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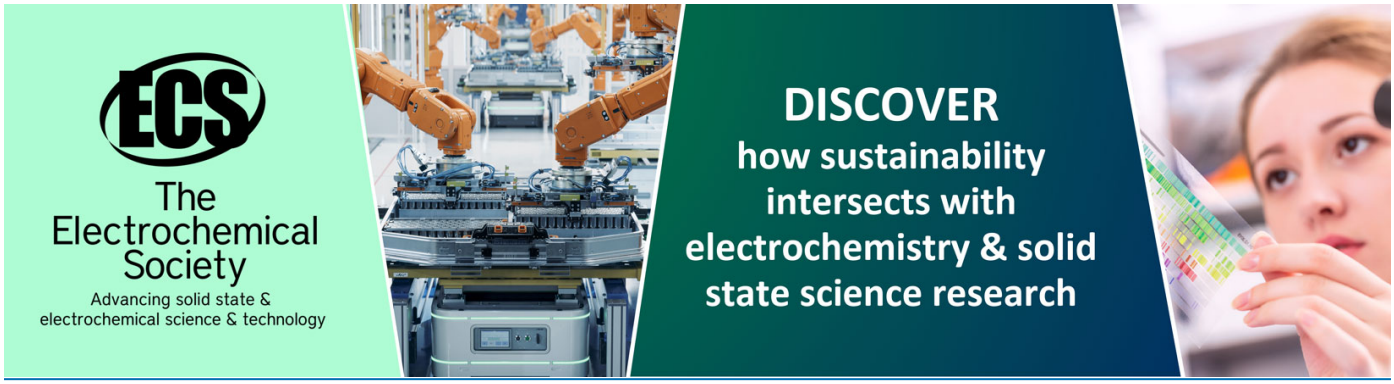

The Improvement of INS Gene Sequences on Rat Model of Type 1 Diabetes in Goat Milk Casein Protein Treatment, but not in Glibenclamide

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Abstract. This study was aimed to determine the effect of CSN1S2 protein from Etawah Crossbred goat milk for repairing DNA sequence of INS gene in T1DM rat model. We divided the experimental rats into control group, diabetes group, control with CSN1S2 protein treatment group, diabetes with CSN1S2 protein treatment group, and diabetes with glibenclamide treatment group. The dose of CSN1S2 protein and glibenclamide used was 800 mg/kg BW and 5 mg/kg BW, respectively. We isolated the DNA from rat pancreas tissue and amplified it with a specific primer of INS gene. Our study investigated that there were transition, transversion, and deletion mutation in diabetic rat. The glibenclamide administration had changed 6 point mutations in INS of the diabetic rat to normal, but it created the other 9 new mutations. The CSN1S2 protein treatment showed mutation repairing in INS of diabetes group. Consequently, CSN1S2 have potential effect as anti-diabetes and is safe to consume than glibenclamide.
Keywords: CSN1S2, insulin gene, T1DM

1. Introduction

Type 1 diabetes (T1DM) is an autoimmune disease leading to the deficiency of pancreatic beta cells when insulin secretion in the islets of Langerhans decrease [1]. Rat insulin is synthesized by two genes, preproinsulin-1 (Ins-1) and preproinsulin-2 (Ins-2) [2]. Preproinsulin-1 (Ins-1) is rodent-specific retrogene and ortholog to human gene. The preproinsulin-1 and preproinsulin-2 encode proinsulin peptide which expressed in pancreas. Insulin action occurs because of the interaction between insulin and its receptors on the target cell membrane. It activates the downstream proteins, such as Insulin Receptor Substrate-1 (IRS-1), Insulin Receptor Substrate-2 (IRS-2) and other proteins [3-4].

