related to phenotype.

Mutation occurring in sequence of IL-10 gene in organ BDM and GDM shows that T2DM may lead to frameshift mutation same amino acid (Ser→Asn, Arg→Thr, Asp→His), but mutation in amino acid number forty-four has different amino acid result, BDM (Glu→Asp), GDM (Glu→Asn). Other frameshift mutations in BDM were (Ser→Arg), GDM (His→Pro, Ala→Gly, Met→Arg, Glu→Thr, Gln→Lys, Pro→Ser, Gln→Arg, His→Cys, Pro→Phe) (Figure 1D).

Bands in the normal rats group (C) and the T2DMrats group (DM) showed different amounts of bands, as a whole, the number of protein bands formed in the normal group was higher than that of the protein band under T2DM. Differences of bands density showed that in organs with T2DM losing some protein when compared with normal. One of the causes of flooding of activated protein differences is due to the condition of insulin resistance in patients with T2DM. Pareire et al (22) resulted that men with T2DM had insulin resistance against protein metabolism. Insulin-resistant, impaired energy inhibits stimulation of protein synthesis. Their study indicated that the clinical entity of T2DM involves defective protein metabolism impaired insulin plus amino acid stimulated protein synthesis in T2DM men may be of clinical importance.

Spots with blue-purple visualization on PVDF membrane indicated that primer antibody and secondary antibody had positive reaction with recombinant IL-10 protein. In this research we evaluated the expression of IL-10 protein by the primary antibody dotblot assay and did not use separated protein according to their molecular weight (23). The dot blot assay also showed colour intensity to determine titer of antigen antibody binding. In the previous study, Rohmah et al (13) showed that the ileum destruction was also related with alteration of inflammatory cytokines, the increasing of cytokine pro-inflammation IL-17 and decreasing of cytokine anti-inflammation IL-10 in Rheumatoid Arthritis model with inflammation response. Interestingly, in this study we found that IL-10 on IDM, BDM, and LiDM (Figure 3B) indicate opposite performance, where earlier inflammatory conditions may provide different performance of IL-10 function. Interestingly on organ IDM, BDM, and LiDM the performance of IL-10 expression increased compare with normal. The increased expression of IL-10 protein correlated with dendritic cell signal transduction in IDM, BDM, and LiDM. IL-10 signaling is targeted toward surplus STAT1 activation, different with STAT3. Signaling from STAT1 causes IL-10 triggered pro-inflammatory responses, supports Th1-like inflammation, processes that favor apoptosis and control of tumour growth leading to increased inflammation in inflammatory diseases (24–26).

5. CONCLUSION

Based on our result, the T2DM animal model cause mutations on IL-10 gene and amino acid sequences of brain and gastric tissues. Those mutations induced the increasing of IL-10 expression level in ileum, brain, liver, but decreasing of IL-10 expression level in heart, lung, kidney and gastric.

REFERENCES

1. Roglic G. Global report on diabetes. Geneva Switzerland: World Health Organization. 2016; (6): 9-12.

2. WHO. Diabetes. WHO. 2017. http://www.who.int/diabetes/facts/world_figures/en/index5.html. Access March, 15th 2018

3. Katsuda Y, Ohta T, Miyajima K, Kemmochi Y, Sasse T, Tong B, Yamada T. Diabetic complications in obese type 2 diabetic rat models. Experimental Animals. 2014; 63(2): 121-132.

4. Elazziz DSA, Hafez MH, Galal NM, Meshaal SS, El Marsafy AM. CD4+ CD25+ cells in type 1 diabetic patients with other autoimmune manifestations. Journal of Advanced Research. 2014; 5(6): 647-655.

5. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia. 2003; 46(1): 3-19.

6. King AJ. The use of animal models in diabetes research: Animal models of diabetes. British Journal of Pharmacology. 2012; 166(3): 877-894.

7. Kusminski CM, Shetty S, Orci L, Unger RH, Scherer PE. Diabetes and apoptosis: lipotoxicity. Apoptosis. 2009; 14(12): 1484-1495.

8. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. The Lancet. 2011; 378(9786): 169-181.

9. Baratawijaya, KG. Imunologi. Dasar Edisi ke-11. Jakarta Fakultas Ke-

10. Iyer SS, Cheng G. Role of interleukin 10 transcriptional regulation in inflammation and autoimmune disease. Critical ReviewsTM in Immuno-

11. Ogawa Y, Duru EA, Ameredes BT. Role of IL-10 in the resolution of airway inflammation. Current Molecular Medicine. 2008; 8(5): 437-445.

12. Yaghini N, Mahmoodi M, Asadikaram GR, Hassanshahi GH, Khorrampelazad H, Arababadi MK. Serum levels of interleukin 10 (IL-10) in patients with type 2 diabetes. Iranian Red Crescent Medical Journal. 2011; 13(10): 751-752.

13. Rohmah RN, Widijanto E, Fatchiyah F. Protective effect of CSN1S2 protein of goat milk on ileum microstructure and inflammation in rat-CFA-induced rheumatoid arthritis. Asian Pacific Journal of Tropical Disease. 2015; 5(7): 564-568.

14. Sambrook J, Russell DW. Molecular Cloning A Laboratory Manual
Differential Expression of IL-10 Gene and Protein in Target Tissues of Rattus Norvegicus Strain Wistar Model Type 2 Diabetes Mellitus (T2DM)

16 Fatchiyah, Arumingtyas, EL., Widyarti, S, Rahayu S. Biologi Molekul-er: Prinsip Dasar Analisis. Jakarta Erlangga. 2011; 104-106.
17 Rohmah RN, Widyasari S, Aulanni’am A, Fatchiyah F. Cloning and Expression of hGAD65 Gene in E. Coli BL21. Indonesian Journal of Biotechnology. 2013; 18(1): 52-57.
18 Strle K, Zhou J, Shen W, Broussard S, Johnson R, Freud G. Interleukin-10 in the brain. Crit Rev Immunol. 2001; 21(5): 427-449.
19 Tseng CH. Metformin reduces gastric cancer risk in patients with type 2 diabetes mellitus. Aging (Albany NY). 2016; 8(6): 1636-1649.
20 Garcia J M, Stillings SA, Leclerc. Role of interleukin-10 in Acute Brain injuries. Frontiers in Neurology. 2017; (8): 1-17.
21 Kryukov GV, Pennacchio LA, Sunyaev SR. Most Rare Missense Alleles Are Deleterious in Humans: Implications for Complex Disease and Association Studies. The American Journal of Human Genetics. 2007; 80(4): 727-739.
22 Pereira S, Marlis EB, Morais J A, Chevalier S, Gougeon R. Insulin Resistance of Protein Metabolism in Type 2 Diabetes. Diabetes. 2008; 57(1): 56-63.
23 Guillenin N, Meunier B, Jurie C, Cassar-Malek I, Hocquette JF, Levéziel H, Picard B. Validation of a dot-blot quantitative technique for large scale analysis of beef tenderness biomarkers. Journal of Physiology and Pharmacology. 2009; (60): 91-97.
24 Mühl H. Pro-inflammatory signaling by IL-10 and IL-22: bad habit stirred up by interferons? Frontiers in Immunology. 2013; 4(18): 1-10.
25 Carey A J, Tan CK, Ulett GC. Infection-induced IL-10 and JAK-STAT A review of the molecular circuitry controlling immune hyperactivity in response to pathogenic microbes. Jak-Stat. 2012; 1(3): 159-167.
26 Latifi SQ, O’Kiordan MA, Levine AD. Interleukin-10 Controls the Onset of Irreversible Septic Shock. Infect Immun. 2002; 70(8): 4441-4446.