POPULATION PHARMACOKINETICS OF REPAGLINIDE IN HEALTHY SUBJECTS AND GENETIC POLYMORPHISMS OF *CYP3A4* AND *CYP2C8*

by

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DEDICATION

Dituju khas buat arwah ayahanda tercinta,
Allahyarham Haji Abu Bakar Ibrahim.....yang sentiasa dalam ingatan. Al-Fatihah (18/10/1993).

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Ruzilawati Abu Bakar,
Mei 2008

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IT2B	Iterative Bayesian Parametric Population Model
ka	Absorption rate constant
kb	Kilobases
KCl	Potassium Chloride
kel	Elimination rate constant
LOD	Limit of detection
LOQ	Limit of quantitation
µl	microlitres
mM	mililitres
min	minutes
mt	mutant
MTBE	methyl t-butyl ether
NONMEM	Nonlinear Mixed Effect Modelling
NPAG	Nonparametric Adaptive Grid
NPEM	Nonparametric Expectation Maximisation
NPML	Nonparametric Maximum Likelihood
OD	Optical Density
PASTRX	A USC*PACK programme used for entry of past therapy data, creating and storing of patient data
PCR	Polymerase Chain Reaction
Pmod	Each fitted Bayesian posterior parameter value in the model
Ppop	Each population (mean) parameter value
QC	Quality control
RFLP	Restriction fragment length polymorphism

LIST OF ABBREVIATIONS

ACN	Acetonitrile
AF	Ammonium formate
AUC	Area under the serum concentration-time curve
AS-PCR	Allele Specific-Polymerase Chain Reaction
bp	Base pair
CDER	Centre for Drug Evaluation Research
CL	Clearance
C_{max}	Peak concentration in serum
Cmod	Concentration in the Bayesian fitted model
Cobs	Observed serum concentration
CV	Coefficient of variation
CYP	Cytochrome P450
CYP2C8	Cytochrome P450 2C8
CYP3A4	Cytochrome P450 3A4
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleoside triphosphate
EDTA	Ethylenediaminetetraacetic acid
FDA	US Food and Drug Administration
G	Gauge
GIT	Gastrointestinal tract
h	Hours
HPLC	High Performance Liquid Chromatography

rpm	Rotation per minute
RSD	Relative standard deviation
SD	Standard deviation
sec	Seconds
SNP	Single Nucleotide Polymorphism
STS	Standard Two-Stage Approach
$t_{1/2}$	Half-life
TE	Tris-EDTA
TBE	Tris-borate
Tm	Melting temperature
t_{max}	time to reach C_{max}
U	Units
USC*PACK	a PC Programme created by the University of Southern California Laboratory of Applied Pharmacokinetics
UV	Ultraviolet
V	Volt
Vd	Volume of distribution
wt	Wild type

Population Pharmacokinetics of Repaglinide in Healthy Subjects and Genetic Polymorphisms of *CYP3A4* and *CYP2C8*

ABSTRACT

Repaglinide is a novel prandial glucose regulator (PGR) for the treatment of type 2 diabetes mellitus. Repaglinide is mainly metabolised in the liver by CYP3A4 and CYP2C8 enzymes. The objective of the present study is to investigate the effects of the *CYP3A4* and *CYP2C8* genotypes on the pharmacokinetics of repaglinide in 121 healthy Malaysian subjects.

The study protocol was approved by our local Research and Ethics Committee, School of Medical Sciences, Universiti Sains Malaysia. Initially, a new HPLC method using a simple liquid-liquid extraction for the determination of repaglinide in human serum was developed and later validated. Then, PCR methods were optimized to detect *CYP3A4* and *CYP2C8* genetic polymorphisms among healthy Malaysian subjects.

Each subject received 4 mg of oral repaglinide. Six blood samples per individual were taken (0 min, 30 min, 60 min, 120 min, 180 min and 240 min) for repaglinide's serum concentration determination by using HPLC. NPAG was then used to determine population pharmacokinetic parameter values of repaglinide.

The developed HPLC method was selective and calibration curves of repaglinide were found to be linear in the concentration range of 20-200 ng/ml. The limits of detection (LOD) and quantification (LOQ) were 10 ng/ml and 20 ng/ml, respectively. The inter-day precision was from 5.21% to 11.84% while the intra-day precision ranged from 3.90% to 6.67%. The inter-day accuracy ranged between

89.95% and 105.75% with the intra-day accuracy ranging from 92.37% to 104.66%. No mutations were detected for the *CYP3A4*4* and *CYP3A4*5* alleles. The allele frequency of the *CYP3A4*18* allele was 2.07%. All five subjects with *CYP3A4*18* mutations were found to be heterozygous. For *CYP2C8,* the allele frequency for both *CYP2C8*2* and **3* was 0.4% while the allele frequency for *CYP2C8*5* was 4.13%. All subjects with mutations were found to be heterozygous.

