การเปรียบเทียบเภสัชจลนศาสตร์ และภาวะพหุสัณฐานของยีน ระหว่างผู้ป่วยโรคเบาหวานชนิดที่ 2 ที่มีและไม่มีภาวะบวมน้ำ เมื่อได้รับยาไทอะโซลิดีนไดโอน

นางสาว วรรณกมล สอนสิงห์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรดุษฎีบัณฑิต
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ปีการศึกษา 2551

COMPARISONS OF PHARMACOKINETICS AND GENETIC POLYMORPHISMS BETWEEN TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT EDEMA CONDITIONS WHEN TREATED WITH THIAZOLIDINEDIONES

Miss Wannakamol Sonsingh

A Dissertation Submitted in Partial Fulfillment of the Requirements
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Thesis Title	COMPARISONS OF PHARMACOKINETICS AND GENETIC POLYMORPHISMS BETWEEN TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT EDEMA CONDITIONS WHEN TREATED WITH THIAZOLIDINEDIONES
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วรรณกมล สอนสิงห์ : การเปรียบเทียบเภสัชจลนศาสตร์ และภาวะพหุสัณฐานของยีนระหว่างผู้ป่วยโรคเบาหวานชนิดที่ 2 ที่มีและไม่มีภาวะบวมน้ำ เมื่อได้รับยาไทอะโซลิดีนไดโอน. (COMPARISONS OF PHARMACOKINETICS AND GENETIC POLYMORPHISMS BETWEEN TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT EDEMA CONDITIONS WHEN TREATED WITH THIAZOLIDINEDIONES) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. ดวงจิตต์ พนมวัน ณ อยุธยา, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม : ผศ. พญ. วัลยา จงเจริญประเสริฐ, 120 หน้า.

การศึกษานี้มีวัตถุประสงค์เพื่อหาความชุก และหาบทบาทของภาวะพหุสัณฐานของยีนที่แตกต่างกันต่อการเกิดภาวะบวมน้ำจากการใช้ยาไทอะโซลิดีนไดโอนในผู้ป่วยโรคเบาหวานชนิดที่ 2 และเพื่อเปรียบเทียบขนาดของยาที่ใช้ และค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของยาพิโอกลิตาโซน ระหว่างผู้ป่วยที่มีและไม่มีภาวะบวมน้ำเมื่อได้รับยาพิโอกลิตาโซน เวชระเบียนของผู้ป่วยเบาหวานที่ใช้ยากลุ่มนี้ ณ โรงพยาบาลรามาธิบดี จำนวน 278 รายถูกนำมาศึกษาทบทวน เพื่อวิเคราะห์หาความชุกตลอดจนปัจจัยทางประชากรต่างๆ ที่อาจจะมีผลกระทบต่อการเกิดภาวะบวมน้ำจากการใช้ยากลุ่มนี้ ผู้ป่วยจำนวน 25 ราย ที่มาติดตามผลการรักษาที่คลินิกผู้ป่วยนอก และยินยอมเข้าร่วมงานวิจัยได้ถูกเก็บตัวอย่างเลือด 2 ชุด เพื่อวิเคราะห์หาระดับยาพิโอกลิตาโซนในเลือด โดยใช้วิธีไฮเพอร์ฟอร์แมนลิคควิดโครมาโตกราฟฟีวิเคราะห์ด้วยแสงอัลตราไวโอเล็ต จากนั้นนำไปคำนวณค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ ตัวอย่างเลือดจากผู้ป่วยจำนวน 134 ราย ได้ถูกนำไปวิเคราะห์หาภาวะพหุสัณฐานของยีนเอ็นโดธอลิน-1 (rs5370) และยีนโซเดียมชาเนลเบต้าซับยูนิต (rs34241435) โดยใช้วิธีการของแทคโพลิเมอร์เรส และระบบไลท์ไซเคอร์ 480 เอสวายบีอาร์ กรีนวันเมสเตอร์

ผลการศึกษาพบความชุกในการเกิดภาวะบวมน้ำจากการใช้ยาไทอะโซลิดีนไดโอน ร่วมกับยาชนิดอื่นในการรักษาโรคเบาหวาน 13.7% (พบ 15.1% ในกลุ่มที่ใช้ยาพิโอกลิตาโซน และ 12.2% ในกลุ่มที่ใช้ยาโรซิกลิตาโซน) ปัจจัยที่มีผลต่อความเสี่ยงในการเกิดภาวะบวมน้ำของผู้ป่วยที่ใช้ยากลุ่มนี้ได้แก่ อายุ เพศ การมีโรคทางระบบหลอดเลือดขนาดใหญ่ร่วมด้วย การใช้ยากลุ่มเอซีอีไอร่วมด้วย การใช้ยาไทอะโซลิดีนไดโอนในขนาดที่สูง และผู้ป่วยที่มีการใช้อินซูลินร่วมด้วย สำหรับค่าพารามิเตอร์ทางเภสัชจลนศาสตร์ของยาพิโอกลิตาโซนพบว่าไม่มีความแตกต่างกันระหว่างกลุ่มที่เกิดภาวะบวมน้ำและไม่บวมน้ำ อย่างไรก็ตามพบว่าค่าคงที่ของการขจัดยา ค่าครึ่งชีวิต และอัตราการขจัดยา ของผู้ป่วยกลุ่มที่ใช้ยาพิโอกลิตาโซนคงตัวอยู่ในขนาดสูง มีความแตกต่างอย่างมีนัยสำคัญ กับค่าพารามิเตอร์ทางเภสัชจลนศาสตร์เหล่านี้ในกลุ่มผู้ป่วยที่ใช้ยานี้คงตัวอยู่ในขนาดต่ำ ผู้ป่วยที่ไม่มีภาวะบวมน้ำและคงขนาดการใช้ยาอยู่ที่ 15 มิลลิกรัม จะมีค่าคงที่ของการขจัดยาและอัตราการขจัดยาน้อยกว่าผู้ป่วยที่จำเป็นต้องคงขนาดใช้ของยาอยู่ที่ 30 มิลลิกรัม ผู้ป่วยเหล่านี้จึงสามารถควบคุมระดับน้ำตาลในเลือดได้ด้วยการใช้ยาพิโอกลิตาโซนในขนาดที่ต่ำกว่า การศึกษาครั้งนี้พบความถี่ของอัลลีลของยีนเอ็นโดธอลิน-1 ในผู้ป่วยคนไทย 32.1% แต่ไม่พบอัลลีลของยีนโซเดียมชาเนลเบต้าซับยูนิต และไม่พบความแตกต่างของความถี่ของภาวะพหุสัณฐานของยีนเอ็นโดธอลิน-1 ระหว่างกลุ่มที่มีบวมน้ำและไม่มีบวมน้ำ ดังนั้นจึงสรุปได้ว่าภาวะพหุสัณฐานของยีนทั้งสองตัวดังกล่าว ไม่มีบทบาทต่อการเกิดภาวะบวมน้ำจากยากลุ่มนี้ในผู้ป่วยเบาหวานไทย

ภาควิชา เภสัชกรรมปฏิบัติ ลายมือชื่อนิสิต .....

สาขาวิชา การบริบาลทางเภสัชกรรม ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก .....

ปีการศึกษา 2551 ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์ร่วม .....

4676959133: MAJOR PHARMACEUTICAL CARE

KEYWORDS: THIAZOLIDINEDIONES / PHARMACOKINETICS / POLYMORPHISM / EDEMA / TYPE 2 DIABETES

WANNAKAMOL SONSINGH: COMPARISONS OF PHARMACOKINETICS AND GENETIC POLYMORPHISMS BETWEEN TYPE 2 DIABETIC PATIENTS WITH AND WITHOUT EDEMA CONDITIONS WHEN TREATED WITH THIAZOLIDINEDIONES. ADVISOR: ASSOC. PROF. DUANGCHIT PANOMVANA NA AYUDHYA, CO-ADVISOR: ASST. PROF. WALLAYA JONGJAROENPRASERT, 120 pp.

The purpose of this study were to determine the prevalence of thiazolidinediones (TZDs)-induces edema, the role of different genetic polymorphisms on TZDs-induced edema in type 2 diabetic patients, and to compare doses and pharmacokinetic (PK) parameters of pioglitazone between patients with and without edema conditions. Medical chart of 278 diabetic patients using TZD at Ramathibodi hospital were reviewed to determine the prevalence of TZDs-induces edema and the demographic factors which might influencing the risk of edema. 25 patients who came to follow-up treatment at the medical outpatient clinic and agree to participate were recruited for pharmacokinetic study, two blood samples were collected from each patient and were analyzed pioglitazone concentrations by HPLC-UV method, then, PK parameters were determined. Blood sample of 134 patients were analyzed for SNPs of endothelin-1 (*ENDO1*; rs5370) and epithelial sodium channel β subunit (*SCNN1B*; rs34241435) by Tag polymerase and LightCycler 480 SYBR Green I Master system.

The prevalence of edema in patients using TZD combined with other anti-diabetic drugs identified in this study was 13.7% (15.1% for pioglitazone and 12.2% for rosiglitazone). Factors influencing the risk of edema in patients who were using TZD were age, sex, macrovascular diseases, ACEI use, high dose of TZD, and co-medication with insulin. The PK parameters of pioglitazone were not different between edema and non-edema groups. However, Ke, $t_{1/2}$, and CL values of patients who were stabilized on high dose of pioglitazone were significantly different from those who were stabilized on low dose of pioglitazone. Lower Ke and CL were found in non-edema patients who stabilized on 15 mg dose of pioglitazone as compared to those who were stabilized on 30 mg dose of pioglitazone, lower dose of pioglitazone were therefore required for controlling blood glucose. This study found the rate of allele frequency of *ENDO1* in Thai patients to be 32.1%, while no allele frequency could be found for *SCNN1B*. The polymorphisms frequency of *ENDO1* showed no significantly different between edema and non-edema groups.

Department : Pharmacy Practice Student's Signature : _____

Field of Study : Pharmaceutical Care Advisor's Signature : _____

Academic Year : 2008 Co-Advisor's Signature : _____

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LIST OF ABBREVIATIONS

ACEIs	Angiotensin converting enzyme inhibitors
ACS	Acute coronary syndrome
ADA	American Diabetes Association
AHA	American Heart Association
ARBs	Angiotensin receptor blockers
BMI	Body mass index
CAD	Coronary artery disease
CCBs	Calcium channel blockers
CHF	Congestive heart failure
CI	Confidence interval
CL	Clearance
ClCr	Creatinine clearance
CVA	Cerebrovascular disease
dl	Diciliters
DM	Diabetes mellitus
ENaC	Epithelial sodium channel
ENDO1	Endothelin-1 gene
et al.	et alii (and others)
FPG	Fasting plasma glucose
g	Grams
Hb	Hemoglobin
HbA_{1c}	Hemoglobin A1c
Hct	Hematocrit
HDL	High-density lipoprotein
hr	Hours
Ke	Elimination rate constant
kg	Kilograms
LDL	Low-density lipoprotein

mg	Milligrams
mg/d	Milligrams per day
MI	Myocardial infarction
ml	Milliliters
mmol/l	Millimole per liter
n	Number of patients
ng	Nanograms
NSAIDs	Non-steroidal anti-inflammatory drugs
p	p-value
PG	Pharmacogenetic
PK	Pharmacokinetic
PPARG	Peroxisome proliferators activated receptor gamma gene
r	Pearson's correlation coefficient
SCNN1B	Epithelial sodium channel β subunit
SD	Standard divation
SNP	Single nucleotide polymorphism
T2DM	Type 2 diabetes mellitus
TC	Total cholesterol
TG	Triglyceride
TZDs	Thiazolidinediones
μg	Micrograms
Vd	Volume of distribution
VEGF	Vascular endothelial growth factor
vs	Versus

CHAPTER I

INTRODUCTION

1.1 Rationale and Significance of the Problem

Thiazolidinediones (TZDs), pioglitazone and rosiglitazone, are insulin sensitizing antidiabetic agents that bind with high affinity to peroxisome proliferators activated receptor-γ (PPAR-γ) in adipose tissue, pancreatic β-cells, vascular endothelium, heart, skeletal muscle, kidney and macrophages [1,2]. The activated PPAR-γ affects the transcription of several genes that regulate glucose and lipid homeostasis [3]. They are highly effective in decreasing blood glucose concentrations for type 2 diabetes mellitus by reducing insulin resistance and improving peripheral glucose disposal [4]. Currently, two TZDs are available for clinical use in Thailand. However, some patients taking either pioglitazone or rosiglitazone suffer from peripheral edema and fluid retention [5-7], which can develop into pulmonary edema or chronic heart failure [8,9]. Edema and fluid retention have emerged as the most common and serious side effect of TZDs and have become the most frequent cause of discontinuation of therapy [10,11].

Previous clinical trials suggest that pioglitazone combined with insulin has higher incidence of edema than pioglitazone combined with oral hypoglycemic agents [5-7]. The highest incidence of edema in all regimens of TZDs therapy was reported in the study in Melbourne based on hospital data [12], of which 33% of peripheral edema was reported in the patients with pioglitazone therapy, and 21% with rosiglitazone therapy. It was severe enough to prompt withdrawal of these drugs at the rate of 7% and 4%, respectively. However, there is still no detail study about the incidence of TZDs-induced edema in Thailand.

Potential mechanisms responsible for TZD therapy associated with edema are not fully understood and are likely to be multifactorial such as increased plasma volume, increased renal sodium reabsorption, reflex sympathetic activation, alteration of intestinal ion transport, and increased production of vascular endothelial growth factor (VEGF), an important angiogenic factor with strong microvascular permeabilizing properties [13,14]. Pioglitazone may cause decreasing in hemoglobin. Across all clinical studies [15,16], mean hemoglobin values declined by 2% to 4% in patients treated with pioglitazone. These changes may be related to increased plasma volume and have not been associated with any significant hematological clinical effects. Recently, studies focused on PPAR-γ activation in the kidney [17] and the epithelial sodium channel [18] in mouse models of PPAR-γ agonist-induced edema.

By reviewing literatures, there is no information about association between pharmacokinetic parameters and TZDs-induced edema, but the incidence of edema relates to dosage of TZDs. A prospective study of Majima et al. [19] evaluated the effect of low-dose pioglitazone (7.5 mg/day) on metabolic control and the incidence of edema compared with a standard dose of pioglitazone (15 mg/day) in 95 Japanese type 2 diabetic patients. The incidence of edema was significantly lower in the low-dose group (2/54) than in the standard dose group (11/41) ($p=0.0014$) while, the change of glucose and lipid control did not differ significantly between the two groups. The American Heart Association (AHA) and the American Diabetes Association (ADA) recommend that if edema occurs and CHF is not presented during TZD therapy, the TZD dosage can be reduced and/or added diuretics [13]. Because edema is a dose-dependent effect, reducing the TZD dosage is a viable option [16]. Thus, the pharmacokinetic parameters such as elimination rate constant, volume of distribution, and clearance of pioglitazone and its metabolites may be related to edema in diabetic patients treated with TZDs.

In genetic variants studies, a variant of PPARG gene was highly interested. The PPARG2 variant contains a common Pro12Ala single nucleotide polymorphism (SNP), substituting alanine for proline at codon 12 in the unique PPARG2 [20]. A study in Korea

showed the relationship of the Pro12Ala polymorphism and rosiglitazone response in type 2 diabetic patients [21]. The Ala12 allele prevalence was 4% in the subjects in the study. The decrease in HbA_{1c} level was significantly greater in patients with the Ala12 allele than in those without the allele (P = 0.015). This study result was inconsistent to the study by Bluher [22]. They reported that the Pro12Ala variant in the PPARG gene did not affect the therapy efficacy of pioglitazone. Although there is no study identify the relationship between the Pro12Ala variant and TZDs-induce edema, the study by Hansen et al. reported the association between the Pro12Ala variant and development of edema in type 2 diabetic patients treated with a dual acting PPAR-γ/α agonist, Ragaglizar [23]. They showed that Pro12Ala variant is the most significant risk factor for edema (hazard ratio 4.42, p = 0.0081), besides, other risk factors for edema found included female gender (hazard ratio 3.34, p = 0.0005) and weight change during treatment (hazard ratio 1.20, p = 0.0017). Whether the Pro12Ala genotype plays a role in the TZDs-induced edema remains to be determined.

Nowadays, the pharmacogenetic studies of TZDs-induced edema implicate to several genes that involved through the pathways of PPAR-γ agonists. In clinical trials on muraglitazar [24], a PPAR-α/γ dual agonist, a total of 213 SNPs in 63 genes were genotyped in 730 participants. SNPs in renin (rs2368564) and endothelin-1 (rs5370) were associated with reduced risk of edema (p = 0.003 and p =0.028, respectively) while a SNP in β1adrenergic receptor (rs1801253) was associated with increased susceptibility to edema (P = 0.034). Moreover, a phase III clinical trial on the PPAR-γ agonist, farglitazar [25] showed that four SNPs in ENaCβ subunit (SCNN1B) were significantly associated with fluid retention (P<0.0005) using 'time to oedema adverse event' as the dependent variable and genotype, sex, farglitazar dose and hypertension history as the independent variables. Thus, it is interesting to determine the association between some SNPs of these genes with edema induced by PPAR-γ agonists in diabetic type 2 in Thai patients.

However, the mechanisms of TZDs-induced edema in diabetic patients are still not fully understood while edema is an important clinical sign of fluid retention can develop into serious pulmonary edema and/or CHF problems. Therefore, this study hypothesized that pharmacokinetics of pioglitazone in the patients and the differences in polymorphism of the endothelin-1 gene could involve in the development of edema in type 2 diabetic patients treated with pioglitazone. Prevalence of TZDs-induced edema and the polymorphisms in these genes in type 2 diabetic patients would be reported.

1.2 Objectives

1. To determine the prevalence of thiazolidinediones-induced edema in type 2 diabetic patients
2. To compare doses and pharmacokinetic parameters of pioglitazone between edematous and non-edematous type 2 diabetic patients treated with pioglitazone
3. To determine the role of different polymorphisms of endothelin-1 (rs5370) gene on pioglitazone-induced edema in type 2 diabetic patients

1.3 Scope of the Study

1.3.1 The population in this study was patients diagnosed with type 2 diabetes, attended the Diabetic Clinic and the General Medicine Clinic at Ramathibodi hospital.

1.3.2 The subjects in this study were patients diagnosed with type 2 diabetes, attended the Diabetic Clinic and the General Medicine Clinic at Ramathibodi hospital during the study period.

1.3.3 Variables in this study consist of;

1.3.3.1 Dependent variable: Edema

1.3.3.2 Independent variables:

1.3.3.2.1 Demographic data: body mass index (BMI), co-morbid conditions, and co-medications

1.3.3.2.2 Blood glucose: fasting plasma glucose (FPG) and glycosylated hemoglobin (HbA_{1c})

1.3.3.2.3 Plasma lipid: low density lipoprotein (LDL), high density lipoprotein (HDL), triglyceride (TG), and total cholesterol (TC)

1.3.3.2.4 Doses of pioglitazone and rosiglitazone

1.3.3.2.5 Insulin therapy

1.3.3.2.6 Pharmacokinetic parameters: elimination rate constant (K_e), volume of distribution (Vd), and clearance (Cl) of pioglitazone.

1.3.3.2.7 Polymorphism of endothelin-1 (rs5370)

1.3.3.2.8 Renal function tests: creatinine clearance (Cl_{Cr})

1.3.3.3 Control variables:

1.3.3.3.1 Underlying diseases: congestive heart failure (CHF), ascites, deep vein thrombosis (DVT), nephritic syndrome, untreated hypothyroid, liver dysfunction (AST or ALT>3 TUL), and renal insufficiency (SCr >1.5 mg/dl)

1.3.3.3.2 Other drugs affecting edema: calcium channel blockers (CCBs); including amlodipine, felodipine, lacidipine, manidipine, and verapamil; NSAIDs, ACE inhibitors (ACEIs), angiotensin-II receptor blockers (ARBs), nitrate drugs, corticosteroid, and furosemide

1.3.3.3.3 Other risk factors: gender and age

1.4 Operational Definition

Type 2 diabetes mellitus:	Non-insulin-dependent diabetes mellitus, diagnosed by American Diabetes Association (ADA) criteria which are [26]; - Fasting plasma glucose (FPG) $\geq$ 126 mg/dl; or - 2-hour post load glucose $\geq$ 200 mg/dl; or - Symptoms of diabetes and random plasma glucose $\geq$ 200 mg/dl.
Edema:	Edema is defined as pitting edema or weight gain that must be more than 3 kg per month after pioglitazone initiation, accompanied by documented worsening signs of volume overload as determined by the practitioners such as worsening peripheral edema, jugular venous distention, ascites, or pulmonary edema.
Creatinine clearance [27]:	A patient's renal function can be estimated by calculating creatinine clearance (Cl_{Cr}) using the Cockroft-Gault equation; Cl_{Cr} (ml/min) = $\frac{(140 - age)\ (IBW)}{(72)(S_{Cr})}$ (if female times 0.85)
Idea body weight (IBW) [27]:	Ideal body weight can be calculated by; IBW (male) = 50 + 2.3 (height in inches over 5 feet), or IBW (female) = 45.5 + 2.3 (height in inches over 5 feet).
Coronary artery disease [28]:	Stable angina, unstable angina, or myocardial infarction (MI) including non-ST-segment-elevation MI (NSTEMI), and ST-segment-elevation MI (STEMI)
Liver dysfunction:	AST and/or ALT > 3 times upper limit
Renal insufficiency:	Serum creatinine > 1.5 mg/dl

1.5 Ethic Consideration

This proposal was approved by the Ethical Committee of the Faculty of Medicine, Ramathibodi hospital, Mahidol University, Bangkok, Thailand. This study used the blood samples of patients treated with pioglitazone to analyze serum pioglitazone concentration and the blood samples of patients treated with pioglitazone or rosiglitazone to investigate polymorphisms of the endothelin-1 gene. However, all patients included in this study must provide written informed consent voluntarily. Patient's medical information was protected confidentially. The results of this study might be published in scientific journals or presented at medical meeting but the patients would not personally be identified.

CHAPTER II

REVIEW OF LITERATURE

2.1 Thiazolidinediones

The succession of TZDs or glitazone drugs that followed, trogitazone, pioglitazone, englitazone, darglitazone, and rosiglitazone have been most extensively reported. Troglitazone was available in USA and in Japan at 1998, but it was withdrawn at 2000 by the FDA of USA because of hepatotoxicity. Moreover, ciglitazone and englitazone were not developed in clinical phase due to adverse effect on the liver. Pioglitazone, darglitazone, and rosiglitazone were into clinical phase [29]. Currently, only pioglitazone and rosiglitazone were available in Thailand which rosiglitazone was approved at 2002 and pioglitazone at 2004.

