Acta Med. Okayama, 2018
Vol. 72, No. 4, pp. 423–426

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Clinical Study Protocol

Study Design of a Phase II Clinical Trial to Assess the Efficacy and Safety of Eperisone in Japanese Type 2 Diabetes Patients with Risk and Non-risk Alleles of *CDKAL1*

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Genetic variation in Cdk5 Regulatory Associated Protein 1-Like 1 (*CDKAL1*) is associated with the development of type 2 diabetes (T2D). Dysfunction of CDKAL1 impairs the translation of proinsulin, which leads to glucose intolerance. Eperisone, an antispasmodic agent, has been shown to ameliorate glucose intolerance in Cdkal1-deficient mice. We have launched a phase II clinical study to investigate the potential anti-diabetic effect of eperisone in T2D patients carrying risk or non-risk alleles of *CDKAL1*. The primary endpoint is the change of hemoglobin A1c (HbA1c) levels. We also examined whether the efficacy of eperisone in T2D patients is associated with CDKAL1 activity.

Key words: diabetes, insulin secretion, single nucleotide polymorphism, glucose

Type 2 diabetes (T2D) is a chronic metabolic disease characterized by the impairment of insulin secretion or insulin action. T2D is caused by both lifestyle and genetic factors. Recent advances in genome-wide association studies (GWASs) have revealed a number of risk genes associated with the development of T2D [1]. Among these genes, Cdk5 Regulatory Subunit Associated Protein 1-Like 1 (*CDKAL1*) is one of the most reproducible risk genes among various ethnic populations [2-5]. T2D patients carrying the risk alleles of *CDKAL1* exhibit a decrease in insulin secretion [5].

CDKAL1 is a methylthiotransferase that catalyzes 2-methylthio (ms^2) modification at position 37 (A37) of cytoplasmic $tRNA^{Lys(UUU)}$ [6]. The ms^2-modification facilitates accurate decoding of the lysine codon, which is important for proinsulin biosynthesis in pancreatic β-cells. Deficiency of Cdkal1 induces the mistranslation of proinsulin, which leads to a shortage of mature insulin and ultimately causes glucose intolerance in animal models [7]. In humans, T2D-associated genetic variations of Cdkal1 are found in the middle region of intron 5. The risk variations lead to aberrant splicing of *CDKAL1* mRNA, which results in decreases of both the CDKAL1 protein level and as its enzymatic activity [8]. Importantly, a decrease of CDKAL1 activity is associated with decreased insulin secretion in non-diabetic individuals [8, 9].

Interestingly, the frequency of *CDKAL1* mutation differs among ethnic groups. As many as 22.4% of Asians carry the risk *CDKAL1* alleles, whereas only 8.8% of Europeans carry them [9]. In line with these

Received January 4, 2018; accepted March 9, 2018.
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Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

data, the majority of Asian T2D patients exhibit reduced insulin secretion without obesity [10]. For the treatment of these patients, insulin secretagogues such as sulfonylureas and glinides have been widely used. Although these agents can rapidly induce insulin secretion, it is known that the long-term usage of sulfonylureas leads to secondary ineffectiveness due to the exhaustion of pancreatic β cells [11]. This phenomenon is particularly problematic in T2D patients with pathogenic mutations of *CDKAL1* because sulfonylureas further amplify the translation of aberrant proinsulin in these patients. Indeed, possession of the risk haplogenotypes of *CDKAL1* is associated with reduced response to sulfonylurea [12]. These results suggest that sulfonylureas or glinides might be unsuitable for the treatment of T2D patients with *CDKAL1* mutations.

In order to develop a new agent that can ameliorate translational deficits, we screened a chemical library in cells with deficiency of 2-thio-modification in tRNA. We identified eperisone, an antispasmodic agent, which effectively improved translation in this type of cellular model (manuscript in preparation). In addition, when eperisone was administered to mice with Cdkal1 knockout in pancreatic β-cells, the drug successfully accelerated insulin secretion and improved glucose intolerance in the mice (manuscript in preparation). Eperisone has already been prescribed for decades in patients with muscle stiffness and pain worldwide. Given the evidence, we have launched a phase II clinical study to evaluate the safety and antidiabetic effect of eperisone in Japanese patients with T2D.