The triggers for T1DM can vary. The environmental and dietary factor may cause someone with the genetic susceptibility to develop this disease. Bovine serum albumin and bovine insulin in cow's milk are the implicated dietary proteins in diabetes development. Bovine insulin activates T-cells in someone with diabetes predisposition. It leads to the destruction of beta-cell through the direct attack from T-killer cells [5]. One of the drugs used in the management of diabetes is glibenclamide. Glibenclamide also known as glyburide is derivative of sufonylurea with sequester characteristic that have a role for hypoglycemia stimulation as farmakogenomic agent [6]. This drug acts by inhibiting ATP-sensitive

potassium channel (K-ATP), then the cells membrane will depolarize and open the calcium channel in β cells pancreas. As a result, the intracellular calcium level will increase and stimulate insulin secretion from granula secretory by exocytosis [7-8]. However, glibenclamide can cause hypoglycaemia as a side effect [9].

The alternative that can be implemented to deal with diabetes management is the consumption of healthy nutrition. Goat milk can act as a potential health-promoting food due to its nutritional properties and its lower allergenicity when compared to cow milk [10]. Etawah crossbred goat from Indonesia has a specific protein in its milk, namely α-s2 casein (CSN1S2) protein. The CSN1S2 administration in rheumatoid arthritis rat can induce the STAT3 gene improvement through frameshift mutation [11]. We predicted that the CSN1S2 protein might affect the repairing mechanism of INS gene in type 1 diabetic rat as well. Therefore, this study aimed to analyse the potential effects of CSN1S2 protein from Etawah crossbred goat milk as antidiabetes through the INS gene sequence improvement.

2. Materials and Methods

2.1. Animal model

We used the 2-3 months old adult Wistar rats (*Rattus norvegicus*) for the experimental animal. Their weight was about 150-200 gram. We purchased the rats from The Integrated Research and Testing Laboratory, Gadjah Mada University. These rats underwent acclimatization for one week. We divided the rats into five groups, namely control (C), control with 800 mg/kg BW of CSN1S2 protein treatment (CM), diabetes (DM), diabetes with 800 mg/kg BW of CSN1S2 teratment (DMM), and diabetes 5 mg/kg BW of glibenclamide treatment (DMG). We induced the rats by 60 mg/kg BW of streptozotocin for T1DM rat model establisment. These animals treatment was evaluated and approved by ethic committee of Brawijaya University (Ethical certificate number: 417 KEP UB).

2.2. Preparation of CSN1S2 protein from Etawah Crossbred goat milk

We isolated the CSN1S2 protein from Etawah Crossbred goat milk. A 250 mL of goat milk was boiled on 40℃ of temperature and added 5 mL of glacial acetic acid. The separation of protein precipitation was done using the mesh of nylon membrane. The protein concentration measurement was performed by NanoDrop spectrophotometer. The CSN1S2 protein was stored at -20℃ [12].

2.3. DNA isolation and amplification

We isolated the DNA from rat pancreas tissue. The tissue was weighed 0,3 gram, then homogenized. The next procedures referred to Fatchiyah *et. al.*, 2011 with few modifications in the extraction buffer [13-14]. The DNA was quantified by using NanoDrop spectrophotometer. The quality of the DNA was evaluated using agarose gel 1% with chemidoc gel imaging. The amplification mix solution composition was referred to Fatchiyah *et. al.*, 2011. We designed the primer based on NCBI sequence. The primer pair for INS was NC_019129.3: rINS-Foward 34-5' AAT GTT CCA ACA TGG CCC TG 3' and rINS-Reverse 333-5' TGC AGC ACT GAT CCA CAA TG 3'. The PCR program for this primer was one cycle for the hot start at 95℃ for 1 minute, then 35 cycles for denaturation at 95℃ for 1 minute, annealing at 51℃ for 1 minute, and extension at 72℃ for 30 seconds. The post extension was at 72℃ for and 10 minutes. The amplification results were tested by agarose gel 1,5%. They could be stored at -20℃ for long-term storage.

2.4. DNA sequencing and analysis

A 5 µl of purified PCR products was mixed with 5 µl of primer. We homogenized the mixture before sequenced it by using ABI 3730xl DNA Sequencer (Koeln, Germany) [15]. The DNA sequences data were aligned and analyzed using multiple alignment program by BioEdit version 7.0 [16].