2.1.1 Mechanism of Action

TZDs are high-affinity ligands for PPAR-γ, a member of the nuclear receptor superfamily of ligand-activated transcription factors that both positively and negatively regulate gene expression in response to the binding of a number of fatty acid metabolites [30]. These receptors are found in various tissues, including skeletal muscle, adipose tissue, heart, large and small intestine, colon, and kidney. The interaction between PPAR agonists and their receptors at nuclear level allow the formation of a complex with another nuclear receptor known as retinoid X receptor (RXR), which is bound with its own ligand, retinoic acid [31]. This heterodimeric complex results in a conformational change of these receptors. The PPAR-RXR indicated as 9-*cis* retinoic acid (RA) recruit coactivator complexes to the target gene to recognize specific DNA response elements (PPAR response elements, PPRE) in the promoter region of target genes, resulting in increased transcription through inherent histone acetylase (HAT)

activity or via interactions with the basal transcription machinery. This complex can turn on or turn off the expression of different genes involved in different metabolic pathways of glucose and lipids metabolism [32]. This mechanism of TZD was activated by PPAR-γ shows in Figure 1 that modified from Reginato MJ. [33].

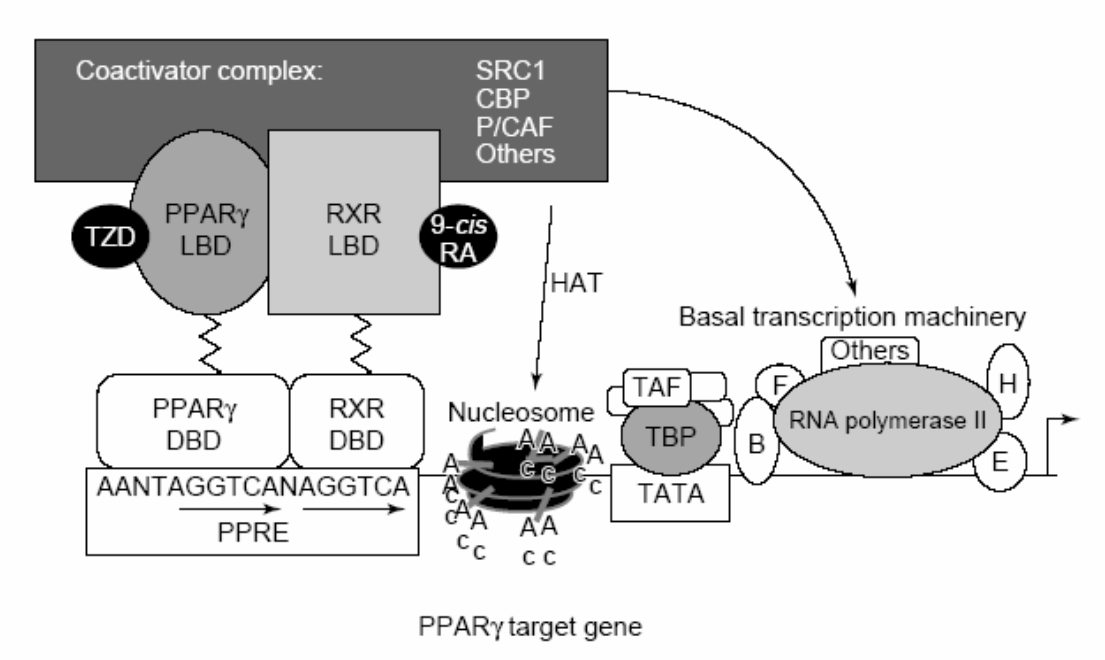

Figure 1 Mechanism of TZD activation of transcription by PPAR-γ

CBP, CREB-binding protein; CREB, cyclic AMP response element-binding protein; DBD, DNA-binding domain; LBD, ligand-binding domain; P/CAF, p300/CBP-associated factor; SRC1, steroid receptor coactivator 1; TAF, TBP-associated factor; TBP, TATA-binding protein.

To date, the PPAR family consists of three subtypes of PPARs encoded by different genes; PPAR-α, -γ, and –β/δ. All three PPAR isoforms have been identified in the nephron. PPAR-α is expressed mainly in tissues where active fatty acid catabolism occurs (e.g., liver, brown fat, kidney, heart, and skeletal muscle), in kidney, it is predominantly expressed in the proximal tubules and medullary thick ascending limbs. PPAR-β/δ is equally expressed in renal cortex and medulla in all segments of nephron. PPAR-γ is restricted largely to white and brown adipose tissue, with lower levels in cardiac and skeletal muscle, in kidney, it is selectively expressed in the medullary

collecting duct, glomeruli and pelvic urothelium. Moreover, PPAR-α and -γ are also expressed in vascular endothelium, vascular smooth muscle, and macrophages/ foam cells [34-36]. In general, PPAR-α regulates genes involved in fatty acid uptake and oxidation, inflammation and vascular function, whereas PPAR-γ regulates genes involved in fatty acid uptake and storage, inflammation, and glucose homeostasis. PPAR-δ regulates genes involved in fatty acid metabolism, inflammation, and macrophage lipid homeostasis [36].

Currently, there are four PPAR-γ isoforms, γ1, γ2, γ3, and γ4, derived from alternative promoter usage. PPAR-γ2 contains an additional 31 amino acids at its N-terminus, but the functional significance is unclear. Interestingly, PPAR-γ2 is found exclusively in adipocytes, whereas PPAR-γ1 is expressed predominantly in adipocytes, but is also expressed in other tissues at low levels such as pancreatic β cells, macrophages, and vascular endothelium [37]. The PPAR-γ3 and PPAR-γ4 mRNA variants, which differ in the 5'-untranslated region, both yield proteins that are identical to PPAR-γ1. PPAR-γ3 expression appears to be restricted to macrophages, adipose tissue and colon, while the tissue distribution of PPAR-γ4 remains to be defined [38].

PPAR-γ agonists promote the differentiation and proliferation of pre-adipocytes with accompanying lipogenesis that enhance the local effects of insulin, and they promote free fatty acid (FFA) uptake and storage in subcutaneous adipose rather than visceral adipose tissue. This reduces FFA levels, with associated reductions in insulin resistance. In addition, activation of PPAR-γ is believed to increase the expression and translocation to the cell surface of the glucose transporters GLUT1 and GLUT4, thus increasing glucose uptake into liver and skeletal muscle cells, respectively, and reducing plasma glucose levels [39]. Some reports indicated that rates of gluconeogenesis in the liver are reduced. Other mechanisms may involve altered release of adipocyte signaling factors such as increased adiponectin, decreased tumor necrosis factor (TNF)-α, decreased interleukin-6 (IL-6), and decreased leptin to modulate the insulin sensitivity of non-adipose tissue [40].

The possible mechanism is that TZD-dependent activation of PPAR-γ induces adipocytes to send an endocrine signal to muscle that enhances insulin action [40], as following;

Via PPAR-γ in adipocytes:

- Direct stimulation of increased glucose disposal in adipocytes
- Stimulation of increased glucose disposal in skeletal muscle
- Reduced tumor necrosis factor a
- Reduced leptin
- Reduced free fatty acids
- Alteration of other adipocyte factors

Via extra-adipocytic PPAR-γ:

- Direct stimulation of increased glucose disposal in skeletal muscle
- Action on other target tissue (such as liver) leading to increased glucose disposal in skeletal muscle

2.1.2 Indications

Rosiglitazone [41] and pioglitazone [15] are an oral antidiabetic agent that acts primarily by decreasing insulin resistance. It is used in the management of type 2 diabetes mellitus. Pharmacological studies indicate that it improves sensitivity to insulin in muscle and adipose tissue, inhibits hepatic gluconeogenesis, and improves glycemic control while reducing circulating insulin levels. The drugs are approved in monotherapy and in combination with a sulfonylurea, metformin, or insulin when diet, exercise, and a single agent do not result in adequate glycemic control. For patients inadequately controlled with a maximum dose of a sulfonylurea or metformin, TZD should be added to, rather than substituted for, a sulfonylurea or metformin.

2.1.3 Dosage and Administration

Rosiglitazone [41] may be administered either at a starting dose of 4 mg as a single daily dose or divided and administered in the morning and evening. For patients who respond inadequately following 8 to 12 weeks of treatment, as determined by reduction in FPG, the dose may be increased to 8 mg daily as monotherapy or in combination with metformin, sulfonylurea, or sulfonylurea plus metformin. The dose of rosiglitazone should not exceed 8 mg daily, as a single dose or divided twice daily. Doses of rosiglitazone greater than 4 mg daily in combination with insulin are not currently indicated. Rosiglitazone may be taken with or without food.

Pioglitazone [15] monotherapy in patients not adequately controlled with diet and exercise may be initiated at 15 mg or 30 mg once daily. For patients who respond inadequately to the initial dose of pioglitazone, the dose can be increased in increments up to 45 mg once daily. For patients not responding adequately to monotherapy, combination therapy should be considered. The dose of pioglitazone should not exceed 45 mg once daily since doses higher than 45 mg once daily have not been studied in placebo-controlled clinical studies.

According to the AHA and the ADA recommendations [13], in patients without clinical data of CHF but with one or more risk factors for its development, as it is the case in CRF patients, therapy with glitazones should be initiated at low doses, i.e, rosiglitazone 4 mg/day and pioglitazone 15 mg/day. The increases in dose should be gradual, with tight monitoring for signs of excessive weight gain, peripheral oedema, and/or CHF.

In the both drugs during combination therapy [42], the dosage of a sulphonylurea may be maintained, but should be decreased if hypoglycemia is reported, while the dosage of metformin is unlikely to need adjustment. The current insulin dosage can be continued after starting a TZD therapy, but dosage should be reduced by 10 to

25% if hypoglycemia is reported. Moreover, dose adjustment of the TZDs in patients with renal insufficiency is not recommended. Therapy with the TZDs should not be initiated if the patient exhibits clinical evidence of active liver disease, increased serum transaminase levels (ALT >2.5 times upper limit of normal at start of therapy), or cardiac failure (NYHA class III-IV). Liver enzyme monitoring is recommended in all patients prior to initiation of therapy with the TZDs and periodically thereafter [15,41],.

2.1.4 TZDs in Diabetic Nephropathy

Recently, Agarwal et al. [43] reported the result of a randomised, open-label, study comparing glipizide with pioglitazone over 16 weeks in 44 type 2 diabetic patients with overt diabetic nephropathy. Glipizide produced a mean increase in proteinuria of 6.1%, whereas pioglitazone therapy was followed by a proteinuria reduction of 7.2%. This difference, however, was not statistically significant. Similar findings have been reported with rosiglitazone. Treatment with rosiglitazone, 4mg twice a day for 52 weeks, was accompanied by a significant reduction (25%) of microalbuminuria in type 2 diabetic patients [44].

More recently, it has been reported that combined therapy with rosiglitazone and metformin was followed by a reduction of microalbuminuria significantly higher than that found with the combination of glyburide and metformin (22.8% vs. 7.1%, p=0.001) in type 2 diabetic patients with microalbuminuria. Thus, TZDs are not need to adjust the dose in diabetic nephropathy patients.

2.2 Effects of Thiazolidinediones on Glycemic Control and Lipid Parameters

TZD drugs are highly effective to decrease blood glucose concentrations for type 2 diabetes mellitus by reducing insulin resistance and improving peripheral glucose disposal [4]. The TZD can decreases fasting plasma glucose and HbA_{1c} levels

when used as monotherapy and in combination with a sulfonylurea, metformin, or insulin. Additionally, the TZD can increase levels of HDL and LDL, but only pioglitazone significantly lower TG levels. The effects of TZDs on glycemic and lipid levels have shown in many clinical trial and studies.

Studies in Pioglitazone

In multicenter double-blind, placebo-controlled clinical trial [6], 408 patients were randomized to receive pioglitazone monotherapy for 26 weeks. There were significant mean percent decreases from baseline in TG, significant mean percent increases from baseline in HDL-C, and only small percent changes in total cholesterol and LDL-C. Other study of pioglitazone-based therapy over 52 weeks in elderly diabetic patients was associated with reductions in HbA_{1c} and FPG values ranging from 1.1% to 1.6% and 2.1 to 2.8 mmol/L, respectively [7]. TG levels decreased $\geq$ 9.6%, and HDL-C levels were significantly improved, but LDL-C levels significantly increased with pioglitazone-based treatment in the elderly patients study.

A double-blind study of pioglitazone in combination with a sulfonylurea [46], there were dose-dependent decreases in the HbA_{1C} levels in both pioglitazone and sulfonylurea groups, which were statistically significance compared with placebo and with baseline. The pioglitazone 30 mg with sulfonylurea group had a statistically significant decrease in the mean TG level, and increases in the mean HDL-C level compared with baseline. There were no significant differences in effect on total and LDL-C levels from baseline in all groups. In Rosenstock et al. study [47], the patients were randomly assigned to pioglitazone 15 or 30 mg daily in combination with their baseline doses of insulin, HbA_{1c} levels improved by 1.0 and 1.3% respectively after 16 weeks of therapy.

Studies in Rosiglitazone

For rosiglitazone effects, Raskin et al. [48] observed a decrease in HbA_{1c} level of 0.6 and 1.2% after 26 weeks of treatment with rosiglitazone 4 and 8 mg respectively. Total cholesterol, HDL and LDL cholesterol levels significantly increased with rosiglitazone therapy. Other study of 16 weeks of rosiglitazone 8 mg [49] showed that LDL-C levels increased by 8%. Similarly to in a long-term study of rosiglitazone [50], LDL-C levels increased from baseline by 6% at 3 months and 8% at 12 months. HDL-C levels increased about 17% over 18 months. Additionally, Phillips et al. [51] found that TG levels increased significantly by 14 to 21% from baseline in patients receiving rosiglitazone for 26 weeks.

Comparison between Pioglitazone and Rosiglitazone

In a multicenter, prospective, double-blind trial, Goldberg et al. [52] compared the lipid effects of pioglitazone and rosiglitazone in subjects with type 2 diabetes (treated with diet alone or oral monotherapy) and dyslipidemia (not treated with any lipid lowering agent). Patients were randomly assigned to receive pioglitazone (n=400) or rosiglitazone (n=402) and were followed for 24 weeks. Mean TG levels in pioglitazone group were significantly reduced by 51.9 ± 7.8 mg/dl, whereas in the rosiglitazone group, TG levels increased by 13.1 ± 7.8 mg/dl ($p<0.001$). Both drugs increased HDL-C, but the magnitude of change was significantly greater with pioglitazone (5.2 ± 0.5 vs. 2.4 ± 0.5 mg/dl; $p<0.001$). The increase in LDL-C was less for pioglitazone compared with rosiglitazone (12.3 ± 1.6 vs. 21.3 ± 1.6 mg/dl; $p<0001$), respectively. Additionally, LDL-C particle concentration was reduced with pioglitazone but increased with rosiglitazone ($p<0.001$) and LDL-C particle size increased more with pioglitazone ($p=0.005$). Thus, pioglitazone compared with rosiglitazone is associated with significant improvements in TG, HDL-C, LDL-C particle concentration, and LDL-C particle size.

2.3 Adverse Events of Thiazolidinediones

2.3.1 Edema

The reasons for fluid retention and peripheral edema with TZD use are not fully understood and are likely to be multifactorial. The increase in plasma volume related to TZDs has already been cited and may result from a reduction in renal excretion of sodium and an increase in sodium and free water retention [53]. TZDs may interact synergistically with insulin to cause arterial vasodilatation, leading to sodium reabsorption with a subsequent increase in extracellular volume, and thereby resulting in pedal edema. Increased sympathetic nervous system activity [54], altered interstitial ion transport [55], alterations in endothelial permeability [56], and PPAR-γ–mediated expression of vascular permeability growth factor (VEGF), an important angiogenic factor with strong microvascular permeabilizing properties [13,14] represent other possible mechanisms for edema with these agents. Therefore, many studies have shown the adverse events of edema and weight gain in TZDs both monotherapy and combinations.

Pioglitazone monotherapy in clinical trial [6] showed the incidence of edema or peripheral edema was only 12 of 329 (3.6%). Whereas a post hoc analysis of data from elderly patients participating in 4 randomized clinical trials in the U.S. [7], rates of edema were 11.7% for pioglitazone monotherapy, 9.5% for combination therapy with a sulfonylurea, and 3.2% for combination therapy with metformin. Additionally, the weight increases of 2.1% for pioglitazone monotherapy and 4.8% for pioglitazone in combination with sulfonylurea. In a prospective study in Europe [57], data collected from four 1-year of double-blind studies, edema was reported in 6.7-8.1% for pioglitazone monotherapy, 6.9% for combination therapy with a sulfonylurea, and 6.3% for combination therapy with metformin. This edema was generally mild or moderate and very rarely resulted in withdrawal of treatment. Moreover, the greatest increase in mean weight was 2.8 kg seen with use of pioglitazone as monotherapy and in combination with a sulfonylurea.

The other double-blind studies, edema was reported in 7.2% for combination therapy of pioglitazone with a sulfonylurea [46], and in 5.9% for combination therapy of pioglitazone with metformin [5]. When pioglitazone was combined with insulin, edema was reported in 15.3% compared with 7.0% of insulin alone [58]. Therefore, the incidence of edema in clinical trials was higher when pioglitazone was combined with insulin, compared with when pioglitazone was used in combination therapy with oral hypoglycemic agents. Similarly in rosiglitazone study, Raskin et al. [48] showed that rosiglitazone 4 or 8 mg per day in combination with insulin was associated with a 13.1% and 16.2% incidence of edema, respectively, compared with 4.7% in those taking insulin alone.

One study based on hospital data showed edema rate higher than the previous studies. The prospective study at Melbourne [12] for all patterns of TZDs therapy found that peripheral edema was noted in 33% of the pioglitazone therapy and 21% of the rosiglitazone therapy (difference not significant). It was severe enough to prompt withdrawal of TZD in 7% and 4%, respectively. This study also showed mean gain was 2.3 kg (range, –5.0 to 19 kg) in the pioglitazone group and 2.9 kg (range, –5.0 to 11.5 kg) in the rosiglitazone group (P=0.95). The edema from TZDs in Niemeyer study [59] also showed the withdrawal of TZD therapy. This study followed for 4–5 months in 116 patients receiving TZD drug, and found 18.1% of patients developed edema. Fifty-three percents of them needed discontinuation of therapy because they were unresponsive to diuretics (furosemide and/or thiazide).

Finally, a meta-analysis of the risk of TZDs-induced edema [60] showed that the pooled odds ratio for TZD induced edema was 2.26 (95%CI: 2.02-2.53). The results yielded a higher risk for developing edema with rosiglitazone (OR=3.75) compared to pioglitazone (OR=2.42). The study also showed the range of weight gain was -0.59 to +3.86 kg for pioglitazone from 12 studies and +1.2 to +5.0 kg for rosiglitazone from 6 studies.

2.3.2 Weight Gain

Previous studies showed weight gain either in monotherapy or in combination with other hypoglycemic therapies. In pioglitazone monotherapy caused median weight gains of 0.9, 1.0, and 2.6 kg at the 15-, 30-, and 45-mg daily doses, respectively [15]. Median weight gains of 2.3 and 3.6 kg occurred when pioglitazone at 15 and 30 mg daily was added to insulin. For a 52-week study comparing rosiglitazone to glyburide [41], a mean weight gain of 1.9 kg was observed in both the glyburide group and the rosiglitazone group at the 4-mg daily dose, and a 2.9-kg weight gain was observed at the rosiglitazone 8-mg daily dose. When coadministered with a sulfonylurea in a 26-week study, rosiglitazone at 4 mg daily was associated with a 1.8-kg weight gain compared with sulfonylurea alone. After 6 months of treatment, weight gains of 4.1 kg and 5.4 kg were encountered when rosiglitazone, at the 4-mg and 8-mg daily doses, respectively, was added to insulin.

The adverse event of weight gain showed dose-dependent of pioglitazone or rosiglitazone. Rosenstock et al. study [58], patients received insulin combined with pioglitazone 15 mg and 30 mg which showed weight gained 2.3 and 3.7 kg respectively. In rosiglitazone therapy, Raskin et al. [48] significant weight gain occurred in all three groups by 0.9 kg in placebo group, 4.0 kg in rosiglitazone 4 mg group, and 5.3 kg in rosiglitazone 8 mg group.

A randomized, double-blind, placebo-controlled trial [61] in 48 men and women with type 2 diabetes who were treated for 24 weeks with 45 mg of pioglitazone or a matching placebo. Weight gain was significantly greater in the pioglitazone-treated group within the first 4 weeks, and although it had increased by 3.88 kg, it had not reached a plateau by the end of the 6-month study. Body weight and fat increased steadily in the patients treated with pioglitazone during the 6 months of the study (+3.9 $\pm$ 3.1 kg at 6 months in the pioglitazone group vs. -0.8 $\pm$ 3.4 kg in the placebo group). Visceral and subcutaneous fat were measured by computed tomography (CT) which

showed that visceral fat did not change significantly in either group, but subcutaneous body fat increased by pioglitazone use. Similarly to some studies [62,63] that showed an increase in subcutaneous fat during treatment with TZDs by using CT.

Consequently, the rise in body weight has been observed in many studies that have treated patients with TZDs. It is possible that a fraction of the change in body weight is due to changes in total body water. This increase in fat is to be expected from the mechanism of action for this drug. Thiazolidinediones are agonists for the PPAR-γ and initiate differentiation of adipocytes from preadipocytes into mature fat cells [64]. Thus, treatment with this class of drugs would be expected to increase the number of small insulin-sensitive fat cells providing a reservoir for storage of fat.

2.3.3 Congestive Heart Failure

Despite these potential benefits, TZD-associated fluid retention and increased risk of CHF have been of concern. In PROactive (Prospective Pioglitazone Clinical Trial in Macrovascular Events) [65], more than 5,000 patients with diabetes were randomized to receive pioglitazone or placebo. Patients with NYHA functional class II–IV CHF were excluded. Hospitalizations related to CHF occurred in 149 of 2,605 patients (5.7%) receiving pioglitazone versus 108 of 2,633 patients (4.1%) in the placebo group ($p = 0.007$). However, mortality rates from CHF did not differ significantly between the groups (0.96% for pioglitazone vs. 0.84% for placebo; $p = 0.639$).

In a retrospective cohort study of a health insurance claims database of 33,544 patients with diabetes, Delea et al. [66] reported that a new diagnosis of CHF was observed more frequently in patients treated with a TZD compared with patients who received other oral hypoglycemic agents (HR 1.7; 95%CI 1.54 to 1.97; $p<0.001$). By 40 months, the adjusted frequency of hospitalization for CHF was 2.5% among patients receiving a TZD and 1.0% among control patients ($p<0.001$). The unadjusted and

adjusted odds ratios for exposure to a TZD were 1.71 (95%CI 1.24 to 2.36) and 1.37 (95%CI 0.98 to 1.92), respectively, in case versus control patients.