Study Design

This study is a non-randomized, single-arm, open-labeled phase II trial investigating T2D patients with and without genetic variants of *CDKAL1*, with the aim of examining the efficacy and safety of eperisone. This is a single-center trial. Fig. 1 provides an overview of the study design.

This study was approved by the Kumamoto University Institutional Review Board prior to study initiation (No. 1927 Advanced, No. 275 Genome). The study was registered with the UMIN Clinical Trials Registry (UMIN-CTR), Japan (UMIN000017926).

Endpoints

The primary endpoint is the change of HbA1c levels during the observation period of 12 weeks. T2D patients enrolled in this study will be administered eperisone for 12 successive weeks, and HbA1c levels will be compared within T2D patient groups carrying risk or non-risk alleles of *CDKAL1*.

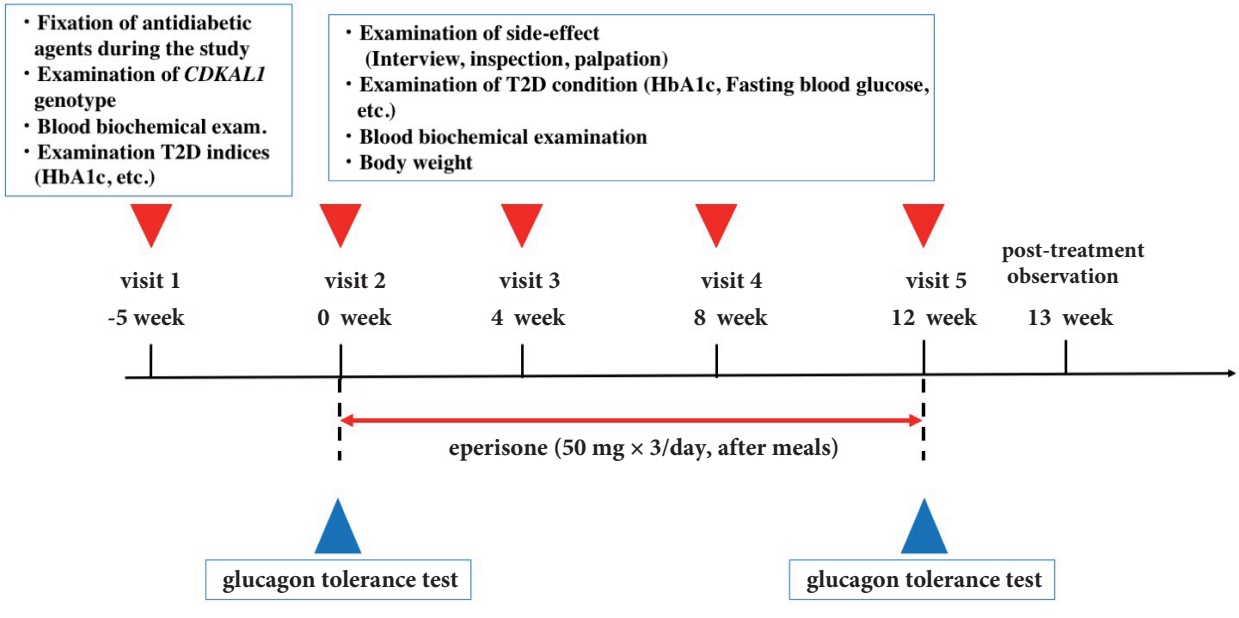

Fig. 1 Overview of the study design.

Secondary endpoints are as follows.

(1) Impact of eperisone in the correlation between the 2-methylthio modification level of $tRNA^{Lys(UUU)}$ and HbA1c levels in all enrolled patients.

(2) Effects of eperisone on glycemic control indices (fasting blood glucose, 1, 5-anhydroglucitol), beta-cell function indices (glucose level during glucagon tolerance test, HOMA-β, proinsulin/insulin ratio), and lipid metabolism indices (total cholesterol, low-density-lipoprotein cholesterol, high-density-lipoprotein cholesterol, triglycerides) in patients carrying risk and non-risk alleles of *CDKAL1*.

(3) Effect of eperisone on the correlation of the 2-methylthio modification level of $tRNA^{Lys(UUU)}$ with the parameters measured in section (2) in all enrolled patients.