No mutation was detected for the *CYP2C8*4* allele. *CYP2C8* and *CYP3A4* genotypes were not significantly associated with changes in the blood glucose lowering effect of repaglinide. On the other hand, the *CYP3A4* genotype significantly influenced repaglinide's pharmacokinetics where the mean elimination rate constant (kel) was 34% lower ($p = 0.04$) and the mean half-life ($t_{1/2}$) was 133% longer ($p = 0.04$) in subjects having the *CYP3A4*1/*18* genotype compared to those having the *CYP3A4*1/*1* genotype.

In conclusion, CYP3A4 activity plays an important role in influencing repaglinide's pharmacokinetics.

Farmakokinetik Populasi Repaglinide di dalam Subjek Sihat dan Folimorfik Genetik *CYP3A4* dan *CYP2C8*

ABSTRAK

Repaglinide adalah sejenis ubat pengawal glukosa prandial untuk merawat penyakit kencing manis jenis 2. Repaglinide dimetaboliskan di dalam hepar oleh enzim CYP3A4 dan CYP2C8. Tujuan kajian ini adalah untuk menyelidik kesan kedua-dua genotip *CYP3A4* dan *CYP2C8* ke atas farmakokinetik repaglinide di dalam 121 subjek sihat.

Kajian ini telah diluluskan oleh Jawatankuasa Etika, Pusat Pengajian Sains Perubatan, Universiti Sains Malaysia. Kaedah HPLC menggunakan pengekstrakan cecair-cecair untuk penganalisaan repaglinide di dalam serum dibangunkan dan disahkan. Kemudian, kaedah PCR diubahsuai untuk menentukan polimorfisma genetik *CYP3A4* dan *CYP2C8* di dalam subjek Malaysia yang sihat. Setiap subjek menerima 4 mg repaglinide secara oral. Enam sampel darah diambil daripada setiap subjek (0 min, 30 min, 60 min, 180 min dan 240 min) untuk analisa HPLC repaglinide di dalam serum.

Kaedah HPLC yang dibangunkan adalah selektif dan keluk kalibrasinya juga adalah linear bagi julat kepekatan repaglinide di antara 20 sehingga 200 ng/ml. Tahap pengesanan paling minimum ialah 10 ng/ml manakala tahap kuantitasi paling minimum ialah 20 ng/ml. Kejituan dalam sehari adalah di antara 5.21% hingga 11.84% manakala kejituan di antara hari mempunyai julat di antara 3.90% sehingga 6.67%. Manakala ketepatan dalam sehari berjulat di antara 89.95% dan

105.75% dan ketepatan di antara hari pula mempunyai julat di antara 92.37% sehingga 104.66%.

Tiada mutasi ditemui untuk alel *CYP3A4*4* dan *CYP3A4*5.* Frekuensi alel *CYP3A4*18* di dalam populasi Malaysia ialah 2.07%. Kelima-lima subjek yang mempunyai mutasi *CYP3A4*18* adalah heterozigot. Frekuensi alel bagi *CYP2C8*2* dan **3* adalah masing-masing 0.4% dan frekuensi alel bagi *CYP2C8*5* ialah 4.13%. Kesemua subjek yang mempunyai mutasi adalah heterozigot. Tiada mutasi ditemui untuk alel *CYP2C8*4.* Genotip untuk *CYP2C8* dan *CYP3A4* tidak signifikan untuk perubahan di dalam kesan penurunan glukosa darah repaglinide. Manakala bagi genotip *CYP3A4* terdapat perbezaan yang signifikan di dalam farmakokinetik repaglinide di mana purata kadar konstan eliminasi repaglinide (kel) adalah 34% lebih rendah ($p = 0.04$) dan jangka masa separuh hayat ($t_{1/2}$) pula adalah 133% lebih panjang ($p = 0.04$) di dalam subjek yang mempunyai genotip *CYP3A4*1/*18* berbanding subjek normal.

Kesimpulannya, aktiviti CYP3A4 memainkan peranan yang penting dalam mempengaruhi farmakokinetik repaglinide.

PUBLICATIONS AND PRESENTATIONS

PUBLICATIONS

Full articles

1) **Ruzilawati, A. B**, Suhaimi A. W., Imran, A., Ismail, Z. and Gan, S. H. (2007). Method development and validation of repaglinide in human plasma by HPLC and its application to pharmacokinetics study, *Journal of Pharmaceutical and Biomedical Analysis* **43(5)**, 1831-1835. (2007 Impact Factor - 2.761).
   - Received **Hadiah Sanjungan USM 2007** (publication category).

2) **Ruzilawati, A. B.**, Mohd Suhaimi A. W. and Gan, S. H. (2007). Genetic polymorphisms of *CYP3A4*: *CYP3A4*18* allele is found in five healthy Malaysian subjects, *Clinica Chimica Acta* **383**,158–162. (2007 Impact Factor - 2.601).
   - Received **Hadiah Sanjungan USM 2007** (publication category).