In contrast, data analysis of 23,440 patients from the Kaiser Permanente Northern California Diabetes Registry [67] showed that hospitalization for CHF was not significantly increased among those receiving pioglitazone relative to sulfonylurea drugs (HR 1.28; 95%CI 0.85 to 1.92). There was a significantly higher incidence among those initiating insulin (HR 1.56; 95%CI 1.00 to 2.45) and lower incidence among those initiating metformin (HR 0.70; 95%CI 0.49 to 0.99).

For the report of CHF rate, the ADOPT (A Diabetes Outcome Progression Trial) study [68], a 4-year of double-blind trial involving 4,360 patients with diabetes who were randomized to receive rosiglitazone, metformin, or glyburide. Interestingly, of the 22 investigator-reported CHF events among 1,456 patients (1.51%) in the rosiglitazone group ($p = 0.26$, comparison between rosiglitazone and glyburide groups). In the DREAM (Diabetes Reduction Assessment with Ramipril and Rosiglitazone Medication) study [69], rosiglitazone significantly increased the risk of new-onset CHF in patients (0.53% of 2,635 pts. receiving rosiglitazone vs. 0.07% of 2,634 pts. receiving placebo; RR 7.0; 95%CI 1.59 to 30.76, $p = 0.01$). An analysis of the RECORD trial (Rosiglitazone Evaluated for Cardiac Outcome and Regulation of Glycemia in Diabetes) [70] after a mean follow-up of 3.75 years also showed an increased risk of CHF (1.71% of 2,220 pts. receiving rosiglitazone vs. 0.76% of 2,227 controls; RR 2.24; 95%CI 1.27 to 3.97). In a prospective study [57], the reports of CHF were rare and occurred in 0.6% of pioglitazone-treated patients and 0.5% of metformin- and gliclazide-treated patients.

However, a retrospective data analysis of 1,668 matched pairs of patients receiving pioglitazone or insulin [71] showed that insulin therapy was associated with a significantly higher incidence of inpatient hospitalizations for CHF than pioglitazone therapy ($P<0.001$). The insulin group also had a higher incidence of CHF ($P=0.014$) and a higher hospitalization rate for CHF ($P=0.021$). The hazard ratio for pioglitazone versus

insulin was 0.501 (95%CI 0.331-0.758; P = 0.001) for a primary or secondary diagnosis of CHF in any setting and 0.263 (95%CI 0.135-0.511; P<0.001) for any occurrence of an inpatient hospitalization for CHF.

Finally, a meta-analysis of pioglitazone trials [72] enrolling 16,390 patients and ranging in duration from 4 months to 3.5 years reported an increased incidence of CHF associated with pioglitazone (2.34% of 8,554 pts. receiving pioglitazone vs. 1.77% of 7,836 pts. in the comparator group; RR 1.41; 95%CI 1.14 to 1.76).

The American Heart Association (AHA) and the American Diabetes Association (ADA) recommend [13] that patients should be evaluated for underlying cardiac disease before being prescribed a TZD. In those with NYHA functional class III or IV CHF, TZDs are contraindicated. Patients with NYHA class I and II CHF, those at risk for CHF, and patients with an ejection fraction less than 40% may start at a low dose of TZD, with slow escalation of the dose over several weeks. TZD-related CHF can occur within weeks to months after the drug is started. If excessive, rapid weight gain (>3 kg within a few weeks) and pedal edema (particularly if the onset is acute, with rapid progression) develop, the presence of CHF should be evaluated. Signs and symptoms of CHF, such as shortness of breath, ankle and leg edema, orthopnea, and paroxysmal nocturnal dyspnea, should be assessed, and an electrocardiogram, echocardiogram, and serum brain natriuretic peptide level (BNP) may be obtained. Inadequate response to diuretics and/or reduction in TZD dose should prompt discontinuation of the TZD, and standard therapy for CHF should be initiated as necessary.

When a TZD is prescribed to patients who do not have established heart disease but have one or more risk factors for CHF (see below), one should consider starting with low doses and increase the dosage gradually as required to optimize glycemic control, while observing for any signs of excessive weight gain, peripheral edema, or CHF.

Risk factors for heart failure in patients treated with TZDs

1. History of heart failure (either systolic or diastolic)
2. History of prior myocardial infarction or symptomatic coronary artery disease
3. Hypertension
4. Left ventricular hypertrophy
5. Significant aortic or mitral valve heart disease
6. Advanced age (>70 years)
7. Long-standing diabetes (>10 years)
8. Preexisting edema or current treatment with loop diuretics
9. Development of edema or weight gain on TZD therapy
10. Insulin co-administration
11. Chronic renal failure (creatinine > 2.0 mg/dl)

2.3.4 Acute Coronary Syndrome and Myocardial Infraction

Recently in 2007, rosiglitazone has been linked to an increased risk of MI. In a meta-analysis of 42 trials, Nissen et al. [73] compared the risk for MI associated with rosiglitazone with that of placebo or other antihyperglycemic agents. Rosiglitazone was associated with a significant 43% increased risk for MI ($p = 0.03$). Moreover, in a subsequent meta-analysis of 4 randomized controlled trials with a follow-up of 1– 4 years and monitored cardiovascular events, Singh et al. [74] showed that rosiglitazone significantly increased the risk of MI compared with control ($p = 0.02$).

Gerrits et al. [75] analyzed the database of a healthcare insurer to ascertain the risk of acute MI associated with pioglitazone relative to rosiglitazone. This retrospective cohort study included 14,807 patients treated with pioglitazone and 15,104 patients treated with rosiglitazone, with a mean follow-up of 1.2 years. Pioglitazone was associated with a lower rate of MI compared with rosiglitazone. In the pioglitazone group, 161 (1.1%) patients were hospitalized for acute MI versus 214 (1.4%) in the rosiglitazone

group (adjusted HR 0.78; 95%CI 0.63 to 0.96), with a 15% decrease in the composite of MI and coronary revascularization (adjusted HR 0.85; 95%CI 0.75 to 0.98).

Similarly, a population-based retrospective study using healthcare databases in Ontario, Lipscombe et al. [76] analyzed information on more than 159,000 elderly (aged>66 y) patients with diabetes followed for a median duration of 3.8 years. The risk of MI among patients treated with TZD monotherapy versus non-TZD oral therapy was examined. The groups were well matched with regard to age, sex, diabetes duration, and history of CVD. Current users of TZD monotherapy had an increased risk of MI compared with users of non-TZD agents (65 cases; adjusted RR 1.40; 95%CI 1.05 to 1.86; p = 0.02). An increased risk of MI was identified only with rosiglitazone (RR 1.76; 95%CI 1.27 to 2.44; p<0.001). Whether pioglitazone is associated with a risk of MI could not be evaluated because of the small number of patients who were prescribed pioglitazone (RR 0.73; 95%CI 0.40 to 1.36; p = 0.33). Moreover, in a meta-analysis conducted by Lincoff et al. [72], MIs occurred in 1.53% of 8,554 patients in the pioglitazone group and 2.03% of 7,836 patients in the comparator group (placebo, metformin, sulfonylurea, rosiglitazone) (p = 0.08). Overall, pioglitazone was associated with a significantly lower risk of death, MI, or stroke, and the magnitude of this effect was observed across trials ranging from 4 months to 3.5 years.

Furthermore, the only completed, prospective, double-blind study that specifically investigated cardiovascular endpoints is the PROactive [65]. The study included 5,238 patients with type 2 diabetes and preexisting CVD who were randomized to receive pioglitazone or placebo added to existing therapy for an average of 2.9 years. Pioglitazone produced a 10% relative risk reduction in the primary endpoint, which included a broad composite of cardiovascular and procedural events; this did not achieve statistical significance (HR 0.90; 95%CI 0.80 to 1.02; p = 0.095). However, the secondary composite endpoint of all-cause mortality, MI, or stroke was significantly reduced by 16% in the pioglitazone arm (HR 0.84; 95% CI 0.72 to 0.98; p = 0.027).

For instance, RECORD [77], a prospective, randomized trial including 4,447 patients with type 2 diabetes, is specifically designed to measure cardiovascular outcomes of rosiglitazone therapy. An interim analysis showed no statistically significant differences between the rosiglitazone group and the control group regarding MI and death from cardiovascular causes or any other causes, however, the final results are due in 2009.

2.4 Pharmacokinetics of Pioglitazone

Overall, the pharmacokinetic parameters of pioglitazone in type 2 diabetic patients are similar to those in healthy volunteers [78]. Steady-state serum concentration of both pioglitazone and its active metabolites are achieved within 7 days. At steady-state, two of the pharmacologically active metabolites of pioglitazone in human, metabolites III (M-III, keto-derivative) and IV (M-IV, hydroxyl-derivative), reach serum concentrations equal to or greater than pioglitazone. In the fasting state, pioglitazone is first measurable in serum within 30 minutes, with peak concentrations observed within 2 hours. Food slightly delays the time to peak serum concentration to 3 to 4 hours. Pioglitazone is highly protein bound (>99%) in human serum with a resulting low volume of distribution (0.63 L/kg in single dose). Metabolites M-III and M-IV also are highly protein bound (>98%). Pioglitazone undergoes extensive hepatic metabolism, predominantly via by the cytochrome P450 2C8 system. It is excreted into the bile either unchanged or as metabolites and eliminated in the feces. Approximately 15% to 30% of the pioglitazone dose is recovered in the urine. The mean serum half-life of pioglitazone and total pioglitazone ranges from 3 to 7 hours and 16 to 24 hours, respectively. Pioglitazone has an apparent clearance (CL/F) calculated to be 5 to 7 L/hr.

Metabolites of pioglitazone are more active and are excreted predominantly in the bile. Both pioglitazone as its metabolites M-III and M-IV do not accumulate in CRF.

Pharmacokinetic profile of pioglitazone was similar in healthy subjects and in patients with moderate and severe renal failure [79].

2.5 Pharmacogenetics in Edema Condition

2.5.1 PPARG gene

PPAR-γ is a transcription factor that belongs to the same family of nuclear receptors as steroid and thyroid hormone receptors. It is activated by fatty acids, prostanoids, and TZDs. In activation, PPAR-γ is heterodimerized with the retinoid X receptor and binds to specific PPAR-responsive elements of DNA to promote transcription of numerous target genes [80]. From differential promoter usage with alternate splicing of the PPARG gene, it gives a variety of mRNA isoforms (PPAR-γ 1-4) but just two receptors (PPAR-γ 1 and PPAR-γ 2). Although the isoform PPAR-γ 1 exhibits widespread expression in the most tissues such as adipose tissue, pancreatic-β cells, macrophages, and vascular endothelium, PPAR-γ 2 is expressed specifically in adipose tissue. PPAR-γ 3 expression appears to be restricted to macrophages, adipose tissue, and colon, while the tissue distribution of PPAR-γ 4 remains to be defined [37].

The most prevalent human PPARG genetic variant reported to date is the Pro12Ala polymorphism, substituting alanine for proline at codon 12 of exon B in the unique PPARG2. The frequencies of Ala allele that differ quite markedly depending on the population are 23% in Hispanic subjects, 12% in Caucasians, 10% in native Americans, 4% in Japanese and Korean, and 1% in Chinese [37,80]. However, a study in an Asian population from Singapore [81] showed that a frequency for Ala12 allele was found 3.2% in Malays, 3.7% in Chinese, and 11.9% in Indians. A meta-analysis [82] using data from 30 independent studies with a total of 19,136 subjects has suggested that subjects with mean body mass index (BMI) value $\geq$ 27 kg/m^2 and Ala12 allele carriers had a significantly higher BMI than non-carriers (P=0.0006). Moreover, other

studies [83] have shown greater sensitivity of Ala carriers to dietary factors such as faster weight regain after a hypocaloric diet, and a stronger relationship between BMI and the ratio of dietary polyunsaturated fat to saturated fat intake.

In type 2 diabetes mellitus (T2DM), a meta-analysis in 2000 [84] showed that the common Pro12Pro significant increases a modest risk of T2DM by odds ratio (OR) of 1.25 (P=0,002). Furthermore, this meta-analysis including five data set demonstrated a significant risk reduction of 21% of T2DM in the Ala12 allele carriers by OR of 0.79 (P=0.00007). One study investigated the relationship between the Pro12Ala variant and response to therapy with TZDs in type 2 diabetic patients. Bluher et al. [85] reported that the Pro12Ala variant dose not affect the therapy efficacy of pioglitazone by defined response rate to a >20% decrease in FPG or a >15% decrease in HbA_{1c}.

Similarly, Snitker et al. [86] using the data from the Troglitazone in Prevention of Diabetes (TRIPOD) study showed that the Pro12Ala variant did not explain the failure of 1/3 of subjects treated with troglitazone to increase their insulin sensitivity. Nevertheless, a study of Kang et al. [21] in Korea showed opposing results from previous studies. Patients with the Pro12Ala variant had a better therapeutic response to rosiglitazone than did patients with the Pro12Pro variant and this study used the same definition of response rate in Bluher study. The different results may be a result of ethnic, demographic, or clinical differences among the patient populations. In addition, a study of Hansen et al [23] investigating a relationship between Pro12Ala variant and PPARγ/α agonist (ragaglitazar)-induced edema in T2DM indicated that the Pro12Ala of PPARG gene is the most important risk factor for ragaglitazar-induced edema by OR of 4.42 (P=0.081) and female gender is the second risk for edema by OR of 3.34 (p=0.0005).

2.5.2 Epithelial Sodium Channel (ENaC)

Nowadays, several recent studies indicated that PPAR-γ may be involved in the regulation of electrolytes and water excretion in distal nephron segments and collecting

ducts, especially sodium transport. It is reasonable to hypothesize that increased sodium and water retention at the renal level plays an important role of edema by activation of TZDs through PPAR-γ [2]. TZDs may interact synergistically with insulin to cause arterial vasodilatation, leading to sodium reabsorption with a subsequent increase in extracellular volume, and resulting in peripheral edema [47].

In animal studies [17.18], they found that mice with collecting duct (CD) knockout of PPAR-γ were resistant to TZDs-induced fluid retention as compared with controls. Furthermore, TZDs stimulated sodium transport in primary cultures of CD cells expressing PPAR-γ and not in cells lacking PPAR-γ. Thus, a PPAR-γ-dependence pathway in regulation of sodium transport in the CD underlies TZD-induced fluid retention and epithelial sodium channel (ENaC) gene as a PPAR-γ target gene in the CD. A study of GI262570 (farglitazar) [87], a potent non-TZD PPAR-γ agonist, in rats showed that plasma volume expansion was accompanied by a significant decrease in plasma potassium concentration, but a significant increase in plasma sodium and chloride concentrations. They suggested that this drug can increase water and sodium reabsorption in distal nephron by stimulating the ENaC and Na, K-ATPase system.

For rosiglitazone study [88], normal rats treated with rosiglitazone had significantly decreased urine volume, urine sodium excretion, creatinine clearance, and urinary sodium-to-creatinine ratio. In double-blind, randomized, placebo-controlled, cross-over study, ten healthy men subjects receiving pioglitazone had significantly decreased urine sodium excretion and lithium clearance; an indirect measurement of renal proximal sodium reabsorption, suggesting an increased reabsorption of sodium in the proximal tubule [89]. However, no clinical study has ever demonstrated the changes of electrolytes by TZDs in diabetic patients with or without edema.

Both fluid retention and overt clinical edema typically developed during the first few months of treatment and was reversible after drug withdrawal. It is reasonable to hypothesize that increased sodium and water retention at the renal level plays an

important role. Several recent lines of evidence indicate that PPARγ may be involved in the fine regulation of electrolytes and water excretion in the distal nephron segments. The genes that seem to be the target for modulation via PPARγ activation are the ones encoding for the epithelium Na channel (ENaCα), the serum and glucocorticoid regulated kinase (SGK1) and Na+, K+-ATPase [90].

2.5.3 Other Genetics Related TZD-Induced Edema

A study of Ruaño et al. [91] analyzed 384 single nucleotide polymorphisms (SNPs) from 222 cardiovascular and metabolic genes in 87 outpatients with type 2 diabetes receiving thiazolidinedione therapy. Physiogenomic analysis was used to discover associations with body mass index (BMI) and edema. The 5 most significant gene associations found between BMI and SNPs were ADORA1, adenosine A1 receptor (rs903361, p=0.0003), PKM2, pyruvate kinase-muscle (rs2856929, p=0.002); ADIPOR2, adiponectin receptor 2 (rs7975375, p=0.007); UCP2, uncoupling protein 2 (rs660339, p =0.008); and APOH, apolipoprotein H (rs8178847, p=0.010). For edema, the 5 most significant gene associations were NPY, neuropeptide Y (rs1468271, p=0.006); GYS1, glycogen synthase 1-muscle (rs2287754, p=0.013); CCL2, chemokine C–C motif ligand 2 (rs3760396, p=0.015); OLR1, oxidized LDL receptor 1 (rs2742115, p=0.015); and GHRH, growth hormone releasing hormone (rs6032470, p=0.023).

In other study of Geese et al. [92], a total of 213 SNPs in 63 genes were genotyped in 730 participants. SNPs in renin (rs2368564) and endothelin-1 (rs5370) were associated with reduced risk of edema (P = 0.003 and P =0.028, respectively) and an SNP in b1 adrenergic receptor (rs1801253) was associated with increased susceptibility to edema (P = 0.034). In genetically susceptible individuals, PPARγ agonists increase renin levels directly by upregulating renin gene expression and indirectly by downregulating endothelin-1 gene expression via AP-1 antagonism [93]. This increase in renin levels activates the renin–angiotensin–aldosterone pathway, thereby increasing expression of epithelial sodium channels in the kidney and ultimately

causing excess sodium and water reabsorption by the kidney, thus contributing to edema (Figure 2). This mechanism suggests that edema caused by PPARγ agonists could be controlled by inhibiting the downstream effects of renin, such as through the use of commonly used antihypertensive medicines including angiotensin receptor blockers, angiotensin-converting enzyme inhibitors, renin inhibitors and mineralocorticoid receptor blockers.

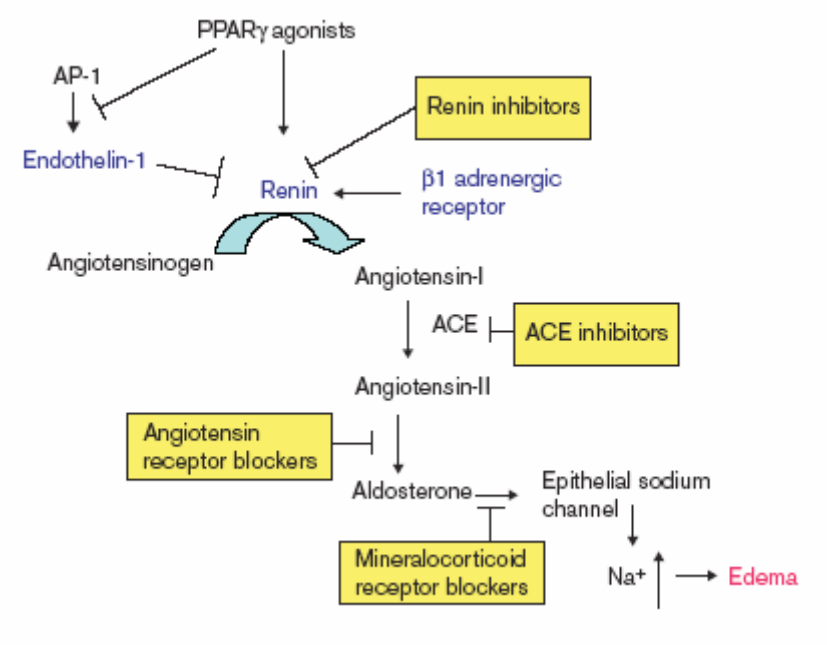

Figure 2 Potential mechanism of edema caused by (PPARγ) agonists.

CHAPTER III

METHODOLOGY

The methodology of this study are presented in 3 phases which are Phase 1: Determining the prevalence of TZDs-induced edema using retrospective study; Phase 2: Comparisons of pharmacokinetic parameters between edema and non-edema type 2 diabetic patients treated with pioglitazone; and Phase 3: Determining the associaiton of SNP rs5370 of endothelin-1 gene (*ENDO1*) and SNP rs34241435 of epithelial sodium channel β subunit gene (*SCNN1B*) and TZDs-induced edema.

3.1 Phase 1: Determining the Prevalence of Thiazolidinediones-Induced Edema Using Retrospective Study

3.1.1 Subjects

This part was a retrospective study by medical chart review for the prevalence of edema in type 2 diabetic patients treated with pioglitazone or rosiglitazone. The subjects of this study were recruited from the Diabetic Clinic, the Family Medicine Clinic, and the General Medicine Clinic at Ramathibodi hospital during 2006-2008. The inclusion and exclusion criterias of the patients were as follow;

Inclusion criteria: patient were included if all of the following conditions were met;

- were diagnosed type 2 diabetes mellitus by the physicians.
- received pioglitazone or rosiglitazone alone or as a combination therapy for more than 3 months.

Exclusion criteria: patient would be excluded if either one of the following conditions occurred;

- inadequate data in medical records such as co-morbid diseases, co-medications, or laboratory tests of glucose controls.
- data and diseases history could not be revealed.
- received continuous corticosteroid or furosemide.
- received CCBs, including amlodipine, manidipine, felodipine, lacidipine, and verapamil within 1 month before receiving TZDs or during TZDs treatment and edema occurred [94].
- received NSAIDs, ARBs, or nitrates within 1 week before receiving TZDs or during TZDs treatment and edema occurred [95-98].
- have at least one of following diseases: CHF, DVT, nephritic syndrome, untreated hypothyroid, ascites, liver dysfunction (AST or ALT >3 TUL), and renal insufficiency (SCr > 1.5 mg/dl).

3.1.2 Sample Size

Sample size for one group study of TZDs-induced edema was calculated based on the proportion of edema reported in the study of Rosenstock et al. [58] as following;

$$N = (Z_{\alpha/2})^2 PQ / d^2$$

Set; Significance level (α) = 0.05 (two-sided); $Z_{\alpha/2} = 1.96$

P = proportion of edema in patients treated with TZDs = 0.153

Q = proportion of non-edema in patients treated with TZDs = 0.847

d = the specified effect size = 30%of P = 0.046

$$N = (1.96)^2 (0.153) (0.847) / (0.046)^2$$
$$= 0.498 / 0.0021$$
$$= 237 \text{ patients}$$

The required sample size for this retrospective study was at least 237 patients.