(4) Occurrence of adverse events, abnormal laboratory test values, and abnormal vital signs.

Eligibility Criteria

The main inclusion and exclusion criteria for patients are listed in Table 1. Written informed consent was obtained from all patients before registration.

Treatment Methods

An overview of this study is shown in Fig. 1. After written informed consent is obtained, blood samples of patients will be taken and subjected to laboratory screening, including screening for the *CDKAL1* genotype (rs7756992), the 2-methylthio modification level of $tRNA^{Lys(UUU)}$, and other T2D indices. Eligible patients will then be assigned to 1 of 3 groups: a risk group, a heterozygous group or a non-risk group, depending on whether they carry two, one or no risk alleles, respectively. One month after the first screening, eligible participants of all 3 groups will receive 150 mg (50 mg × three times after meals) eperisone daily for 12 weeks. Participants will visit the hospital for examinations and blood tests every 4 weeks. If participants show any sign consistent with the withdrawal criteria shown in Table 2, the eperisone treatment will be discontinued.

Statistical Considerations

Based on the frequency of *CDKAL1* alleles as described above, the target number of participants has been set at 100, including 25 participants in the *CDKAL1* risk group, 50 participants in the *CDKAL1* heterozygous group, and 25 participants for the *CDKAL1*

Table 1 Patient eligibility

Inclusion criteria

1. Patients diagnosed with T2D.
2. Age: 20–75 years.
3. T2D patients with mono or combination therapy using the following oral hypoglycemic agents: sulfonyl urea, glinides, α-GI, biguanides, thiazolidinedione, and DPP-4 inhibitors.
4. HbA1c: 6.2–8.5%.
5. Rate of HbA1c change ≦ 1.0%, and no change of treatment for 3 months prior to the day of registration.
6. For female patients, agreement to use contraception during the period of this study.
7. Adequate ability to understand and follow the protocol as judged by investigators.
8. Provision of written informed consent before any medical screening.

Exclusion criteria

1. Recurrent severe hypoglycemia or hypoglycemic unawareness.
2. Severe liver dysfunction. (AST ≧ 100IU/L, ALT ≧ 100 IU/L)
3. Previous history of severe allergy to pharmaceutical products.
4. Potential pregnancy.
5. Incapability to understand or follow the protocol due to psychological illness (including moderate or severe dementia) as judged by investigators.
6. Hospitalization during the period of observation.
7. Treatment with prohibited drugs.
8. Prescription of eperisone within 4 weeks prior to the day of registration.
9. Treatment with methocarbamol.
10. Participation in other clinical studies at the time of registration.
11. Any conditions that investigators consider to be inappropriate for this study.

Table 2 Withdrawal Criteria

1. Withdraw of consent.
2. Violation of protocol.
3. Difficulty continuing the administration of eperisone due to adverse events.
4. HbA1c > 9.0%.
5. Severe hypoglycemia.
6. Death.
7. Loss of contact.
8. Informed by Research Ethics Committee.
9. Pregnancy.
10. Any condition that investigators consider to be grounds for withdrawal.

non-risk group. The data will be analyzed using Windows SAS ver. 9.3 or higher, and plotted using Microsoft Office Excel 2007 or a more recent version. Significance will be assigned to values of $p < 0.05$ is in the two-tailed test and $p < 0.25$ in the one-tailed test. For calculation of the inter-group equilibrium regarding the background factors, p values < 0.15 will be considered significant.

Acknowledgments This work is supported by a Grant-in-Aid for Research on the Development of New Drugs from the Japan Agency for Medical Research and Development (AMED) (16ak0101023h0003).

In addition to the authors, The EDIT Study Group includes: Takeshi Nishikawa MD PhD, Noboru Furukawa MD PhD, Takeshi Matsumura MD PhD, Hiroyuki Motoshima MD PhD, Tatsuya Kondo MD PhD, Junji Kawashima MD PhD, Daisuke Kukidome MD PhD, Shinsuke Iwashita MD, Motoyuki Igata MD PhD, Norio Ishii MD PhD, Hiroyuki Kinoshita MD PhD, Shuuji Kawasaki MD PhD, and Akiko Satou MD (Kumamoto University Hospital).

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