3. Results and Discussion

The INS DNA sequences from our samples was aligned with the sequence from NCBI database with gene ID: NM_019129. The aligned sequences was started from nucleotide 65 to 463. There were some

frameshift mutations in nucleotides of all treatments. Transition mutations had been occurred in CM group from $_{102}$C to $_{102}$T, $_{106}$T to $_{106}$C and deletion sequence in nucleotides 100, 101, and 114. The sequence of DM group showed transversion mutations in $_{99}$C to $_{99}$G, $_{112}$C to $_{112}$G, and $_{115}$A to $_{115}$C, transition mutation in nucleotide $_{102}$C to $_{102}$T and also deletion sequences in nucleotide 97, 100, 101, 114 and 124. The CSN1S2 protein treatment with the dose of 800 mg/kg BW in diabetic rat was able to improve the DNA sequence of INS. In addition, the DMM group had similar nucleotide sequence compared to CM group, but it had transversion mutation in $_{96}$G to $_{96}$C. Glibenclamide has been known as the common drug for diabetes mellitus patient management. However, this drug treatment had changed many of INS DNA sequence in diabetic rats. The DMG group underwent DNA sequence alteration, namely deletion in nucleotides 101, 114 and 115, transversion mutations in $_{99}$C to $_{99}$G, $_{108}$G to $_{108}$T, $_{109}$G to $_{109}$T, $_{111}$G to $_{111}$T, $_{121}$G to $_{121}$T, and, $_{125}$A to $_{125}$C, and $_{127}$G to $_{127}$C, and transition mutation $_{103}$C to $_{103}$T and $_{128}$C to $_{128}$T (Figure 1).

Mutations caused by streptozotozin can be repaired through the base excision repair (BER). The administration of CSN1S2 protein as nutrition for rats with T1DM might induce the DNA polymerase β, then the BER mechanism in INS gene would be stimulated. This repairing mechanism happened through the elimination of 5'deoxyribose phosphate (5'dRP) compound and catalyzation of DNA synthesis in single-nucleotide-gap-filling by lyase activity which stimulated by DNA polymerase β [17-18]. The glibenclamide treatment to animals with DM as a synthetic drug may cause the condensation and DNA fragmentation in cells and lead to specific apoptosis in β cells pancreas [19-20]. In other study, mutation in insulin gene occurred in normal animal treated with CSN1S2 protein. It can be prevented by consuming the whole milk which contain vitamins, several kinds of protein, mineral, and conjugated linoleic acid [21]. Therefore, the consumption of Etawah Crossbred goat milk for a normal condition with appropriate concentration also give a good impact.

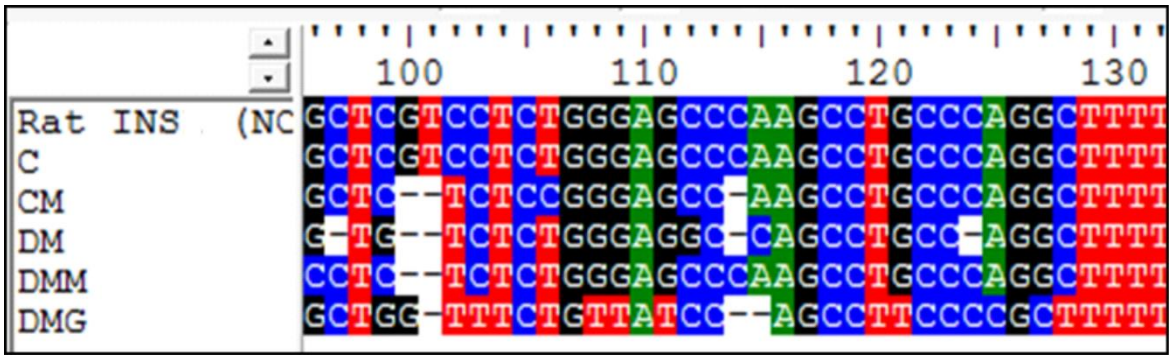

Figure 1. The DNA sequences of INS in all rat groups.

4. Conclusion

According to this research, we concluded that CSN1S2 protein from Etawah Crossbred goat milk can affect the repairing mechanism of gene damage. This protein plays role as anti-diabetes for repairing the damage in insulin gene of T1DM rat model.

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