3.1.3 Data Collection

Data from medical records were collected for information on patient demographics (i.e. age, gender, weight, and BMI), duration of diagnosed diabetes, co-morbid diseases, co-medication, doses and duration of pioglitazone or rosiglitazone receiving, laboratory tests (i.e. FPG, HbA_{1c}, SCr, urine protein, and urine creatinine), and edema status. The data collection form is presented in **Appendix A.**

3.1.4 Data Analysis

Patient characteristics and laboratory data were presented as mean and standard deviation. The edema status was analyzed to the prevalence of TZDs-induced edema in frequency and percentage. Comparisons of gender, co-morbid diseases, co-medication, and doses of pioglitazone or rosiglitazone between edema group and non-edema group were analyzed by Chi-square test. Comparisons of weight, BMI, and laboratory tests between the two groups were analyzed by Mann-Whitney U test or Student's t test. The difference mean of laboratory tests between baseline and after TZDs use were analyzed by Paired t-test. The relationship between the factors in edema condition of TZDs use was analyzed by Logistic Regression. Data were analyzed using computer programs SPSS for windows version 16.0.

3.2 Phase 2: Comparisons of Pharmacokinetic Parameters between Edema and Non-Edema Type 2 Diabetic Patients Treated with Pioglitazone

3.2.1 Subjects

This part was a retrospective cross-sectional study to compare pharmacokinetic parameters between edema and non-edema diabetic patients who have been treated with pioglitazone. The subjects consisted of type 2 diabetic patients from the Diabetic Out Patient Clinic, the Family Medicine Clinic, and the General Medicine Clinic at

Ramathibodi hospital. The subjects were recruited from consecutive patients appointed for the follow-up treatment at the hospital during December 2, 2007 to December 31, 2008. The inclusion and exclusion criterias of the patients were as follow;

Inclusion criteria: patient would be included if all of the following conditions were met;

- were diagnosed type 2 diabetes mellitus by the physicians.
- received pioglitazone alone or as a combination therapy for more than 3 months and continuously used the drug during the study.
- agreed to participate in this study and provided written informed consent.

Exclusion criteria: patient would be excluded if either one of the following conditions occurred;

- received continuous corticosteroid or furosemide.
- received CCBs, including amlodipine, manidipine, felodipine, lacidipine, and verapamil within 1 month before receiving TZDs or during TZDs treatment and edema occurred [94].
- received NSAIDs, ARBs, or nitrates within 1 week before receiving TZDs or during TZDs treatment and edema occurred [95-98].
- have at least one of following diseases: CHF, DVT, nephritic syndrome, untreated hypothyroid, ascites, liver dysfunction (AST or ALT >3 TUL), and renal insufficiency (SCr > 1.5 mg/dl).

All patients were separated into edema group or non-edema group by edema status.

3.2.2 Data Collection

The researcher reviewed patient's medical records to recruit patients who met inclusion and exclusion criteria. The patients were contacted by phone at one week and two days before the appointment for follow-up at the clinic to confirm their appointment and to invite them to participate in this study. They were explained about the study on the day of the recruitment and if agreed and provided written informed consent they were included in this study.

Demographic data, co-morbid diseases, co-medication, edema status, and laboratory tests were recorded. Blood samples were obtained at the first time after overnight fasting, the total amount drawn was 25 ml (15 ml for the first collection and 10 ml for the second collection), and they were separated into several different tubes as follow;

- 2 ml of was sent for FPG determination.
- 3 ml was sent for HbA_{1c} determination.
- 10 ml was put in a plastic tube containing lithium heparin for investigation of a trough concentration of pioglitazone ($C_{ss\ min}$) in patients receiving pioglitazone.
- 10 ml of the 2^{nd} blood sample were drawn after taking pioglitazone for 4 to 6 hours, to investigate a peak concentration of pioglitazone ($C_{ss\ max}$).

3.2.3 Laboratory Assays

The blood samples used for pharmacokinetic study were centrifuged at 4°C for 10 minutes at 4,500 rpm, and the serum was transferred into a cryogenic vial and was stored at -80°C until analyzed. The serum pioglitazone concentration was measured by high-performance liquid chromatography using ultraviolet detector (HPLC-UV). Method for determination of pioglitazone concentration was modified from Sripalakit et al. study as described in **Appendix B** [99].

3.2.4 Pharmacokinetic Parameters Calculation

The pharmacokinetic parameters of each patient were calculated individually. The pharmacokinetic parameters calculated included the elimination rate constant (K_e), the volume of distribution (Vd), and the clearance (Cl) values. They were calculated according to the following equations;

$$K_e = \ln (C_{ss\ max}/C_{ss\ min}) / \Delta t$$

$$Vd = (S)(F)(D)(e^{-K\tau}) / (C_{ss\ min})(1- e^{-K\tau})$$

$$Cl = (K_e)(Vd)$$

$C_{ss\ max}$ = the maximum serum drug concentration at steady state

$C_{ss\ min}$ = the minimum serum drug concentration at steady state

Δt = the time interval between Css max and Css min.

S = the salt form of a drug, here, free form is used, S = 1

F = the bioavailability factor, total drug was assumed to absorb, F = 1

D = the dose administered

τ = tau; the dosing interval (hr)

$(1- e^{-K\tau})$ = the fraction of drug that is eliminated within one dosing interval.

3.2.5 Data analysis

Demographic data, co-morbid diseases, co-medication, and variables of laboratory test were presented in descriptive statistics, such as, frequency, percentage, mean, and standard deviation. Comparisons between dichotomous variables (gender, co-morbid diseases, co-medication, and TZDs doses) and dichotomous variables (edema status) were compared by Chi-square test.

The values of pharmacokinetic parameters and creatinine clearance were tested normal distribution by Kolmogorov-Smirnov tests. Comparisons of the values between

different edema status groups would be performed by nonparametric tests, i.e., Mann-Whitney's U test if they were not normally distributed or parametric tests, i.e., Student's t test would be used if the data were normally distribution. All tests of significance were two-tailed at significant level 0.05 (α=0.05). Data were analyzed using statistical SPSS program, version 16.0.

3.3 Phase3: Determining the Association of SNP rs5370 of *ENDO1* and SNP rs34241435 of *SCNN1B* and TZDs-Induced Edema Status

3.3.1 Subjects

This part was a retrospective cross-sectional study by comparing SNPs of endothelin-1 and epithelial sodium channel β subunit genes between edema and non-edema of diabetic patients treated with TZDs. The subjects of this study were type 2 diabetic patients treated at the Diabetic Clinic, the Family Medicine Clinic, and the General Medicine Clinic at Ramathibodi hospital. The subjects were recruited from consecutive patients appointed for the follow-up treatment at the hospital during December 2, 2007 to December 31, 2008. The inclusion and exclusion criterias of the patients were as follow;

Inclusion criteria: patient would be included if all of the following conditions were met;

- were diagnosed type 2 diabetes mellitus by the physicians.
- received pioglitazone or rosiglitazone alone or as a combination therapy for more than 3 months.
- agreed to participate in this study and provided written informed consent.

Exclusion criteria: patient would be excluded if either one of the following conditions occurred;

- received continuous corticosteroid or furosemide.
- received CCBs, including amlodipine, manidipine, felodipine, lacidipine, and verapamil within 1 month before receiving TZDs or during TZDs treatment and edema occurred [94].
- received NSAIDs, ARBs, or nitrates within 1 week before receiving TZDs or during TZDs treatment and edema occurred [95-98].
- have at least one of following diseases: CHF, DVT, nephritic syndrome, untreated hypothyroid, ascites, liver dysfunction (AST or ALT >3 TUL), and renal insufficiency (SCr > 1.5 mg/dl).

All patients were separated into edema group or non-edema group by edema status.

3.3.2 Sample Size

Sample size for comparisons of variants in the interested gene between edema and non-edema groups was calculated based on the proportion of T allele in endothelin-1 gene in Geese et al. study [24] as following;

$$N = \left[Z_{\alpha/2} \sqrt{2PQ} + Z_{\beta} \sqrt{P_1Q_1 + P_2Q_2} \right]^2 \Big/ d^2$$

Set; Significance level (α) = 0.10 (one-tailed); $Z_\alpha = 1.28$

Power (β) = 0.20 (one-sided); $Z_\beta = 0.84$

P_1 = proportion of T allele of endothelin-1 gene in edema group = 0.56

P_2 = proportion of T allele of endothelin-1 gene in non-edema group = 0.41

$Q_1 = 1 - P_1 = 1 - 0.56 = 0.44$

$Q_2 = 1 - P_2 = 1 - 0.41 = 0.59$

$P = ½(P1 + P2) = ½(0.44 + 0.59) = 0.515$

$Q = 1 - P = 1 - 0.515 = 0.485$

$d = |P1 - P2| = |0.56 - 0.41| = 0.15$

$$N = \left[1.28\sqrt{2\,(0.515)(0.485)} + 0.84\sqrt{(0.56)(0.44)+(0.41)(0.59)}\right]^2 \Big/ (0.15)^2$$

$$= (0.90 + 0.59)^2 / (0.15)^2$$

$$= 95 \text{ patients / group}$$

The required sample size for this phase in each group was at least 95 patients.

3.3.3 Data Collection

The researcher reviewed patient's medical records to recruit patients who met inclusion and exclusion criteria. The patients were contacted by phone at one week and two days before the appointment for follow-up at the clinic to confirm their appointment and to invite them to participate in this study. They were explained about the study on the day of the recruitment and if agreed and provided written informed consent they were included in this study.

Demographic data, co-morbid diseases, co-medication, edema status, and laboratory tests were recorded. Total amount of 15 ml of blood samples were obtained at the first time after overnight fasting.

- 2 ml of was sent for FPG determination.
- 3 ml was sent for HbA_{1c} determination.
- 10 ml was sent for DNA extraction and genotying of SNP rs5370 of *ENDO1* (rs5370) and SNP rs34241435 of *SCNN1B*.

3.3.4 Laboratory Assays

Serum FPG and HbA_{1c} were assessed by standard automated enzymatic method and cation exchange high-performance liquid chromatography method, respectively, at Ramathibodi Hospital Central Laboratory.

For pharmacogenetic study, the blood samples were centrifuged at 25°C for 10 minutes at 3,500 rpm. A medium layer of buffy coat was collected into microtubes and the DNA was extracted by the in-house method of Ramathibodi hospital which has been applied from the phenol – chloroform - isoamyl alcohol method [100] **(Appendix C)**. The extracted DNA was stored at 4°C until genotyping. SNP genotyping for rs5370 and rs34241435 were done [24,101] as described in **Appendix D.**

3.3.5 Data analysis

Demographic data, co-morbid diseases, co-medication, and variables of laboratory test were presented in descriptive statistics, such as, frequency, percentage, mean, and standard deviation. Comparisons between dichotomous variables (gender, co-morbid diseases, co-medication, TZDs doses, and different SNPs) and dichotomous variables (edema status) were compared by Chi-square test. All tests of significance were two-tailed at significant level 0.05 (α=0.05). Data were analyzed using statistical SPSS program, version 16.0.

CHAPTER IV

RESULTS

The results of this study are presented in 3 parts which are (1) Determining the prevalence of TZDs-induced edema using retrospective study; (2) Comparisons of pharmacokinetic parameters between edema and non-edema type 2 diabetic patients treated with pioglitazone; and (3) Determining the associaiton of SNP rs5370 of endothelin-1 gene (*ENDO1*) and SNP rs34241435 of epithelial sodium channel β subunit gene (*SCNN1B*) and TZDs-induced edema.

4.1 Determining the Prevalence of Thiazolidinediones-Induced Edema Using Retrospective Study

4.1.1 Included and Excluded Patients

Total numbers of screened patients were 446 patients. They were excluded of 168 patients (37.7%) and were included into this phase of the study of 278 patients (62.3%), as shown in table 1.

Table 1 The numbers of included and excluded patients

	Number of screened patients (N=446)	% of total
Excluded patients (n=168)		
- Inadequate data	121	27.1
- Data could not be revealed	8	1.8
- Corticosteroid used	2	0.4
- Furosemide used	8	1.8
- CCBs	5	1.1
- CHF	5	1.1
- Liver dysfunction	4	0.9
- Renal insufficiency	15	3.4
Included patients	278	62.3

4.1.2 Patients Characteristics

The total number of medical chart of the patients reviewed in this phase of study was 278. Of these, 139 patients received pioglitazone and 139 received rosiglitazone therapy. Table 2 shows baseline characteristics of patients in both pioglitazone and rosiglitazone groups. There were no significant differences between pioglitazone and rosiglitazone groups in baseline general characteristics except for TG. The mean TG at baseline in the pioglitazone group was significantly higher than the rosiglitazone group.

Co-morbid diseases of the 278 patients were shown in Table 3. There were no differences in co-morbid diseases between the two groups, except for nephropathy. The pioglitazone group had significantly higher percentage of nephropathy than the rosiglitazone group

Table 2 Baseline characteristics of patients

Characteristics	Pioglitazone group (n=139)	Rosiglitazone group (n=139)	Total (N=278)	p-value
Sex				0.966
- female	64.7% (90)	66.2% (92)	65.5% (182)	
- male	35.3% (49)	33.8% (47)	34.5% (96)	
Age (years)	58.30 ± 10.00	58.35 ± 9.91	58.24 ± 10.04	0.849
Weight (kg)	66.44 ± 11.20	65.36 ± 11.75	65.90 ± 11.47	0.440
BMI (kg/m^2)	26.24 ± 4.00	26.47 ± 4.22	26.34 ± 4.09	0.709
Duration of DM (years)	9.77 ± 5.52	10.10 ± 5.62	9.39 ± 5.57	0.621
Duration of TZD (years)	3.86 ± 2.04	4.06 ± 1.88	3.96 ± 1.96	0.401
FPG (mg/dl)	196.30 ± 54.12	198.51 ± 55.56	197.43 ± 54.77	0.742
HbA_{1c} (%)	9.41 ± 1.56	9.35 ± 1.45	9.38 ± 1.50	0.780
Cl_{Cr} (ml/mim)	73.38 ± 23.72	70.59 ± 22.94	72.14 ± 23.35	0.439
TC (mg/dl)	191.49 ± 37.31	195.88 ± 42.60	193.73 ± 40.05	0.449
LDL (mg/dl)	109.61 ± 32.50	115.88 ± 33.36	112.61 ± 32.99	0.164
HDL (mg/dl)	44.00 ± 12.83	46.47 ± 10.83	45.23 ± 11.91	0.176
TG (mg/dl)	195.02 ± 109.58	160.06 ± 107.07	182.99 ± 135.56	0.026

Data are mean ± standard deviation, except for sex

Table 3 Co-morbid diseases at baseline

Co-morbid diseases	Pioglitazone group (n=139)	Rosiglitazone group (n=139)	Total (N=278)	p-value
Coronary artery disease	10.1% (14)	8.6% (12)	9.4% (26)	0.680
Stroke	3.6% (5)	8.6% (12)	6.1% (17)	0.080
Hypertension	71.2% (99)	69.8% (97)	70.5% (196)	0.793
Dyslipidemia	83.5% (116)	83.5% (116)	83.5% (232)	1.000
Diabetic nephropathy [a]	37.4% (52)	22.3% (31)	29.8% (83)	0.018
- Macroproteinuria	24.5 % (34)	15.1% (21)	19.8% (55)	
- Microproteinuria	12.9 % (18)	7.2% (10)	10.1% (28)	
Diabetic retinopathy [b]	25.2% (35)	23.0% (32)	24.1% (67)	0.858
Diabetic neuropathy [c]	23.0% (32)	18.0% (25)	20.5% (57)	0.577
Foot ulcer	5.8% (8)	2.2% (3)	4.0% (11)	0.124

[a] Total missing data of diabetic nephropathy was 11.5%(32)

[b] Total missing data of diabetic retinopathy was 35.3%(98)

[c] Total missing data of diabetic neuropathy was 65.8%(183)

Most patients received basic antihyperglycemic drugs, with 91.0% receiving sulfonylureas, 81.3% receiving metformin, but only 11.9% receiving insulin and 11.2% receiving alpha-glucosidase inhibitors (Table 4). For antihypertensive drugs, ACEIs were the most common co-medications of the patients while diuretics were dispended in 26.6% of the patients. Statins were the most often use anti-dyslipidemia. There were no significant differences in co-medications used between pioglitazone and rosiglitazone groups.

Initial dose of TZD drugs were presented in Table 5 by dividing into low dose and high dose. Comparison of the percentage of high and low doses between pioglitazone and rosiglitazone groups showed no significant difference. The regimens of antihyperglycemic drugs at baseline between the two groups were similarly.

Table 4 Co-medications at baseline

Medications	Pioglitazone group (n=139)	Rosiglitazone group (n=139)	Total (N=278)	p-value
Sulfonylureas	89.9% (125)	92.1% (128)	91.0% (253)	0.529
Metformin	81.3% (113)	81.3% (113)	81.3% (226)	1.000
α-Glucosidase inhibitors	10.1% (14)	12.2% (17)	11.2% (31)	0.568
Insulin	12.9% (18)	10.8% (15)	11.9% (33)	0.578
Diuretics	27.3% (38)	25.9% (36)	26.6% (74)	0.786
ACEIs	43.2% (60)	38.8% (54)	41.0% (114)	0.464
ARBs	13.7% (19)	11.5% (16)	12.6% (35)	0.588
Beta-blockers	35.3% (49)	30.9% (43)	33.1% (92)	0.444
Nitrates	0.7% (1)	4.3% (6)	2.5% (7)	0.120
Alpha blockers	2.9% (4)	5.0% (7)	4.0% (11)	0.356
Ca channel blockers	25.2% (35)	23.7% (33)	24.5% (68)	0.780
Statins	76.3% (106)	77.0% (107)	76.6% (213)	0.887
Fibrates	15.1% (21)	15.1% (21)	15.1% (42)	1.000

Table 5 Initial thiazolidinediones dose and regimens of antihyperglycemic drugs at baseline (N=278).

Antihyperglycemic drugs	Pioglitazone group (n=139)	Rosiglitazone group (n=139)
Thiazolidinediones **		
- Low dose [a]	74.8% (104)	81.3% (113)
- High dose [b]	25.2% (35)	18.7% (26)
Oral antihyperglycemic	87.1% (121)	89.2% (124)
- Oral monotherapy	15.1% (21)	15.1% (21)
- Double-agent therapy	65.5% (91)	65.5% (91)
- Triple-agent therapy	6.5% (9)	8.6% (12)
Insulin monotherapy	1.4% (2)	1.4% (2)
Combination therapy [c]	10.8% (15)	9.4% (13)
No drug	0.7% (1)	0% (0)

** p=0.192 (comparison between the two TZD drugs.)

[a] Low dose was 7.5 or 15 mg/day for pioglitazone and 2 or 4 mg/day for rosiglitazone.

[b] High dose was > 15 mg/day for pioglitazone and > 4 mg/day for rosiglitazone.

[c] Oral antihyperglycemic drugs plus insulin.

4.1.3 Prevalence of Edema, Congestive Heart Failure (CHF), and Acute Coronary Syndrome (ACS)

Prevalence of edema was noted in 13.7% of patients treated with TZDs and the mean duration of TZDs used until edema was 8.09 ± 8.62 months. Pioglitazone-induced edema was 15.1% and rosiglitazone-induced edema was 12.2%. The percentage of edema was not significant difference between the two groups. The edema was severe enough that prompt withdrawal of pioglitazone was recorded in 12 of 21 patients (57.1%), and prompt withdrawal of rosiglitazone was recorded in 9 of 17 patients (52.9%). These percentages of prompt withdrawals were no significant difference (p=0.796).

CHF was noted 3.2% and the mean duration of TZDs used until CHF was diagnosed was 1.53 ± 1.04 years. The percentage of CHF occurred was not significant difference between pioglitazone and rosiglitazone groups. Prompted withdrawal of pioglitazone was performed in all 5 patients and in 2 of 4 patients in rosiglitazone group.

ACS was noted in 5.4%, while 8 of the 15 patients were pervious CAD. There was not significant difference between pioglitazone and rosiglitazone groups. The mean duration of TZDs used until diagnosed of ACS was 1.74 ± 1.49 years. These adverse events of the patient treated with TZDs were presented in Table 6.

Table 6 Prevalence of edema, congestive heart failure, and acute coronary syndrome during treatment with TZD

	Pioglitazone group (n=139)	Rosiglitazone group (n=139)	Total (N=278)	p-value
Edema	15.1% (21)	12.2% (17)	13.7% (38)	0.485
CHF	3.6% (5)	2.9% (4)	3.2% (9)	0.488
ACS	5.0% (7)	5.8% (8)	5.4% (15)	0.791

4.1.4 Comparisons between Edema and Non-Edema Groups

Among the 278 patients included into the study, edema was recorded in 38 patients as presented in Table 7. The edema group composed of 33 female (86.8%) and 5 male (13.2%), whereas non-edema group composed of 149 female (62.1%) and 91 male (37.9%). Female in edema group was significantly higher than in non-edema group (p=0.003). A risk of edema in female was significantly higher than in male (OR=4.73; 95% CI, 1.80 to 12.42). Moreover, there were significant difference between the edema and non-edema groups in age, duration of TZDs use, HbA_{1c}, and Cl_{Cr}. Age and HbA_{1c} in the edema group were significantly higher than in the non-edema group. In contrast, mean duration of TZDs use and Cl_{Cr} in the edema group were significantly lower than in the non-edema group.

Table 7 Baseline characteristics of patients in edema group and non-edema group (N=278)

Characteristics	Edema group (n=38)	Non-edema group (n=240)	p-value
Sex			0.003
- female	86.8% (33)	62.1% (149)	
- male	13.2% (5)	37.9% (91)	
Age (years)	61.97 ± 9.22	57.75 ± 9.94	0.015
Weight (kg)	66.81 ± 10.10	65.76 ± 11.68	0.610
BMI (kg/m^2)	27.29 ± 3.97	26.17 ± 4.10	0.183
Duration of DM (years)	10.58 ± 5.58	9.83 ± 5.57	0.444
Duration of TZD (years)	2.80 ± 1.76	4.14 ± 1.93	<0.001
FPG (mg/dl)	202.95 ± 55.78	196.55 ± 54.68	0.511
HbA_{1c} (%)	9.91 ± 1.66	9.30 ± 1.47	0.048
Cl_{Cr} (ml/mim)	60.56 ± 20.70	73.74 ± 23.26	0.006
TC (mg/dl)	203.27 ± 38.85	192.23 ± 40.15	0.192
LDL (mg/dl)	120.60 ± 33.18	111.32 ± 32.86	0.153
HDL (mg/dl)	45.42 ± 10.91	45.20 ± 12.10	0.934
TG (mg/dl)	193.14 ± 136.14	175.10 ± 104.33	0.415

Data are mean ± standard deviation, except for sex

The comparisons of the baseline characteristics of patient who treated with pioglitazone or rosiglitazone between edema and non-edema groups were showed in Table 8. In patients treated with pioglitazone, there were significant differences in age, duration of TZD used, and HbA_{1c} between edema and non-edema groups, while in patients treated with rosiglitazone, there were significant differences in sex, duration of TZD used, and Cl_{Cr} between edema and non-edema groups. A risk of edema in female who treated with rosiglitazone was significantly higher than in male who treated with rosiglitazone (OR=9.68; 95% CI, 1.24 to 75.47).

To check for possible confounding factors, we compared co-medications, and co-morbid diseases between edema and non-edema groups as shown in Table 9.

There were 26.3% of the patients in edema group and 9.6% in non-edema group who used a TZD combined with insulin for the treatment of diabetes. These was significant difference in the numbers of patients who used a TZD combined with insulin between the edema and non-edema groups (p=0.003). The risk of edema when the patients treated with a TZD combined with insulin was significantly higher than the risk of the patients treated without insulin (OR=3.37; 95% CI, 1.45 to 7.80). Similarly, these were 60.5% of the patients in edema group and 37.9% in non-edema group who used ACEI as a co-medication with a TZD (p=0.008). The patients who used ACEI with a TZD had higher risk of developing edema than the patients who used a TZD without ACEI (OR=2.51; 95% CI, 1.25 to 5.06). Other co-medications including diuretic were not significantly different between the edema and non-edema groups.

The percentages of co-morbid diseases were compared between edema and non-edema groups. We examined in terms of microvascular diseases and macrovascular diseases. Microvascular diseases were patients who had at least one of nephropathy, retinopathy, or neuropathy, while macrovascular diseases were patients who had at least one of CVD, stroke, or foot ulcer.

Table 8 Comparisons the baseline characteristics of patient treated with pioglitazone or rosiglitazone between edema and non-edema groups

Characteristics	Pioglitazone (N=139)			Rosiglitazone (N=139)		
	Edema (n=21)	Non-edema (n=118)	p-value	Edema (n=17)	Non-edema (n=122)	p-value
Sex			0.092			0.009
- female	81.0% (17)	61.9% (73)		94.1% (16)	62.3% (76)	
- male	19.0%(4)	38.1% (45)		5.9% (1)	37.7% (46)	
Age (years)	62.62 ± 9.33	57.53 ± 9.96	0.031	61.18 ± 9.29	57.96 ± 9.97	0.211
Weight (kg)	65.59 ± 9.04	66.58 ± 11.56	0.715	68.34 ± 11.39	64.96 ± 11.79	0.282
BMI (kg/m^2)	26.45 ± 2.73	26.20 ± 4.22	0.822	28.42 ± 5.11	26.13 ± 3.98	0.083
Duration of DM (yrs)	10.71 ± 6.43	9.60 ± 5.36	0.397	10.41 ± 4.49	10.06 ± 5.78	0.809
Duration of TZD (yrs)	2.96 ± 2.10	4.02 ± 1.99	0.027	2.612 ± 1.25	4.26 ± 1.87	< 0.001
FPG (mg/dl)	210.45 ± 58.90	193.78 ± 53.11	0.206	194.12 ± 52.24	199.12 ± 56.19	0.729
HbA_{1c} (%)	10.35 ± 1.76	9.28 ± 1.49	0.014	9.46 ± 1.48	9.33 ± 1.45	0.762
Cl_{Cr} (ml/mim)	63.39 ± 19.64	74.63 ± 24.02	0.091	57.30 ± 22.19	72.62 ± 22.39	0.027
TC (mg/dl)	202.31 ± 41.07	189.27 ± 36.38	0.204	204.80 ± 37.12	194.86 ± 43.25	0.487
LDL (mg/dl)	122.17± 36.83	107.20 ± 31.25	0.073	118.25 ± 28.21	115.57 ± 34.10	0.795
HDL (mg/dl)	47.31 ± 11.28	43.24 ± 13.12	0.255	41.63 ± 9.68	46.97 ± 10.88	0.185
TG (mg/dl)	215.22 ± 166.29	190.48 ± 93.17	0.549	157.00 ± 50.69	160.46 ± 112.57	0.920

Data are mean ± standard deviation, except for sex.

Microvascular diseases were noted in 83.3% of the patients in edema group, and 70.1% in non-edema group, which showed no significant difference (p=0.108). Macrovascular diseases were noted in 42.1% of the patients in edema group and 14.2% in non-edema group, which also showed significant difference (p<0.001). The risk of edema in the patients with macrovascular diseases was significantly higher than the risk of the patients without those diseases (OR=4.41; 95% CI, 2.10 to 9.22).

Table 9 Comparisons of co-medications and co-morbid diseases between edema and non-edema groups (N=278)

	Edema group (n=38)	Non-edema group (n=240)	p-value
Co-medications			
Sulfonylureas	86.8% (33)	91.7% (220)	0.334
Metformin	71.1% (27)	82.9% (199)	0.081
α-Glucosidase inhibitors	13.2% (5)	10.8% (26)	0.672
Insulin	26.3% (10)	9.6% (23)	0.003
Diuretics	28.9% (11)	26.3% (63)	0.727
ACEIs	60.5% (23)	37.9% (91)	0.008
ARBs	10.5% (4)	12.9% (31)	0.798
Beta-blockers	44.7% (17)	31.3% (75)	0.101
Nitrates	5.3% (2)	2.1% (5)	0.246
Alpha blockers	7.9% (3)	3.3% (8)	0.179
Ca channel blockers	36.8% (14)	22.5% (54)	0.056
Statins	86.8% (33)	75.0% (180)	0.109
Fibrates	23.7% (9)	13.8% (33)	0.112
Co-morbid diseases			
Microvascular diseases [a]	83.3% (30)	70.1% (115)	0.108
Macrovascular diseases	42.1% (16)	14.2% (34)	<0.001

[a] Total missing data of microvascular diseases was 28.1%(78)

The comparisons of co-medications and co-morbid diseases of patients who treated with pioglitazone or rosiglitazone between edema and non-edema groups were showed in Table 10. In patients treated with pioglitazone, there were significant differences in co-medication with statins and macrovascular diseases between edema and non-edema groups. The patients who used statins with a TZD had higher risk of developing edema than the patients who used a TZD without statins, but no significant difference (OR=7.44; 95%CI, 0.96 to 57.75). The risk of edema in the patients with macrovascular diseases was significantly higher than the risk of the patients without those diseases (OR=4.78; 95%CI, 1.74 to 13.16).

In patients treated with rosiglitazone, there were significant differences in insulin, ACEIs, and macrovascular diseases between edema and non-edema groups, and nearly significant difference in microvascular diseases between the two groups (p=0.053). The risk of edema when the patients treated with a TZD combined with insulin was significantly higher than the risk of the patients treated without insulin (OR=4.67; 95%CI, 1.37 to 15.93). The patients who used ACEIs with a TZD had higher risk of developing edema than the patients who used a TZD without ACEIs (OR=4.57; 95%CI, 1.51 to 13.85). The risk of edema in the patients with macrovascular diseases was significantly higher than the risk of the patients without those diseases (OR=4.04; 95%CI, 1.36 to 12.00).

Table 10 Comparisons of co-medications and co-morbid diseases of patients treated with pioglitazone or rosiglitazone between edema and non-edema groups

	Pioglitazone (N=139)			Rosiglitazone (N=139)		
	Edema (n=21)	Non-edema (n=118)	p-value	Edema (n=17)	Non-edema (n=122)	p-value
Co-medications						
Sulfonylureas	90.5% (19)	89.8% (106)	1.000	82.4% (14)	93.4% (114)	0.135
Metformin	76.2% (16)	82.2% (97)	0.546	64.7% (11)	83.6% (102)	0.091
α-Glucosidase inhibitors	14.3% (3)	9.3%(11)	0.445	11.8% (2)	12.3% (15)	1.000
Insulin	23.8% (5)	11.0% (13)	0.151	29.4% (5)	8.2% (10)	0.021
Diuretics	28.6% (6)	27.1% (32)	0.891	29.4% (5)	25.4% (31)	0.770
ACEIs	52.4% (11)	41.5% (49)	0.355	70.6% (12)	34.4% (42)	0.004
ARBs	14.3% (3)	13.6% (16)	1.000	5.9% (1)	12.3% (15)	0.693
Beta-blockers	42.9% (9)	33.9% (40)	0.429	47.1% (8)	28.7% (35)	0.125
Nitrates	0% (0)	0.8% (1)	1.000	11.8% (2)	3.3% (4)	0.157
Alpha blockers	4.8% (1)	2.5% (3)	0.485	11.8% (2)	4.1% (5)	0.205
CCBs	38.1% (8)	22.9% (27)	0.139	35.3% (6)	22.1% (27)	0.236
Statins	95.2% (20)	72.9% (86)	0.026	76.5% (13)	77.0% (94)	1.000
Fibrates	19.0% (4)	14.4% (17)	0.525	29.4% (5)	13.1% (16)	0.138
Co-morbid diseases						
Microvascular diseases [a]	80.0% (16)	77.4% (65)	1.000	87.5% (14)	62.5% (50)	0.053
Macrovascular diseases	42.9% (9)	13.6% (16)	0.003	41.2% (7)	14.8% (18)	0.015

[a] Total missing data of microvascular diseases was 25.2%(35) in pioglitazone group, and 30.9% (43) in rosiglitazone group

Comparing the percentages of edema in patients treated with pioglitazone and in patients treated with rosiglitazone, there was not significant difference (p=0.485) (Table 11). There was significant difference in the percentage of edema between the patients treated with low dose and the patients treated with high dose of TZDs (p=0.010). The risk of edema when the patients treated with high dose of TZD was significantly higher than the risk of the patients treated with low dose of TZD (OR=3.15; 95%CI, 1.53 to 6.48). When further categorized into subgroups of pioglitazone and rosiglitazone, significant difference in the percentage of edema was also found between patients treated with high and low doses of pioglitazone (p=0.010), but the difference in percentage of edema between patients treated with high and low doses of rosiglitazone were not significant difference (p=0.091). The risk of edema when the patients treated with high dose of pioglitazone was significantly higher than the risk of the patients treated with low dose of pioglitazone (OR=3.38; 95%CI, 1.29 to 8.86).

Table 11 Comparisons of different type and dose of thiazolidinediones between edema and non-edema groups

	Edema group (n=38)	Non-edema group (n=240)	p-value
Type of TZDs (N=278)			0.485
- pioglitazone (n=139)	15.1% (21)	84.9% (118)	
- rosiglitazone (n=139)	12.2% (17)	87.8% (122)	
Dose of TZDs (N=278)			0.001
- low dose [a] (n=217)	10.1% (22)	89.9% (195)	
- high dose [b] (n=61)	26.2% (16)	73.8% (45)	
Pioglitazone (N=139)			0.010
- low dose (n=104)	10.6% (11)	89.4% (93)	
- high dose (n=35)	28.6% (10)	71.4% (25)	
Rosiglitazone (N=139)			0.091
- low dose (n=113)	9.7% (11)	90.3% (102)	
- high dose (n=26)	23.1% (6)	76.9% (20)	

[a] Low dose was 7.5 or 15 mg/day for pioglitazone and 2 or 4 mg/day for rosiglitazone.

[b] High dose was > 15 mg/day for pioglitazone and > 4 mg/day for rosiglitazone.

4.1.5 Effects of Thiazolidinediones on Weight Gain, Glucose Controls, and Lipid Profiles

Measurements of weight, BMI, FPG, HbA_{1c}, TC, LDL, HDL, and TG during the first 6 months of TZD treatment were available for some patients as presented in Table 12 and Figure 3. Almost all variables mentions showed significant differences between the values at baseline and after TZDs has been used for 6 months, except for TC and HDL levels. Weight, BMI, and LDL values were significantly increased, opposite to FPG, HbA_{1c}, and TG values which were significantly decreased. The mean weight gain was 1.87 $\pm$ 2.37 kg, and the mean increasing of BMI was 0.77 $\pm$ 0.91 kg/m^2.

Table 12 Comparisons of weight, glucose controls, and lipid profiles in patients using thiazolidinediones between baseline and at month-6

	No. of patients	Baseline	month-6	P-value
Weight (kg)	265	65.61 $\pm$ 11.38	67.48 $\pm$ 11.80	<0.001
BMI (kg/m^2)	174	26.23 $\pm$ 4.03	27.01 $\pm$ 4.24	<0.001
FPG (mg/dl)	201	198.88 $\pm$ 57.78	150.01 $\pm$ 49.76	<0.001
HbA_{1c} (%)	131	9.30 $\pm$ 1.51	8.18 $\pm$ 1.38	<0.001
TC (mg/dl)	161	193.30 $\pm$ 37.59	189.78 $\pm$ 37.21	0.229
LDL (mg/dl)	177	112.51 $\pm$ 32.86	117.53 $\pm$ 33.72	0.040
HDL (mg/dl)	134	45.07 $\pm$ 12.19	45.15 $\pm$ 13.81	0.928
TG (mg/dl)	159	177.87 $\pm$ 98.05	149.24 $\pm$ 83.26	<0.001

Data are mean $\pm$ standard deviation.

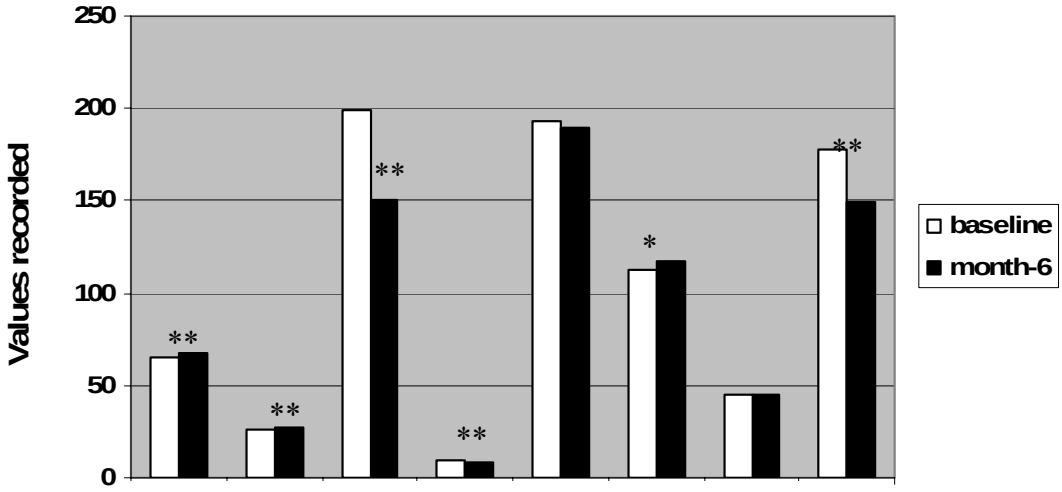

Figure 3 Comparisons of body weight (BW), BMI, FPG, HbA_{1c}, and lipid profiles between baseline and at month-6 after receiving TZD drugs.

* P<0.05 and ** P<0.001.

Comparisons of weight, BMI, FPG, HbA_{1c}, TC, LDL, HDL, and TG between at baseline and at month-6 of pioglitazone or rosiglitazone treatment were presented in Table 13 and 14, respectively. In pioglitazone treatment, almost all variables mentions showed significant differences between the values at baseline and after TZDs has been used for 6 months, except for TC and LDL levels. Weight, BMI, and HDL values were significantly increased, opposite to FPG, HbA_{1c}, and TG values which were significantly decreased.

In rosiglitazone treatment, almost all variables mentions also showed significant differences between the values at baseline and after TZDs has been used for 6 months, except for TC, LDL, and TG levels. Weight and BMI values were significantly increased, opposite to FPG, HbA_{1c}, and HDL values which were significantly decreased.

Table 13 Comparisons of weight, glucose controls, and lipid profiles in patients using pioglitazone between baseline and at month-6

	No. of patients	Baseline	month-6	P-value
Weight (kg)	130	65.87 ± 11.02	67.95 ± 11.30	<0.001
BMI (kg/m^2)	94	26.03 ± 3.88	26.85 ± 3.98	<0.001
FPG (mg/dl)	101	198.58 ± 55.13	151.41 ± 45.09	<0.001
HbA_{1c} (%)	76	9.29 ± 1.52	8.02 ± 1.33	<0.001
TC (mg/dl)	77	191.66 ± 38.06	186.21 ± 35.09	0.206
LDL (mg/dl)	89	107.46 ± 32.73	111.35 ± 30.09	0.254
HDL (mg/dl)	61	42.90 ± 12.96	45.74 ± 15.40	0.031
TG (mg/dl)	78	203.19 ± 111.30	152.90 ± 75.91	<0.001

Data are mean ± standard deviation.

Table 14 Comparisons of weight, glucose controls, and lipid profiles in patients using rosiglitazone between baseline and at month-6

	No. of patients	Baseline	month-6	P-value
Weight (kg)	135	65.36 ± 11.75	67.03 ± 12.29	<0.001
BMI (kg/m^2)	80	26.47 ± 4.22	27.20 ± 4.55	<0.001
FPG (mg/dl)	100	199.18 ± 60.62	148.61 ± 54.26	<0.001
HbA_{1c} (%)	56	9.29 ± 1.51	8.39 ± 1.43	<0.001
TC (mg/dl)	84	194.81 ± 37.32	193.06 ± 38.96	0.662
LDL (mg/dl)	88	117.61 ± 32.38	123.77 ± 36.14	0.080
HDL (mg/dl)	73	46.89 ± 11.27	44.66 ± 12.41	0.028
TG (mg/dl)	81	153.49 ± 76.40	145.72 ± 90.11	0.347

Data are mean ± standard deviation.

Among the 95 patients whose BW had been recorded at each visit, those were 21 patients who were found that their weight gain were higher than 2 kg per month after receiving TZDs (excluding the edema case). Eleven patients were in the pioglitazone group while ten patients were in the rosiglitazone group.

Moreover, comparisons of the changes in values during 6 months between pioglitazone and rosiglitazone groups were revealed. They showed that all variables, except for TG and HDL, were not significantly differences (Table 15). The decrease in TG levels was significantly stronger in pioglitazone group than in rosiglitazone group (p=0.003). The HDL levels was increased in pioglitazone group, but was decreased in rosiglitazone group, therefore, the change in the HDL level turned out to be significances different (p=0.002). The range of weight gain was -0.40 to +4.56 kg in pioglitazone group (n=130), and –0.58 to +3.92 kg for rosiglitazone group (n=135).

Table 15 Comparisons of the changes in weight, glucose controls, and lipid profiles between pioglitazone and rosiglitazone groups

	No. of patients (Pio/Rosi)	Pioglitazone group	Rosiglitazone group	P-value
Weight (kg)	130/135	2.08 ± 2.48	1.67 ± 2.25	0.162
BMI (kg/m^2)	94/80	0.82 ± 0.86	0.72 ± 0.99	0.510
FPG (mg/dl)	101/100	-47.18 ± 59.42	-50.57 ± 66.52	0.703
HbA_{1c} (%)	75/56	-1.28 ± 1.46	-0.90 ± 1.38	0.137
TC (mg/dl)	77/84	-5.45 ± 37.54	-1.75 ± 36.61	0.527
LDL (mg/dl)	89/88	3.89 ± 31.95	6.16 ± 32.59	0.640
HDL (mg/dl)	61/73	2.84 ± 10.03	-2.23 ± 8.52	0.002
TG (mg/dl)	78/81	-50.29 ± 104.37	-7.78 ± 74.03	0.003

Data are mean ± standard deviation.

4.1.6 Association between General Demographic Factors and Edema

We examined factors that affected edema conditions by using logistic regression analysis. Logistic regression analysis was used to estimate odds ratio and significance of each factor which were showed in Table 16.1. The factors included sex, age, duration of DM, macrovascular diseases, co-medication with insulin, co-medication with ACEIs, and low or high dose of TZDs used. There were 6 factors which were significantly included into the equation. The prediction equation for probability of odds of edema conditions in patients treated with TZD was as following;

Log (odds of edema) = 51.71 + 0.96**A** + 6.76**B** + 5.87**C** + 3.90**D** + 5.17**E**+ 3.31**F**

A = age (years)

B = sex (male = 0, female = 1)

C = macrovascular diseases (no = 0, yes = 1)

D = co-medication with ACEIs (no = 0, yes = 1)

E = low or high dose of TZDs used (low = 0, high = 1)

F = co-medication with insulin (no = 0, yes = 1)

This equation can accurately predict the edema conditions for 34.6% of patients treated with TZDs.

In pioglitazone group, there were 3 factors included into the equation which were sex, macrovascular diseases, and low or high dose of pioglitazone used, and the prediction equation of probability of odds of edema conditions in patients treated with pioglitazone was as following;

Log (odds of edema) = 4.22 + 4.49**B** + 5.18**C** + 4.24**E**

B = sex (male = 0, female = 1)

C = macrovascular diseases (no = 0, yes = 1)

E = low or high dose of TZDs used (low = 0, high = 1)

This equation can accurately predict the edema conditions for 22.8% of patients treated with pioglitazone.

In rosiglitazone group, the 4 factors included into the equation which were sex, macrovascular diseases, co-medication with ACEIs, and low or high dose of rosiglitazone used and the prediction equation of probability of odds of edema conditions in patients treated with rosiglitazone was as following;

Log (odds of edema) = 7.53 + 19.04**B** + 8.31**C** + 5.58**D** + 6.31**E**

B = sex (male = 0, female = 1)

C = macrovascular diseases (no = 0, yes = 1)

D = co-medication with ACEIs (no = 0, yes = 1)

E = low or high dose of TZDs used (low = 0, high = 1)

This equation can accurately predict the edema conditions for 37.2% of patients treated with rosiglitazone.

When we compared odds ratio of each risk factor of edema between unadjusted and adjusted covariates, the OR of adjusted covariates were higher in all risk factors than the OR of unadjusted covariates, as shown in Table 16.2.

Table 16.1 Multivariate analysis of the general demographic factors associations with edema

Factors	Odds ratio of Pioglitazone (95% CI) (n=139)	p-value	Odds ratio of Rosiglitazone (95% CI) (n=139)	p-value	Odds ratio of total (95% CI) (N=278)	p-value
Age	-	-	-	-	0.96 (0.92 - 1.00)	0.031
Sex (male=0 vs. female=1)	4.49 (1.22 - 16.59)	0.024	19.04 (1.85 - 195.88)	0.013	6.76 (2.21 - 20.72)	0.001
Macrovascular diseases (no=0 vs. yes=1)	5.18 (1.73 - 15.52)	0.003	8.31 (2.07 - 33.35)	0.003	5.87 (2.46 - 14.00)	< 0.001
ACEIs use (no=0 vs. yes=1)	-	-	5.58 (1.61 - 19.37)	0.007	3.90 (1.68 - 9.03)	0.002
Dose of TZDs use (low=0 vs. high=1)	4.24 (1.44 - 12.49)	0.009	6.31 (1.54 - 25.83)	0.010	5.17 (2.17 - 12.31)	< 0.001
Insulin use (no=0 vs. yes=1)	-	-	-	-	3.31 (1.25 - 8.76)	0.016

Table 16.2 Comparisons of odds ratio of the general demographic factors associations with edema between unadjusted and adjusted covariates

Factors	Pioglitazone (n=139)		Rosiglitazone (n=139)		Total (N=278)	
	Unadjusted OR	Adjusted OR	Unadjusted OR	Adjusted OR	Unadjusted OR	Adjusted OR
Age	-	-	-	-	-	0.96
Sex (male=0 vs. female=1)	-	4.49	9.68	19.04	4.73	6.76
Macrovascular diseases (no=0 vs. yes=1)	4.78	5.18	4.04	8.31	4.41	5.87
ACEIs use (no=0 vs. yes=1)	-	-	4.57	5.58	2.51	3.90
Dose of TZDs use (low=0 vs. high=1)	3.38	4.24	-	6.31	3.15	5.17
Insulin use (no=0 vs. yes=1)	-	-	4.67	-	3.37	3.31

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4.1.7 Cessation of Treatment

Of the 278 patients treated with TZDs, 20.9% was withdrawn from the treatment (30 of the 139 patients who were taking pioglitazone and 28 of the 139 patients who were taking rosiglitazone). The reasons for ceasing the treatment with TZDs in these patients were due to disagreement to continue after receiving the information that TZDs may induce MI (15), peripheral edema (14), non-response (9), CHF (6), weight gain (3), liver dysfunction (2), CRF (2), ACS (1), and other reasons (6) which including anemia (1), CA (1), too expensive (2), and unclear reason (2). The reasons for withdrawal and the changes of TZD type are shown in Table 17. The withdrawal of TZD due to edema was 5.0% and from CHF was 2.2%. The most common reason to switch from rosiglitazone to pioglitazone during April 2007 was the awareness of the risk of MI from TZDs (29).

Table 17 Reasons for withdrawal from TZD or change to different type of TZD

Reasons	Withdrawal of TZD	Change from rosiglitazone to pioglitazone	Change from pioglitazone to rosiglitazone
Awareness of TZD-induced MI	5.4% (15)	20.9% (29)	0
Edema	5.0% (14)	0	2.2% (3)
Non-response	3.2% (9)	0	2.2% (3)
CHF	2.2% (6)	2.2% (3)	0
Weight gain	1.1% (3)	0.7% (1)	0
Liver dysfunction	0.7% (2)	0.7% (1)	0.7% (1)
CRF	0.7% (2)	0	0
ACS	0.4% (1)	0	0
Others	2.2% (6)	0.7% (1)	0.7% (1)
Total	**20.9% (58)**	**25.2% (35)**	**5.8% (8)**

4.2 Comparisons of Pharmacokinetic Parameters between Edema and Non-Edema Type 2 Diabetic Patients Treated with Pioglitazone

4.2.1 Validation of HPLC-UV of Pioglitazone in Plasma

The calibration curve was linear over the pioglitazone concentration range 20 to 3000 ng/ml in human plasma. Table 18 summarized the accuracy and precision of the calibration curve. Lower limit of quantification (LLOQ) of pioglitazone in plasma was verified as 20 ng/ml, as this was the lowest concentration assessed at which the accuracy was between 80 and 120%, and precision was within 20%.

Table 18 Accuracy and precision of calibration standards of the method for determining the concentration of pioglitazone in plasma samples (n=10)

Known concentration (ng/ml)	Concentration found (Mean ± S.D.; ng/ml)	Accuracy (%RD)	Precision (%CV)
3000	3021.55 ± 27.44	0.7	0.9
1500	1506.01 ± 34.42	0.4	2.3
800	807.78 ± 17.55	1.0	2.2
300	296.79 ± 7.65	-1.1	2.6
150	153.15 ± 6.11	2.1	4.0
50	52.75 ± 5.05	5.5	9.6
20	20.55 ± 1.54	2.7	7.5

Table 19 shows the individual calibration equations of pioglitazone from 10 replicate experiments. The equation of the curve, obtained by a least-squares method, was $y = 0.0015x - 0.0633$ (where y is the peak area ratio of the analyte to internal standard and x is the concentration of the analyte). The correlation coefficient (r^2) of the calibration curve generated during the validation was 0.9998 for the analyte.

Table 19 Linearity obtained after least-squares regression analysis of the method for determining pioglitazone in plasma samples

Calibration curve	Slope	Intercept	R^2
1	0.0015	0.0485	0.9999
2	0.0015	0.0399	0.9999
3	0.0015	0.0556	0.9995
4	0.0015	0.0782	0.9992
5	0.0015	0.0727	1.0000
6	0.0015	0.0597	0.9999
7	0.0015	0.0468	0.9999
8	0.0014	0.0717	0.9996
9	0.0014	0.0860	0.9994
10	0.0014	0.0371	0.9998
Mean	**0.0015**	**0.0633**	**0.9998**

The results for accuracy and precision at concentrations of 20–3000 ng/ml for pioglitazone are presented in Table 20. The intra-day accuracy and precision varied between 1.92 and 6.80%, and between 1.45 and 7.95%, respectively. The inter-day accuracy and precision ranged from 0.37 to 2.89% and from 2.91 to 5.18, respectively. For acceptable intra-day and inter-day values, accuracy, presented in relative standard deviation (RD), and coefficient of variation (CV) values should be <15% over the calibration range, except at the LLOQ, where accuracy should be between 80 and 120% and CV should not exceed 20%.

The recovery of pioglitazone in the solid phase extraction (SPE) procedure from 1ml of plasma was measured at 7 different concentrations over the calibration range used. Table 21 shows the absolute recovery, expressed as a percentage, obtained for both pioglitazone and internal standard of rosiglitazone. The recoveries ranged from 96.6 to 106.3% with a CV between 2.3 and 7.1%. A recovery of 98.8% was obtained for the internal standard.

Table 20 Accuracy and precision of the method for determining the concentration of pioglitazone in plasma samples (n=5)

Known concentration (ng/ml)	Concentration found (Mean ± S.D.; ng/ml)	Accuracy (%RD)	Precision (%CV)
Intra-day			
75	77.59 ± 6.17	3.45	7.95
1000	1067.96 ± 34.50	6.80	3.23
2400	2445.97 ± 35.54	1.92	1.45
Inter-day of 3-day			
75	76.84 ± 3.99	2.45	5.19
1000	1028.85 ± 29.99	2.89	2.91
2400	2408.86 ± 70.89	0.37	2.94

Table 21 Absolute recovery of the method for determining the concentration of pioglitazone in plasma samples (n=5)

Concentration	Absolute recovery (mean ± S.D.; %)	Precision %CV
Pioglitazone (ng/ml)		
20	101.4 ± 6.5	6.4
50	101.0 ± 2.3	2.3
150	96.6 ± 6.8	7.1
300	106.3 ± 2.8	2.6
800	103.8 ± 5.8	5.6
1500	101.8 ± 6.5	6.4
3000	100.4 ± 4.8	4.8
Rosiglitazone (μg/ml)		
50	98.8 + 5.7	5.7

Analyte stability in plasma was tested using low concentration of quality control sample (low QCs) and high concentration of quality control (high QCs) for 3 freeze–thaws, long-term, short-term and post-preparative stabilities. The freeze–thaw stability of the analyte was determined over 3 freeze–thaw cycles within 3 days. In each freeze–thaw cycle, the spiked plasma samples were frozen for 24 hours at -80 ˚C and thawed at room temperature. The long-term stability was evaluated after keeping the plasma samples frozen at -80 ˚C for 2 and 6 months.

For the short-term stability, frozen plasma samples were kept at room temperature for 6 and 24 hours before sample preparation. The stability of the prepared plasma samples was tested after keeping the samples at room temperature for 6 and 24 hours. The samples were analyzed and the results were compared with those obtained for freshly prepared samples. For the acceptance criterion of stability, the deviation compared to the freshly prepared standard should be within $\pm$15%.

Plasma samples of pioglitazone of 2 concentrations (75 and 2400 ng/ml) were used for stability experiments. Stability was assessed under a variety of conditions and the maximum period of confirmed stability is presented in Table 22. The deviation of the mean test responses were within $\pm$15% of appropriate controls in all stability tests of pioglitazone in human plasma.

Table 22 Stability of pioglitazone in human plasma

Stability (n = 3)	Concentration (mean ± S.D.; ng/ml)	
	Low QCs 75	High QCs 2400
Freeze–thaw stability		
Initial	70.14 ± 1.99	2419.84 ± 105.48
Measured	69.75 ± 6.64	2326.87 ± 137.62
Deviation (%)	-0.56	-4.00
Long-term stability		
Initial	70.14 ± 1.99	2419.84 ± 105.48
Measured at m-2	70.54 ± 13.71	2463.61 ± 42.69
Measured at m-6	70.79 ± 6.43	2411.03 ± 89.89
Deviation (%)	+0.91	-0.37
Short-term stability		
Initial	75.86 ± 10.37	2574.61 ± 104.19
Measured at h-6	74.40 ± 13.50	2500.71 ± 81.30
Measured at h-24	72.70 ± 3.17	2437.20 ± 111.11
Deviation (%)	-4.34	-5.64
Post-preparative stability		
Initial	70.14 ± 1.99	2419.84 ± 105.48
Measured at h-6	71.19 ± 8.78	2432.26 ± 92.22
Measured at h-24	74.77 ± 4.58	2490.10 ± 22.19
Deviation (%)	+6.19	+2.82

The quality controls of stability in pioglitazone and rosiglitazone stock solution were examined. The stock solutions 50 μg/ml were kept at -4 °C in refrigerator for 8 weeks and were evaluated at 2, 4, and 8 weeks that showed in Table 23. For the acceptance criterion of stability, and precision in the stability of stock solution should be within ±15%.

Table 23 Stock stability of standard solutions

	Peak area (N=3)	Stability (%RD)	Precision (%CV)
Pioglitazone solution			
50 μg/ml			
- at week-0	93.80	na.	5.38
- at week-2	93.22	+0.25	2.66
- at week-4	92.36	-0.66	1.31
- at week-8	88.88	-2.22	2.63
Rosiglitazone solution			
50 μg/ml			
- at week-0	19.08	na.	5.63
- at week-2	19.15	-4.56	3.63
- at week-4	19.52	-2.71	7.22
- at week-8	19.26	+3.29	1.33

4.2.2 Characteristics of Patients Enrolled for Pharmacokinetic Study

Twenty five patients treated with pioglitazone were recruited for pharmacokinetic study. Of these, 6 patients had edema and 19 patients had no report of edema. Table 24 shows baseline characteristics of the patients in edema and non-edema groups. There were no significant differences between the two groups in general baseline characteristics, except for sex which cannot be compared between the two groups because of the low number of subjects in edema group.

Table 24 Baseline characteristics of the patients recruited for pharmacokinetic study

Characteristics	Edema group (n=6)	Non-edema group (n=19)	Total (N=25)	p-value
Sex				1.000
- female	100% (6)	89.5% (17)	92.0% (23)	
- male	0% (0)	10.5% (2)	8.0% (2)	
Age (years)	59.33 ± 5.00	56.11 ± 9.20	56.00 ± 8.4	0.424
Weight (kg)	72.52 + 11.61	68.53 + 10.94	69.49 + 11.00	0.451
BMI (kg/m^2)	29.80 + 4.54	27.41 + 2.53	27.99 + 3.19	0.112
Duration of DM (years)	12.83 ± 5.88	9.21 ± 3.05	10.08 ± 4.08	0.560
Duration of TZD (years)	4.53 ± 1.69	4.92 ± 1.60	4.82 ± 1.60	0.619
FPG (mg/dl)	235.50 ± 40.82	193.39 ± 51.39	203.92 ± 51.58	0.830
HbA_{1c} (%)	8.40±1.00	8.49±1.17	8.47+1.11	0.860
Cl_{Cr} (ml/min)	58.51 + 17.12	69.69 + 23.24	67.01 + 22.14	0.290
TC (mg/dl)	232.50 ± 34.38	199.09 ± 34.53	210.88 ± 37.22	0.076
LDL (mg/dl)	133.33 ± 37.36	120.17 ± 28.28	124.56 ± 31.12	0.414
HDL (mg/dl)	51.67 ± 6.82	44.55 ± 9.92	47.06 ± 9.40	0.140
TG (mg/dl)	220.00 ± 163.15	196.00 ± 95.64	204.00 ± 117.83	0.696

Data are mean ± standard deviation except sex.

Co-morbid diseases and co-medication of the 25 patients were shown in Table 25. There were not significant differences in patients with co-morbid diseases and using co-medications between edema and non-edema groups. However, the percentages of patients with co-morbid diseases of hypertension, diabetic nephropathy, and microvascular diseases were higher in the edema group than the non-edema group for nearly significant differences.

Table 25 Co-morbid diseases and co-medications used of patients recruited for pharmacokinetic study

	Edema group (n=6)	Non-Edema group (n=19)	Total (N=25)	P-value
Co-morbid diseases				
CAD	0% (0)	5.3% (1)	4.0% (1)	1.000
Stroke	0% (0)	10.5% (2)	8.0% (2)	1.000
Hypertension	100.0% (6)	52.6% (10)	64.0% (16)	0.057
Dyslipidemia	83.3% (5)	68.4% (13)	72.0% (18)	0.637
Diabetic nephropathy	83.3% (5)	31.6% (6)	44.0% (11)	0.056
- Macroproteinuria	50.0% (3)	26.3% (5)	32.0% (8)	
- Microproteinuria	33.3% (2)	5.3% (1)	12.0% (3)	
Diabetic retinopathy [a]	66.7% (4)	43.8% (7)	50.0% (11)	0.635
Diabetic neuropathy [b]	100.0% (4)	66.7% (4)	80.0% (8)	0.467
Microvascular diseases	100.0% (6)	52.6% (10)	64.0% (16)	0.057
Macrovascular diseases	33.3% (2)	15.8% (3)	20.0% (5)	0.562
Pioglitazone				0.344
- Low dose of pioglitazone	50.0% (3)	26.3% (5)	32.0% (8)	
- High dose of pioglitazone	50.0% (3)	73.7% (14)	68.0% (17)	
Co-medications				
Sulfonylureas	100.0% (6)	94.7% (18)	96.0% (24)	1.000
Metformin	100.0% (6)	63.2% (12)	72.0% (18)	0.137
Insulin	0% (0)	36.8% (7)	28.0% (7)	0.137
Diuretics	50.0% (3)	15.8% (3)	24.0% (6)	0.125
ACEIs	66.7% (4)	42.1% (8)	48.0% (12)	0.378
ARBs	16.7% (1)	0% (0)	4.0% (1)	0.240
Beta-blockers	50.0% (3)	15.8% (3)	24.0% (6)	0.125
Nitrates	0% (0)	5.3% (1)	4.0% (1)	1.000
Alpha blockers	16.7% (1)	0% (0)	4.0% (1)	0.240
Ca channel blockers	33.3% (2)	15.8% (3)	20.0% (5)	0.562
Statins	83.3% (5)	47.4% (9)	56.0% (14)	0.180
Fibrates	16.7% (1)	5.3% (1)	8.0% (2)	0.430

[a] Total missing data of diabetic retinopathy was 12%(3)

[b] Total missing data of diabetic neuropathy was 60%(15)

4.2.3 Comparisons of Pharmacokinetic Parameters between Edema and Non-Edema Groups

Two plasma samples of each patient were analyzed for their pioglitazone concentrations by HPLC-UV method. These pioglitazone concentrations were used for the calculation of the pharmacokinetic parameters, i.e., K_e, $t_{1/2}$, Vd, and CL. The pharmacokinetic parameters and characteristics of individual patients treated with pioglitazone were presented in Table 26. When the pharmacokinetic parameters were compared between edema and non-edema groups, they were not significant differences as shown in Table 27.

Table 26 Pharmacokinetic parameters and characteristics of individual patients treated with pioglitazone (N=25)

Subject no.	Weight (kg)	BMI (kg/m^2)	Cl_{Cr} (ml/min)	HbA_{1c} (%)	Dose/day (mg)	C_{ssmax} (ng/ml)	C_{ssmin} (ng/ml)	Ke (hr^{-1})	$t_{1/2}$ (hr)	Vd (L)	Vd (L/kg)	CL (L/hr)
Non-edema group (n=19)												
1	66.0	26.4	122.0	9.0	60	1116	447	0.1017	6.82	56.23	0.85	5.72
2	44.8	28.2	52.2	9.8	30	1988	1381	0.0567	12.21	22.26	0.50	1.26
3	71.2	27.8	76.8	8.0	30	1295	162	0.1022	6.78	17.41	0.24	1.78
4	76.1	26.2	41.7	8.2	30	2792	868	0.0607	11.42	10.50	0.14	0.64
5	59.5	24.0	75.5	10.9	30	801	231	0.0624	11.10	37.39	0.63	2.33
6	80.3	31.8	88.6	6.3	30	2073	60	0.1946	3.56	4.72	0.06	0.92
7	73.0	30.4	53.0	7.1	30	786	156	0.0729	9.50	40.42	0.55	2.95
8	70.4	27.9	73.1	7.9	30	728	144	0.0977	7.09	22.07	0.31	2.16
9	51.6	22.9	45.7	8.1	15	1128	613	0.0350	19.80	18.58	0.36	0.65
10	87.4	29.2	95.5	8.4	30	2017	343	0.1042	6.65	7.81	0.09	0.81
11	55.8	25.0	45.9	8.5	15	1049	505	0.0372	18.65	20.63	0.37	0.77
12	59.7	25.0	60.8	8.2	30	865	301	0.0546	12.69	36.80	0.62	2.01
13	72.0	29.2	58.6	8.1	15	832	379	0.0357	19.39	29.15	0.40	1.04
14	91.5	26.4	122	9.0	30	1565	120	0.1228	5.65	13.86	0.15	1.70

Table 26 Pharmacokinetic parameters and characteristics of individual patients treated with pioglitazone (N=25) (continued)

Subject no.	weight (kg)	BMI (kg/m^2)	Cl_{Cr} (ml/min)	HbA_{1c} (%)	Dose/day (mg)	Cssmax (ng/ml)	Cssmin (ng/ml)	Ke (hr^{-1})	$t_{1/2}$ (hr)	Vd (L)	Vd (L/kg)	CL (L/hr)
Non-edema group (n=19) (continued)												
15	68.2	30.8	113	10.9	30	1830	160	0.1455	4.76	5.89	0.09	0.86
16	74.9	28.4	84.4	8.8	30	1305	123	0.1248	5.55	12.83	0.17	1.60
17	53.0	29.1	53.9	9.4	15	1982	1254	0.0275	25.18	12.78	0.24	0.35
18	65.0	23.9	76.9	8.6	15	930	570	0.0278	24.96	27.78	0.43	0.77
19	57.5	29.3	64.3	7.0	30	2028	191	0.1283	5.40	7.58	0.13	0.97
Edema groups (n=6)												
20	80.4	31.6	65.3	7.6	15	881	138	0.0890	7.78	14.54	0.18	1.29
21	82.4	33.4	45.8	8.6	45	997	338	0.0595	11.64	41.95	0.51	2.50
22	80.5	34.2	49.5	7.9	15	736	132	0.0968	7.16	12.34	0.15	1.19
23	65.1	27.5	88.7	7.2	30	1730	30	0.2306	3.00	3.96	0.06	0.91
24	74.0	30.2	42.2	9.6	30	687	313	0.0431	16.09	52.90	0.71	2.28
25	52.7	21.9	59.6	9.5	7.5	451	147	0.0554	12.52	18.38	0.35	1.02

Table 27 Comparisons of pharmacokinetic parameters between edema and non-edema groups

PK parameters	Edema group (n=6)	Non-edema group (n=19)	Total (N=25)	p-value
K_e (hr^{-1})	0.10±0.07	0.08±0.05	0.09±0.05	0.629
$t_{1/2}$ (hr)	9.70±4.64	11.43±6.91	10.70±5.98	0.574
Vd (L)	24.01±19.06	21.30±13.77	21.26±15.11	0.704
Vd/F (L/kg)	0.33±0.25	0.33±0.22	0.32±0.23	0.952
CL (L/hr)	1.53±0.68	1.54±1.23	1.51±1.13	0.985

Data are mean ± standard deviation.

4.2.4 Comparisons of Pharmacokinetic Parameters between Patients Treated with Low and High Doses of Pioglitazone

We compared pharmacokinetic parameters, Cl_{Cr}, HbA_{1c}, and BMI between patients treated with low and high doses of pioglitazone. The Ke, $t_{1/2}$, and CL of pioglitazone were significant differences between the two groups as shown in Table 28.

Table 28 Comparisons of pharmacokinetic parameters and patient characteristics between low dose (≤15 mg/d) and high dose (>15 mg/d) of pioglitazone

PK parameters / Characteristics	Low dose group (n=8)	High dose group (n=17)	p-value
Ke (hr^{-1})	0.05 ± 0.03	0.10 ± 0.05	0.012
$t_{1/2}$ (hr)	16.93 ± 7.06	8.23 ± 3.71	0.010
Vd (L)	19.27 ± 6.39	23.21 ± 14.48	0.421
Vd/F (L/kg)	0.31 ± 0.11	0.34 ± 0.26	0.670
CL (L/hr)	0.89 ± 0.31	1.85 ± 1.22	0.039
Cl_{Cr} (ml/min)	58.21 ± 10.86	71.15 ± 25.03	0.085
HbA_{1c} (%)	8.16 ± 0.74	8.62 ± 1.24	0.351
BMI (kg/m^2)	27.25 ± 4.44	28.33 ± 2.50	0.534

Data are mean ± standard deviation.

When we observed the pharmacokinetic parameters in individual patients (Table 26), the patients of case number 9, 11, 13, 17, and 18 had longer half-life of pioglitazone than the patients of case number 20 and 22, although they were received the same 15 mg dose of pioglitazone per day.

Furthermore, when the edema cases were excluded and we compared the pharmacokinetic parameters between the patients received pioglitazone of 15 and 30 mg per day, we found significantly differences in the Ke, $t_{1/2}$, and CL as shown in Table 29. The patients received 15 mg of pioglitazone had higher levels of the $t_{1/2}$ than the patients received 30 mg of pioglitazone. In contrast, the patients received 30 mg of pioglitazone had higher levels of the Ke and CL than the patients received 15 mg of pioglitazone, while the HbA_{1c} between the both groups were not significant difference. Interestingly, some patients received the same dose of pioglitazone, but had long half-life than the others. This difference might be related to CYP2C8 variant that affected CL and half-life of pioglitazone.

Table 29 Comparisons pharmacokinetic parameters between patients treated with 15 mg and 30 mg of pioglitazone (the edema cases were excluded)

PK parameters / Characteristics	Pioglitazone 15 mg/d (n=5)	Pioglitazone 30 mg/d (n=13)	p-value
C_{max} (ng/ml)	1184.20 ± 460.05	1544.08 ± 642.09	0.273
C_{min} (ng/ml)	664.02 ± 341.34	326.15 ± 377.03	0.100
Ke (hr^{-1})	0.03 ± 0.01	0.10 ± 0.04	<0.001
$t_{1/2}$ (hr)	21.60 ± 3.20	7.9 ± 3.1	<0.001
Vd (L)	21.78 ± 6.76	18.43 ± 12.58	0.584
Vd/F (L/kg)	0.36 ± 0.07	0.28 ± 0.21	0.273
CL (L/hr)	0.72 ± 0.25	1.53 ± 0.70	0.002
Cl_{Cr} (ml/min)	58.27 + 13.20	70.06 + 21.68	0.277
HbA_{1c} (%)	8.06 + 0.63	8.62 + 1.34	0.388
BMI (kg/m^2)	26.05 + 3.00	28.01 + 2.32	0.157

Data are mean ± standard deviation.

4.3 Determining the Association of SNP rs5370 of *ENDO1* and SNP rs34241435 of *SCNN1B* and TZDs-Induced Edema Status

4.3.1 Characteristics of Patients Recruited for Pharmacogenetic Study

The total number of patients included into pharmacogenetic study was 134. Of these, 23 patients had edema and 111 patients did not have edema.

Table 30 shows the characteristics of patients with edema and no edema. There were significant differences between edema and non-edema groups in age, Cl_{Cr}, TC, and LDL. The mean age, TC and LDL in the edema group were significantly higher than the non-edema group, while Cl_{Cr} in the edema group was significantly lower than non-edema group.

Table 30 Characteristics of patient recruited into pharmacogenetic study

Characteristics	Edema group (n=23)	Non-edema group (n=111)	Total (N=134)	p-value
Sex				0.083
- female	82.6% (19)	64.0% (71)	67.2% (90)	
- male	17.4% (4)	36.0% (40)	32.8% (44)	
Age (years)	62.39 ± 8.53	56.61 ± 9.94	57.61 ± 9.93	0.011
Weight (kg)	68.52 ± 12.09	68.02 ± 12.66	68.11 ± 12.52	0.860
BMI (kg/m^2)	28.35 ± 4.72	26.90 ± 4.44	27.17 ± 4.51	0.165
Duration of DM (years)	10.57 ± 5.88	9.58 ± 5.18	9.75 ± 5.30	0.421
Duration of TZD (years)	2.90 ± 1.81	3.69 ± 1.82	3.55 ± 1.84	0.061
FPG (mg/dl)	210.00 ± 57.51	190.55 ± 48.40	194.03 ± 50.45	0.102
HbA_{1c} (%)	9.94 ± 1.87	9.11 ± 1.25	9.24 ± 1.40	0.097
Cl_{Cr} (ml/mim)	56.82 ± 18.32	75.87 ± 24.05	72.29 ± 24.20	0.001
TC (mg/dl)	211.67 ± 39.09	182.15 ± 38.83	187.99 ± 40.43	0.005
LDL (mg/dl)	127.58 ± 33.58	108.19 ± 32.67	111.57 ± 33.49	0.021
HDL (mg/dl)	43.94 ± 8.63	42.10 ± 11.22	42.46 ± 10.74	0.529
TG (mg/dl)	219.37 ± 160.13	176.35 ± 84.37	185.04 ± 104.54	0.271

Data are mean ± standard deviation, except for sex

Co-morbid diseases and co-medications of the patients were shown in Table 31 and 32, respectively. The patients with co-morbid diseases of hypertension, nephropathy, and foot ulcer were significant differences between the edema and non-edema groups. The edema group had significantly higher percentage of hypertension, nephropathy, and foot ulcer than the non-edema group. The patients using co-medications were not significant differences between the edema and the non-edema groups

Table 31 Co-morbid diseases of patients recruited for pharmacogenetic study

Co-morbid diseases	Edema group (n=23)	Non-edema group (n=111)	Total (N=134)	p-value
Coronary artery disease	18.2% (4)	5.4% (6)	7.5% (10)	0.061
Stroke	8.7% (2)	4.5% (5)	5.2% (7)	0.344
Hypertension	91.3% (21)	64.0% (71)	68.7% (92)	0.010
Dyslipidemia	95.7% (22)	82.0 % (91)	84.3% (113)	0.124
Diabetic nephropathy [a]	59.1% (13)	28.6% (28)	34.2% (41)	0.006
- Macroproteinuria	31.8% (7)	19.4% (19)	19.4% (26)	
- Microproteinuria	27.3% (6)	9.2% (9)	11.2% (15)	
Diabetic retinopathy [b]	61.1% (11)	36.4%(16)	43.5% (27)	0.074
Diabetic neuropathy [c]	88.9% (8)	61.5% (16)	68.6% (24)	0.217
Foot ulcer	17.4% (4)	0% (0)	3.0% (4)	0.001

[a] Total missing data of diabetic nephropathy was 10.4%(14)

[b] Total missing data of diabetic retinopathy was 53.7%(72)

[c] Total missing data of diabetic neuropathy was 73.9%(99)

Table 32 Co-medications of patients recruited for pharmacogenetic study

Medications	Edema group (n=23)	Non-edema group (n=111)	Total (N=134)	p-value
Sulfonylureas	87.0% (20)	93.4% (99)	92.2% (119)	0.383
Metformin	65.2% (15)	84% (89)	80.6% (104)	0.076
α-Glucosidase inhibitors	8.7% (2)	6.6% (7)	7.0% (9)	0.662
Insulin	17.4% (4)	8.1% (9)	9.7% (13)	0.247
Diuretics	30.4% (7)	27.0% (30)	27.6% (37)	0.739
ACEIs	60.9% (14)	60.9% (14)	47.0% (63)	0.144
ARBs	17.4% (4)	7.2% (8)	9.0% (12)	0.126
Beta-blockers	43.5% (10)	24.3% (27)	27.6% (37)	0.061
Nitrates	8.7% (2)	0.9% (1)	2.2% (3)	0.076
Alpha blockers	8.7% (2)	1.8% (2)	3.0% (4)	0.136
Ca channel blockers	39.1% (9)	21.6% (24)	24.6% (33)	0.076
Statins	87.0% (20)	75.7% (84)	77.6% (104)	0.238
Fibrates	26.1% (6)	12.6% (14)	14.9% (20)	0.113

Compared the percentages of edema in patients treated with pioglitazone to patients treated with rosiglitazone, there was no statistically significant difference (p=0.554), as shown in Table 33. The percentage of edema in high dose of TZDs was higher significantly than in low dose of TZDs (p=0.047). When categorized into subgroups of pioglitazone and rosiglitazone, the high dose group of both pioglitazone and rosiglitazone showed higher percentage of edema as compared to the low dose group. Moreover, the patients treated with rosiglitazone were nearly significant difference between low dose and high dose of rosiglitazone (p=0.053). Table 34 also showed that the frequency of edema was increased when the dose of either pioglitazone or rosiglitazone was increased.

Table 33 Compared different type and dose of thiazolidinediones between edema and non-edema groups for patients recruited for pharmacogenetic study

	Edema group (n=23)	Non-edema group (n=111)	p-value
Type of TZDs (N=134)			0.554
- pioglitazone (n=86)	18.6% (16)	81.4% (70)	
- rosiglitazone (n=48)	14.6% (7)	85.4% (41)	
Dose of TZDs (N=134)			0.047
- low dose [a] (n=105)	13.3% (14)	86.7% (91)	
- high dose [b] (n=29)	31.0% (9)	69.0% (20)	
Pioglitazone (N=86)			0.340
- low dose (n=59)	15.6% (10)	84.4%(54)	
- high dose (n=22)	27.3% (6)	72.7% (16)	
Rosiglitazone (N=48)			0.053
- low dose (n=42)	9.8% (4)	90.2% (37)	
- high dose (n=6)	42.9% (3)	57.1% (4)	

[a] Low dose was 7.5 or 15 mg/day for pioglitazone and 2 or 4 mg/day for rosiglitazone.

[b] High dose was > 15 mg/day for pioglitazone and > 4 mg/day for rosiglitazone.

Table 34 Frequency of edema when different doses of pioglitazone or rosiglitazone were treated

Treatment (number of patients)	Number of edema cases (%)
Pioglitazone 15 mg (64)	10 (15.6%)
Pioglitazone 30 mg (21)	5 (23.8%)
Pioglitazone 45 mg (1)	1 (100.0%)
Rosiglitazone 4 mg (41)	4 (9.8%)
Rosiglitazone 8 mg (7)	3 (42.9%)
Total (134)	**23 (17.2%)**

4.3.2 Comparisons of Gene Variants between Edema and Non-Edema Groups

Distribution of SNP in the endothelin-1 gene (rs5370) of the patients in edema and non-edema groups was shown in Table 35. The rs5370 in the *ENDO1* gene showed genotype G/G of wild type and G/T or T/T of allele variant, which showed the allele frequency of the *ENDO1* gene of 32.1%. Therefore, SNP in the epithelial sodium channel β subunit (rs34241435) of the 134 patients showed only genotype T/T of wild type (100%). Thus, we cannot compare the edema condition between the different variants of the rs34241435 in the *SCNN1B* gene.

Table 35 Distribution of SNP of endothelin-1 gene (rs5370) in edema and non-edema groups

SNP of Endothelin-1 (rs5370)	Edema group (n=23)	Non-edema group (n=111)	Total (N=134)
G/G	43.5% (10)	45.9% (51)	45.5% (61)
G/T	52.2% (12)	43.2% (48)	44.8% (60)
T/T	4.3% (1)	10.8% (12)	9.7% (13)

Comparisons of edema condition between the different variants of the *ENDO1* gene which were presented in Table 36. These were no statistically significant difference between the wild type and the allele variant of the *ENDO1* gene (p=0.829).

Table 36 Comparisons of edema condition between wild type (G/G) and allele type (G/T or T/T) of endothelin-1 genotype (rs5370)

	G/G wild type (n=61)	G/T or T/T allele (n=73)	p-value
Edema conditions (N=134)			0.829
- Edema group (n=23)	16.4% (10)	17.8% (13)	
- Non-edema group (n=111)	83.6% (51)	82.2% (60)	

CHAPTER V

DISCUSSION

The discussions of this study are presented in 3 parts which are (1) Determining the prevalence of TZDs-induced edema using retrospective study; (2) Comparisons of pharmacokinetic parameters between edema and non-edema type 2 diabetic patients treated with pioglitazone; and (3) Determining the associaiton of SNP rs5370 of endothelin-1 gene (*ENDO1*) and SNP rs34241435 of epithelial sodium channel β subunit gene (*SCNN1B*) and TZDs-induced edema.

5.1 Determining the Prevalence of Thiazolidinediones-Induced Edema Using Retrospective Study

5.1.1 Prevalence of Edema, Congestive Heart Failure, and Acute Coronary Syndrome

This study demonstrates the prevalence of edema in type 2 diabetic patients with TZD therapy. This study identified the prevalence of edema in patients using TZDs combined with other anti-diabetic drugs was 13.7% while other studies reported prevalence of edema in the range of 5.9%-16.2% which depended on the designation of therapy of TZD monotherapy or combinations in each study [6,7,57,46,48,58]. However, the percentage of prevalence of edema reported in this study was about half of what was reported (24%) in the previous retrospective study at a tertiary hospital by Hussein et al. [12]. This discrepancy may have resulted from our control of confounding factors such as baseline of CHF and renal insufficiency, and concurrent use of NSAIDs and CCBs. Moreover, the lower prevalence of the edema might be from underestimation because this study collected data only from recording of physicians in medical charts. Similarly, for each TZD drug, the percentage of pioglitazone-induced edema was 15.1%,

and rosiglitazone-induced edema was 12.2% in this study, which were lower than the prevalence reported (33%, and 21%, respectively) in Hussein et al. study [12].

Patients treated with pioglitazone were found higher edema than patients treated with rosiglitazone in this study which was similarly to the study of Hussein et al. [12]. In contrast, the meta-analysis [60] shown the result of a higher risk of edema with rosiglitazone than with pioglitazone (OR=3.75). One reason of this controversial might be due to the switching of rosiglitazone to pioglitazone or starting with pioglitazone more often than with rosiglitazone due to the information of increasing risk of MI while treated with rosiglitazone which spread out during the beginning of this study. About 55% of the patients with edema in this study withdrew from the TZD treatment, which is concurrent with the withdrawal rate of 53% reported in the study by Delea et al. [66].

The duration of TZD used until edema occurred in most patients was within 3-6 months, the duration which the AHA and the ADA recommended to carefully monitored for edema and fluid retention in diabetic patients treated with TZDs [13]. This study found that the 75th percentile of the duration of TZDs used until edema presented was 10 months while the duration until edema presented in patients under the 75th percentile was 2-3 years after TZDs used, which appeared to be longer as comparing to those reported by the AHA and the ADA. This might be related to the presence of edema for some patients were not recorded until the patients had CHF. On average, in majority of the patients, edema was presented within 6 months of TZDs used.

The prevalence of new onset of CHF was noted to be 3.2%, which were higher than the prevalence of 2.5% reported in the study by Delea [66]. The prevalence of CHF in patients treated with pioglitazone was 3.6% and in patients with rosiglitazone was reported to be 2.9%. The prevalence of CHF in patients treated with pioglitazone reported in previous study was 0.6-5.7% [57,65,72], while in patients treated with rosiglitazone was 0.53-1.51% [68-70] . The study patients who developed CHF

discontinued TZDs at the rate of 77.8%. The discontinuation rate of TZDs was similar to the rate (80%) reported in the study by Hussein et al. [12].

The prevalence of ACS in this study was 5.4% in patients treated with TZDs. However, about 53% of these patients had history of CAD before using the medications and had a recurrent event while using the TZDs (5% reported in pioglitazone and 5.8% in rosiglitazone). Previous studies reported lower prevalence of ACS in patients treated with pioglitazone of 1.1-1.53% [72,75], but another study by Dormandy et al. [65] reported that pioglitazone decreased risk of MI by 16%. ACS prevalence in patients treated with rosiglitazone reported by Gerrits et al. [75] was 1.4%. In 2007, the results from meta-analysis study found that rosiglitazone increased risk of MI by 43% [73]. Thus, the FDA had to recommend for awareness of this risk by the update information to healthcare professionals. However, preliminary results from the study by RECORD [77] in measuring CVD outcomes of rosiglitazone showed that there was no difference of CVD outcomes between patients using pioglitazone and patients using rosiglitazone. The final results of the RECORD study will be presented in 2009. We strongly recommend that TZD be used with great caution in patients with sign of volume overload or edema, and patient who previously had CAD or IHD.

5.1.2 General Demographic Factors Affecting Edema

The results of this study showed that in patients with TZDs being female and older age increased risk of TZDs induced edema than being male and at younger age. The results of this study were concurrent with the results of a meta-analysis study [102]. Duration of TZD used was reported to be shorter in patients who developed edema compared with those of the patients without edema event. Patients who developed edema and CHF often withdrawal from the TZD treatment once the condition occurred might result in an average of shorter duration of TZD used.

Although, duration of diabetes of the patients were not significant influencing to edema, we found that the patients had higher level of HbA_{1c} would be more edema than the patients had lower level of HbA_{1c}. Therefore the severity of diabetes that represented in higher level of HbA_{1c} might be relate to edema occurred from TZDs using.

Patients with nephropathy had higher chance of developing edema than patients without this concurrent disease. DM patients who had nephropathy might relate to the excretion of water and sodium from the body. Moreover, this study found that Cl_{cr} at baseline of the patients who developed edema was lower than those of the patients without edema event, which could be interpreted that patients who had nephropathy or had abnormal Cl_{cr} would related to water retention and edema. Although TZDs are mainly metabolized by hepatic enzymes and are excreted unchanged through kidney only about 15-30% [78] and dose adjustment is not necessary in patients with DN [43-45], it should be recommended to take TZD with caution in DM patients with DN and low Cl_{cr}.

Regarding the use of TZD in combined with insulin, previous studies found that patients co-medicated TZD with insulin had higher risk of developing edema than patients treated with TZD combined with other oral anti-diabetic drug or with TZD alone [48, 58]. This study found that high dose of TZD had greater risk of developing edema compared with lower dose TZD, which confirmed that edema in patients using TZD was dose-dependent [19].

The patients co-medicated with CCB were nearly significant higher edema than the patients treated without CCB, even thought the exclusion criteria included patients used CCB within 1 month before receiving TZD and edema occurred. We found that when the patients received only CCB or TZD, they did not present edema. If the two drugs were used together, the patients were higher the risk of edema. This relation between co-medication with CCB and edema might be reason from the synergistic

reaction between TZD and CCB induced edema, however, the mechanism is not clearly understood.

Previous studies had also reported age, sex, dose of TZDs, and co-medicated with insulin to be the factors affecting edema, however, this study found macrovascular diseases and co-medicated with ACEI to be the additional factors affecting edema. The macrovascular diseases including CVD, stoke, and foot ulcer which related to dysfunction of vascular membrane might result in change of water transporting through vascular membrane and induce edema. This study found that the patients with nephropathy were significant higher edema than the patients without nephropathy. Some diabetic patients were received co-medication with ACEI for improving protienuria in diabetic nephropathy. Therefore the patients who received co-medication with ACEI were significant higher edema than the patients treated without ACEI.

Factors affecting edema in patients using pioglitazone were different from those factors affecting edema in patients using rosiglitazone. Sex, macrovascular diseases, and dose of pioglitazone affected edema event in patients using pioglitazone, while sex, macrovascular diseases, ACEI use, and dose of rosiglitazone had significant effects on edema in patients using rosiglitazone after those factors were adjusted by other factors of patient characteristics. ACEI had no significant effect on edema in patients using pioglitazone while it showed some significant effect on edema in patients using rosiglitazone. Patients who developed edema co-medicated with ACEI in a higher percentage in the rosiglitazone group as compared to that in the pioglitazone group (70.6% vs.52.4%).

5.1.3 Effects of TZD on Weight Gain, Glucose Controls, and Lipid Profile

BW and BMI increased during the first 6 months of TZD used on an average of 1.87 kg and 0.77 kg/m^2, respectively. The maximum increase in BW was 4.56 kg in patients using pioglitazone, and 3.92 kg in patients using rosiglitazone. The results of

this study were concurrent with the report of BW increase in previous studies [48, 58, 61, 75], which might be explained by the stimulation of PPAR-gamma resulting in the changes from adipocytes to mature fat cells [64].

The effects of TZD on glucose control from this study showed significant improvement of HbA_{1c} level after the 1st month of TZD used. The average reduction of HbA_{1c} reported in this study was 1.12% even no other lifestyle restriction or interventions were provided for the subjects. The results from this study support that TZD had an impact on glucose control.

The study found that TZD affected lipid profiles of the patients. The LDL level increased while TG decreased after the use of TZD, although, about 90% of these patients were prescribed lipid-lowering therapy. TG levels had greater reduction in patients using pioglitazone than those of the patients using rosiglitazone. On the other hand, HDL levels increased in pioglitazone group while HDL levels decreased in rosiglitazone group. The results are similar to the findings from the study by the pharmaceutical company [4] and other studies [7, 47, 48, 49] which reported the increase in HDL and LDL but the level of TG was decreased.

5.1.4 Cessation of Treatment

One fourth of the patients who withdrawn from TZD treatment reported the reason was due to being informed that TZD increases risk of developing MI. The reason for switching to different type of TZD was also from the awareness of the risk of MI from TZD. The information of the risk of MI came from the study of Nissen et al. [73] who reported that rosiglitazone increased the risk of MI by 43% from meta-analysis study. The second common reason for withdrawal of TZD was due to the edema condition (24.1%).

5.2 Comparisons of Pharmacokinetic Parameters between Edema and Non-Edema Type 2 Diabetic Patients Treated with Pioglitazone

5.2.1 Comparisons of Pharmacokinetic Parameters in Edema Conditions

The study results showed that the PK parameters were not statistically significant different between edema and non-edema groups. However, subjects recruited might not be the real representation of the edema group since patients who got severe edema usually withdrawal from pioglitazone therefore, blood samples could not be obtained. Moreover, blood samples of patients in the edema group usually obtained from patients with minor edema only therefore, they can continue on pioglitazone with reduced dose.

5.2.2 Comparisons of Pharmacokinetic Parameters in Differences Doses of Pioglitazone

However, dose-related effects on edema were confirmed, high dose of TZD increased risk of edema compared with low dose of TZD. This study found the differences of Ke, $t_{1/2}$, and Cl between patients using high dose- and low dose-pioglitazone. Comparisons of PK parameters in non-edema patients using pioglitazone showed that Ke and Cl of the patients who were stable in the 15 mg dose group, were lower than those of the patients who were stable at 30 mg dose, resulting in the lengthening of $t_{1/2}$ of the drug and in turn, lower dose of the drug was required to control the blood sugar level of the patient. Moreover, C_{max} and C_{min} of the high and low dose groups were not significantly different even thought the dosage given were double indicated that different rate of drug metabolism might exist between these two groups. Plausible explanations of the drug elimination might be genetic manifestation, of which might involve CYP2C8 genotypes. Previous studies [103-105] found that patients with CYP2C8*3 variants produced higher CL of repaglinide, rosiglitazone, and pioglitazone than the patients with CYP2C8*1 variants that were wild type, resulting in shorter half-life of the drugs. Further genetic analyses in these patients are important to identify the types of gene related to the effectiveness and the side effects of these drugs.

In addition, the HbA_{1c} of the high and low dose groups were not significantly different indicated that the drug concentrations of the two groups might not be different or the sensitivity of the receptor of the patients in the two groups might be different.

According to the results of the pharmacokinetic study, the patients who received pioglitazone and had analyzed data of PK parameters of pioglitazone could be determined their individual dose of pioglitazone. The patients who had longer $t_{1/2}$ and lower CL could be received the low dose of pioglitazone for controlling their blood glucose, which decreased the risk of edema from receiving the high dose of pioglitazone.

5.3 Determining the Association of SNP rs5370 of *ENDO1* and SNP rs34241435 of *SCNN1B* and TZDs-Induced Edema Status

5.3.1 Prevalence of Different Variants of the *ENDO1* (rs5370) and the *SCNN1B* (rs34241435) Genes

This study found the allele frequency of the *ENDO1* (rs5370) in Thai patients to be 32.1%, which was similar to the prevalence in Chinese population (30%), but was higher than in White population of Geese et al. study (19.3%) [24]. However, this study did not find the allele frequency of the *SCNN1B* (rs34241435) because all of the patients had only wild type of this genotype.

5.3.2 Effects of Different Variants of the *ENDO1* (rs5370) on Edema Conditions

This study did not find any differences of SNP between edema and non-edema groups which might be related to the small number of subjects with edema could be recruited in this study. The study by Geese [24], which was an RCT, recruited a large number of subjects with edema (edema 155 vs.non-edema117) and found the

differences of this SNP. Although, the small sample size of patients in the edema group was not the required (95 patients/group), the numbers of patients in non-edema group was reached the requirement (111 patients). The patients in non-edema group showed that the distribution of SNPs of the *ENDO1* was equally in the wild type and the allele type of the *ENDO1.* Moreover, the blood samples of the patients in the edema group might be represented to real edema patients because the blood samples came from the patients who had both severe and mild edema conditions from TZDs. Thus, the SNP of the *ENDO1* in Thai patients might be not related to edema from TZDs.

In addition, comparisons of patient characteristics between the edema and non-edema groups in the pharmacogenetic study (N=134) and in the retrospective study (N=278) were similarly in age, nephropathy, and Cl_{Cr} level. Our study found that advanced age, patients with nephropathy, and patients with lower Cl_{Cr} level were more edema than younger, patients without nephropathy, and patients with higher Cl_{Cr} level.

5.4 Limitations of this Study

This study has several limitations. First, the number of subjects with edema was not sufficient for the PK and the PG studies. The significant limitation of the PK study from the small sample size being recruited was due to the spread information during the beginning of the study period that TZDs could induce MI. Many patients requested to switch to other anti-diabetic drugs. In addition, patients who had edema usually would be withdrawal from pioglitazone treatment resulting in small number of subjects being available for PK study.

For limitation of sample size of PG study, sample size of edema group (n=23) was smaller than it was determined (n=95) based on the study by Geese et al. study [24]. The difference of this study and the reference study included the study design, the

recruitment setting, and race of the patients. From this limitation, the study might yield no differences of the SNP between edema and non-edema groups.

The second limitation, the blood samples of many patients could not be obtained or the patients which were required for the PK and PG parts refused to participate, especially the patients who developed edema usually will result in the cessation of TZDs or the patients would more often refused to participate, therefore, blood samples could not be obtained. This might result in some bias in the result of the PK and PG studies.

The third limitation, the blood samples of the patients recruited might not be the real representation of the edema group since patients who got severe edema usually withdrawal from TZDs therefore, blood samples could not be obtained. Therefore, blood samples of patients in the edema group usually obtained from patients with minor edema which they can continue on TZDs with reduced dose.

In addition, recruitment of the patients in the PK study and PG study were not reached the expectation because diabetic patients at the study site were outpatients and difficult to contact directly to the patients because of wrong telephone number in their medical charts.

CHAPTER VI

CONCLUSION

This study identified the prevalence of edema in patients using TZDs combined with other anti-diabetic drugs to be 13.7%. Pioglitazone-induced edema was 15.1%, and rosiglitazone-induced edema was 12.2%. The prevalence of new onset of CHF was noted to be 3.2%, and the prevalence of ACS was 5.4% in patients treated with TZDs. The factors affecting the risk of edema in the patients using TZD were age, sex, macrovascular diseases, co-medicated with ACEI, high dose of TZD, and co-medicated with insulin. Moreover, the effects of TZD on glucose and lipid profile showed that the average reduction of HbA_{1c} was 1.12% and the LDL and HDL levels were increased while the TG level was decreased after the use of TZD. BW and BMI increased during the first 6 months of TZD used in an average of 1.87 kg and 0.77 kg/m^2, respectively.

The study results suggested that the PK parameters of pioglitazone were not different in edema and non-edema group. However, dose-related effects on edema were confirmed, of which high dose of TZD increased risk of edema compared with low dose of TZD. Comparisons of PK parameters of pioglitazone in non-edema patients showed that patients who were stabilized at 15 mg dose, their Ke and CL were lower than those of the patients who were stabilized at 30 mg dose, resulting in the lengthening of $t_{1/2}$ of the drug and the drug effectiveness in controlling blood glucose could be reach at the lower dose of pioglitazone. No significant different in C_{max} and C_{min} even though the dose of the high and low dose groups was double (15 mg and 30 mg) indicated that some genetic difference in the metabolizing enzyme might exist among patients. This study found the allele frequency of the endothelin-1 gene in Thai patients to be 32.1%. This study did not find any differences of SNP between edema and non-edema groups.

Limitation of this study was due to the small sample size of the edema patients, which could be recruited into the study. Further study in a larger number of patients is required before any definite conclusion could be confirmed genetic study on the metabolizing enzyme and its association with pharmacokinetic parameters might be benefit.

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APPENDICES

APPENDIX A

Data Collection Form

Data Collection Form

Case No. _____ visit 1 date_____

A. Patient characteristics HN_____

Name:_____ Tel._____

1.Age (years)		1.Age_____				
2.Sex	1.female 2.male	2.Sex	1._____	2._____		
4.Height (cm)		4.Ht_____				
5. BMI (kg/m^2)		5.BMI_____				
6.Duration of DM at the time of receiving TZD (years)		6.Dur _____				

B. Complications and other diseases

7.Diabetic nephropathy	0=no DN 9=missing lab protnuria 1=microalbuminuria (30-300 mg/d) 2=macroalbuminuria (>300 mg/d)	7.DN	0._____	1._____	2._____	9._____
8.Renal disease	0=no renal insufficiency SCr = …… 1=renal insuff. SCr = ……. 9=missing lab SCr	8.Renal	0. _____	1._____	9._____	
9.Hypertension		9.HT_____ yr				
10.Dyslipidemia		10.DLP_____ yr				
11.Coronary artery disease		11.CAD_____ yr				
12.Peripheral vascular disease		12.PVD_____ yr				
13.CVA		13.CVA_____ yr				
14.Others		14.Oth_____				

C. Other diabetic drugs

15.SU		15.Sul_____				
16.Repaglinide (Novonorm)		16.Rep _____				
17.Metformin		17.MFM_____				
18.Glucosidase inhibitor:Glucobay, Basen		18.Glul_____				
19.Insulin		19.Ins_____				

D. Oral hypertensive drug

20.Thiazide		20.Hctz_____				
21.Moduretic		21.Mod_____				
22.ACE-inhibitor		22.ACEI_____				
23.ARB		23.ARB _____				
24.Beta-blocker		24.B-b_____				
25.Vasodilators	1.Hydralazine 2.Minoxidil 3.Nitrate	25.Vas_____	1.Hsz_____	2.Mzd_____	3.Nit:_____	
26.Central acting		26.Cent_____				
27.Alpha blockers		27.Alp-b_____				
28.Carvedilol: Dilatrend		28.Cvd_____				
29.Spironolactone		29.Aldc_____				
30.Calcium channel blockers		30.CCB _____				

E. Lowering lipid drugs

31.Statins	31.Sta _____	1.Simv_____	2.Atro/Li____	3.Flu/Le_____	4.Prav/Me___	5.Ros/Cr_____
32.Fibrates	32.Fi_____	1.Gem_____	2.Fen_____	3.Bez_____		
33.Nicotinic acid	33.Nic_____					
34.Bile acid sequestration	34.Bil_____					
35.Ezetimib	35.Eze_____					

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F. ADR of TZD

36.Edema 1.yes 2.No 36.Ede 1._____ 2._____

If yes; at date_____ Duration of TZD use =_____ yrs.

37.ACS: unstable angina, STEMI, NSTEMI 37.ACS 1._____ 2. _____

If yes; at date_____ Duration of TZD use =_____ yrs.

If no;F/U until date_____ Duration of TZD use = _____ yrs.

If yes; Lab

37.1.CK 37.1.CK_____

37.2.CK MB 37.2.CK MB_____

37.3.Trop-T 37.3.Trop-T_____

37.4.Cr 37.4.Cr_____

37.5.CAG 1.yes 2.No 37.5.CAG 1._____ 2._____

37.6.Echo 1.yes 2.No 37.6.Echo 1._____ 2._____

If yes; EF = _____

38.CHF 1.yes 2.No 38.CHF 1._____ 2._____

If yes; at date_____ Duration of TZD use =_____ yrs.

G.TZD drugs

39.Stop 1*drug 39.Stop1 1._____ 2._____

If yes; at date_____ Duration of TZD use =_____ yrs. Because of_____

Change TZD drug to_____ __at date_____

40.Stop 2*drug 40.Stop2 1._____ 2._____

If yes; at date_____ Duration of TZD use =_____ yrs. Because of_____

41.Last day of follow-up at date_____

Total duration of TZD use=_____yrs.

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Record data of each visit for before and after receiving TZDs within 6 months

				Blood							Urine			Drug		
Visit	Date	M	BW	FPG	HbA_{1c}	CHOL	LDL	HDL	TRIG	Cr	Micro alb	Dip.	Cr	Micro alb	TZD dose (mg/d)		Insulin
			(Kg)	(mg/dl)	(%)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/dl)	(mg/day)	prot.	(mg/dl)	(mg/dl)	Actos	Avandia	(U/day)
BL																	
1																	
2																	
3																	
4																	
5																	
6																	
7																	
now																	

Note:

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APPENDIX B

Determination of Pioglitazone Concentration in Plasma

Determination of Pioglitazone Concentration in Plasma by HPLC-UV

(Applied from Sripalakit et al. study)

1. Equipments

1.1. Li-heparin tube 6 ml for blood sample

1.2. Cryogenic vial and Cryogenic box for plasma sample at -80 °C

1.3. SPE column, Strata C18-T

1.4. Nylon syringe filter (0.45 μm)

1.5. Nylon membrane filter, 0.45 μm, 47 mm

1.6. Tip 200, 1000 μl

1.7. Poly-spring glass inserts 200 μl

1.8. Glass vial with cap 2 ml

1.9. Guard cartridge (7.5 mm x 4.6 mm, 5 μm) with guard-holder

1.10. Apollo C18 column (250 mm x 4.6 mm, 5 μm)

1.11. A centrifuge

1.12. A Genic-2 vortex mixer

1.13. An analytical balance

1.14. A pH meter

1.15. A HPLC system

1.16. Automatic pipettes

1.17. Glass pipettes

1.18. Plastic pipettes

1.19. Volumetric glass set

1.20. Measuring glass set

2. Reagents

2.1. Pioglitazone HCl (>99% purity)

2.2. Rosiglitazone (>99% purity)

2.3. KH_2PO_4 (analytical grade)

2.4. K_2HPO_4 (analytical grade)

2.5. Ortho-phosphoric acid (analytical grade)

2.6. Methanol (HPLC grade)

2.7. Acetonitrile (HPLC grade)

2.8. Purified water (HPLC grade)

2.9. Human fresh frozen plasma (FFP) 6 packs

3. Preparations of Solutions

3.1. Stock standard solutions

3.1.1. Stock standard pioglitazone (1 mg/ml)

Pioglitazone HCl was weighted of 0.0275 gm and was put in a 25 ml of volumetric flask. Added acetonitrile into the flask until the volume was 25 ml.

3.1.2. Stock internal standard rosiglitazone (1 mg/ml)

Rosiglitazone was weighted of 0.0132 gm and was put in a 10 ml of volumetric flask. Added acetonitrile into the flask until the volume was 10 ml.

3.2. Intermediate standard solution of pioglitazone (50 μg/ml)

The stock standard pioglitazone (1 mg/ml) was pipetted of 0.5 ml into a 10 ml of volumetric flask. Added mobile solution into the flask until the volume was 10 ml.

3.3. Working standard solutions

3.3.1. Working standard rosiglitazone (50 μg/ml)

The stock internal standard rosiglitazone (1 mg/ml) was pipetted of 0.5 ml into a 10 ml of volumetric flask. Added mobile solution into the flask until the volume was 10 ml.

3.3.2. Working standard pioglitazone for calibration curve

Working standard pioglitazone was prepared into 2 concentration of 3000 and 1000 ng/ml. The intermediate standard solution of pioglitazone (50 μg/ml) was pipetted 300 μl (0.3 ml) and 100 μl

(0.1 ml) in 5 ml of 2 volumetric flasks. Added mobile solution into each flask until the volume was 5 ml.

3.4. Mixed phosphate buffer (pH 2.6, 10 mM)

KH_2PO_4 was weighted of 1.36 gm and K_2HPO_4 was weighted of 1.74 gm, and then they were put in 1000 ml of volumetric flask. Added water for HPLC into the flask until the volume around 800 ml and dissolved to solution. Adjusted the solution with ortho-phosphoric acid for pH 2.6, and put the water until the volume was1000 ml. The solution was stored at 4 °C and protected from light with aluminum foil.

3.5. Mobile phase

Mobile phase for diluting standard solutions was consisted of acetonitrile, PO_4 buffer, and methanol in the ratio of 12, 48, and 40, respectively.

3.6. Solutions for sample extraction

3.6.1. KH_2PO_4 (0.1 M)

KH_2PO_4 was weighted of 13.61 gm and was put in 1000 ml of volumetric flask. Added water for HPLC into the flask until the volume was 1000 ml.

3.6.2. K_2HPO_4 (0.1 M)

K_2HPO_4 was weighted of 17.42 gm and was put in 1000 ml of volumetric flask. Added water for HPLC into the flask until the volume was 1000 ml.

4. Procedure of Plasma Extraction

4.1. Venous blood sample (10 ml) was centrifuged at 3500 rpm, 4 °C for 15 minutes, after that plasma was removed and stored in a cryogenic vial at - 80 °C until was analyzed.

4.2. The SPE column was pre-activated with 1 ml of ACN and 1 ml of KH_2PO_4 solution (0.1 M).

4.3. Plasma sample (or working pioglitazone in plasma) of 1 ml was added 70 μl of the internal standard Rosiglitazone (50 μg/ml) and 500 μl of KH_2PO_4 (0.1 M) in micro tube 2 ml, and was vortex-mixed briefly.

4.4. The mixture was applied to the activated SPE column. Solution from the SPE column was waste.

4.5. The column was washed with 2 ml of MeOH and KH_2PO_4 (0.1 M) in ratio of 30:70 followed by 1 ml of K_2HPO_4 (0.1 M), and the eluted was leave. Solution from the SPE column was waste. The column was dried for 5 to 10 minutes.

4.6. The analysts were eluted with 500 μl of ACN-water (40:60) followed by 500 μl of ACN-water (50:50).

4.7. The total eluted was filtered through a 0.45 μm, nylon disposable syringe filter.

4.8. The last eluted 100 μl was injected into the HPLC system. All system is in tank pump.

4.9. The HPLC system was a flow rate of 1.2 ml/min and UV detection was performed at 269 nm.

APPENDIX C

Determination of DNA Extraction

DNA extraction by the in-house method of Ramathibodi hospital

(Applied from the phenol–chloroform-isoamyl alcohol method)

1. Equipments

1.1. Plastic tubes with EDTA solution

1.2. Microtubes

1.3. Plastic pipettes

1.4. Automatic pipettes with plastic tip

1.5. A centrifuge

1.6. A vortex meter

1.7. An incubator with water bath

1.8. A rotator in hood chamber

2. Reagents

2.1. Reagent A consists of 0.5 mM EDTA pH 8.0, 5 mM NaCl, and 5% (w/v) sucrose

2.2. Reagent B consists of 50 mM Tris-HCl pH 8.25, 25 mM EDTA, 25 mM NaCl, and 0.1 mg/ml proteinase K

2.3. Na perchlorate

2.4. Chloroform

2.5. 100% (v/v) absolute alcohol

2.6. 70% (v/v) alcohol

2.7. Tris-EDTA buffer consists of 1 mM tris-HCl pH 8.0 and 1 mM EDTA pH 8.0

3. Procedure

3.1. The blood sample of 10 ml in EDTA-tube was centrifuged at 3,500 rpm, at room temperature, for 10 minutes.

3.2. Carefully removed a medium layer of white blood cell (WBC) to microtube by plastic pipette.

3.3. Reagent A of 500 μl was put in the tube of WBC for cleaning the WBC. The tube was vortexed gently and was centrifuged at 14,000 rpm for 5 minutes.

3.4. Carefully drained the supernatant by plastic pipette. The WBC pellet was at the bottom of the tube.

3.5. Repeatedly cleaned the WBC pellet with reagent A until the supernatant was clear.

3.6. Reagent B of 340 μl and Na perchlorate of 100 μl were put in the tube of cleaned WBC, and the tube was vortexed briefly to break WBC that looked as clear solution.

3.7. The tube of solution was incubated in water bath at 37 °C for 20 minutes, and then at 65 °C for 20 minutes.

3.8. The tube of solution was added chloroform of 80 μl. The tube was rotated in hood chamber for 20 minutes and was centrifuged at 14,000 rpm for 5 minutes. Following the centrifugation, two distinct phases should be seen. The DNA was contained in the upper layer, while the chloroform containing mostly proteins was in the bottom layer.

3.9. Carefully removed the supernatant of DNA to new microtube by plastic pipette.

3.10. Cold ethanol of 800 μl was added to the microtube with DNA to precipitate DNA pellet, and the microtube was centrifuged at 14,000 rpm for 10 minutes.

3.11. Carefully drained the supernatant by plastic pipette. The DNA pellet was at the bottom of the microtube.

3.12. The DNA pellet in the microtube was washed with 70% ethanol of 500 μl, and the microtube was centrifuged at 14,000 rpm for 5 minutes.

3.13. Carefully drained the supernatant by plastic pipette. The DNA pellet was at the bottom of the microtube and was left to dry at room temperature.

3.14. Tris-EDTA buffer of 100 μl was added to dissolve the DNA pellet, and the suspended DNA was stored at 4 °C until SNPs analysis.

APPENDIX D

Determination of Genotype Analysis

Genotype Analysis

1. Equipments

1.1. Microtubes

1.2. LightCycler® Multiwell Plate-96 (20 μl)

1.3. Automatic pipettes with plastic tip

1.4. A speed mixer

1.5. A centrifuge

1.6. A LightCycler® 480 SYBR Green I Master system

1.7. LightCycler® 480 Multiwell Sealing Foil

1.8. Electrophoresis system

1.9. Water bath

1.10. Fluorescence chamber

2. Reagents

2.1. DNA samples of 50 ng/μl

2.2. LightCycler® Master Mix

2.3. Primer HPLC purification 150-300 bp.

2.4. Water, PCR-grade

2.5. Agar for gel electrophoresis

3. Procedure

3.1. SNP genotyping were done using for allele specific kinetic real-time PCR and adding SyBrGreen® dye for PCR reaction detection analysed with Tagman® machine

3.2. Primers of SNP rs5370 and rs34241435 were designed by primer 3 program as shown in following table;

SNP	Forward primer	Reverse primer
rs5370	ATCCCAAGCTGAAAGGCAAG	AGTCAGGAACCAGCAGAGGA
	ATCCCAAGCTGAAAGGCAAT	
rs34241435	CCTGAGGCTAGAGCACAGGT	CTCTGGGCAAGTTGGTGAT
		CTCTGGGCAAGTTGGTGAC

3.3. DNA genotyping for each SNP was done as the following step;

3.3.1. Total amount of 200 ng of DNA sample from each subject were pipetted into LightCycler® Plate-96.

3.3.2. Prepare PCR primer by diluting the primers into 1/10.

3.3.3. Prepared the PCR Mixture for 15 μl reaction as followed

- Water, PCR-grade 3 μl
- PCR primer, 10x concentration 2 μl
- Master Mix, 2x concentration 10 μl

3.3.4. The PCR Mix of 15 μl was added to the DNA sample in each well of LightCycler® Plate-96, and sealed the Multiwell Plate with LightCycler® 480 Multiwell Sealing Foil.

3.3.5. The Multiwell Plate was mixed carefully by speed mixer at 2,000 rcf, 23°C, for 2 minutes.

3.3.6. Load the Multiwell Plate into the LightCycler® 480 system and started the PCR program.

3.3.7. Set the PCR program

- pre-incubation at 95°C for 5 minutes
- amplification at 95°C for 10 minutes
- melting curve at 60°C-65°C for 1 hour
- cooling down at 40°C for 10 minutes

Step 3.3.1 - 3.3.7 were applied for each SNP allele.

3.4. Confirmation of the SNPs

SNP genotyping were confirmed by selected subjects with different genotyping for capillary direct sequencing as followed;

3.4.1. New PCR primers were designed for direct sequencing to cover the target alleles by Primer 3 program as shown in following table;

SNP	Forward primer	Reverse primer
rs5370	AGGTCGGAGACCATGAGAAA	AGTCAGGAACCAGCAGAGGA
rs34241435	CCTGAGGCTAGAGCACAGGT	CCCCCATCACATCCACAC

3.4.2 The DNA with PCR Mix after amplification for 45 cycles was run in gel electrophoresis for 2 hours.

3.4.3. The gel was labeled color with ethylium bromide by soaking in a bath of ethylium bromide for 10 minutes, and washed off in water bath for 15 minutes.

3.4.4. The detected SNPs was confirmed by labeled color was seen in fluorescence chamber.

Biography

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1989-1994 B.Pharm., Chiang Mai University, Chiang Mai, Thailand

Research

2000–Present: Faculty of Pharmacy Project at Chiang Mai University, "Drug Interaction Monitoring for In-Patients at Maharaj Nakron Chiang Mai Hospital"

1995-1997: M.Sc. Thesis, "Drug Use Evaluation of H_2-Receptor Antagonists at Lerdsin Hospital "

Teaching

1997–Present: Lecturer in Faculty of Pharmacy, Chiang Mai University

Guest Speaker

June 15, 2002: Adverse Drug Reactions Related to Hepatotoxicity at Nan Hospital, Nan, Thailand

May 25, 2002: Adverse Drug Reactions Related to Hepatotoxicity at Nakorn Ping Hospital, Chiang Mai, Thailand

April 30, 2001: Documentation for Pharmaceutical Care and SOAP System in 8th Clinical Pharmacy Workshop at Lotus Pangsaunkaew Hotel, Chiang Mai, Thailand

April 2, 2001: Drug Interactions and Drug-Induced Hepatotoxicity in ADR Monitoring Training for Pharmacists at Lamphun Hospital, Lamphun, Thailand