Abstracts

1) **Ruzilawati, A. B.**, Gan, S. H., Mohd. Suhaimi, A. W. and Ismail, Z. Method development and validation of HPLC method for repaglinide in human plasma.*The Malaysian Journal of Medical Sciences*, Vol. 13, No. 2, July 2006.

2) **Ruzilawati, A. B.**, Mohd. Suhaimi, A. W., Imran, A., Ismail, Z. and Gan, S. H. Validated HPLC method for determination of repaglinide in human plasma using liquid-liquid extraction. *British Journal of Pharmacology- in press.*

3) **Ruzilawati, A. B.**, Mohd. Suhaimi, A. W. and Gan, S. H. Effects of *CYP3A4*18* on repaglinide's pharmacokinetics. *Drugs Metabolism Reviews*, Vol. 40 (Supplement 1), 2008. (2006 Impact Factor – 6.238).

ORAL AND POSTER PRESENTATIONS AT SCIENTIFIC MEETINGS

1) **Ruzilawati, A. B.,** Gan, S. H. and Ismail, Z.
Method development and validation of HPLC method for repaglinide in human plasma.
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2) **Ruzilawati, A. B.,** Mohd. Suhaimi, A. W., Imran, A., Ismail, Z. and Gan, S. H.
Validated HPLC method for determination of repaglinide in human plasma using liquid-liquid extraction.
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3) **Ruzilawati, A. B.,** Mohd. Suhaimi, A. W. and Gan, S. H.
Genetic polymorphisms of *CYP3A4* in healthy Malaysian subjects.
Presented at 7th National Congress on Genetics, Renaissance Hotel, Kota Bharu, Kelantan, 5-7 May, 2007. (oral presentation).

4) **Ruzilawati, A. B.,** Mohd. Suhaimi, A. W. and Gan, S. H.
CYP2C8 genotyping among healthy subjects in Malaysia.
Presented at 7th National Congress on Genetics, Renaissance Hotel, Kota Bharu, Kelantan, 5-7 May, 2007. (poster presentation).

5) **Ruzilawati, A. B.,** Mohd. Suhaimi, A. W., and Gan, S. H.
Effects of *CYP3A4*18* on repaglinide's pharmacokinetics.
Presented at 10th European Regional Meeting, International Society for the Study of Xenobiotics (ISSX), Vienna, Austria,18-21 May 2008. (poster presentation).

6) **Ruzilawati, A. B.,** Mohd. Suhaimi, A. W. and Gan, S. H.
CY3A4 and *CYP2C8* genotypes and blood glucose levels in healthy subjects administered with repaglinide.
Presented at 2nd National Conference on Medical Laboratory Sciences, Grand Riverview Hotel, Kota Bharu, Kelantan, 4-6 November 2008. (poster presentation).

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Diabetes mellitus

1.1.1 Classification of Diabetes mellitus

Diabetes means high blood sugar (glucose) or hyperglycaemia due to absolute or relative lack of insulin or insulin resistance (Tattersall, 1986, Rang & Dale, 1993). According to Rang & Dale (1993), hyperglycaemia occurs because the liver and skeletal muscles cannot store glycogen and the tissues are unable to utilise glucose.

It is the leading cause of kidney failure (DeFronzo, 1995) and blindness (Neely *et al.*,1998). It is also a major risk factor for heart diseases and stroke (Mafauzy, 2006a, Pertussona *et al.*, 2008). In Malaysia, the First National Health and Morbidity Survey (NHMS 1) conducted in 1986 reported a prevalence of diabetes of 6.3% and in the Second National Health and Morbidity Survey (NHMS 2) in 1996, the prevalence had risen to 8.2% (Zaini, 2000, Mafauzy, 2006b). In a study in Kelantan, the prevalence was reported to be higher at 10.5% (Mafauzy *et al.*, 1999)

Basically, there are two types of diabetes, Type 1 and Type 2 (Tattersall, 1986, Kilo & Williamson, 1987, Rang & Dale, 1993). Both have similar symptoms but their causes are different (Kilo & Williamson, 1987).

In Type 1 or juvenile onset diabetes, the insulin-producing β-cells of pancreas (i.e the Islet of Langerhans) are destroyed and produce little or no insulin at all. This type of diabetes is most common in the young. It is also known as Insulin-Dependent Diabetes Mellitus (IDDM) since the only treatment is by injection of insulin because all of the β-cells have been destroyed.

Type 2 or maturity onset diabetes is also known as Non-Insulin-Dependent Diabetes Mellitus. It occurs mainly in people over the age of 35 and usually in those who are fat (Kilo & Williamson, 1987). Their β-cells of pancreas can produce some insulin but it is insufficient. NIDDM can be treated either by reducing the body's need for insulin (by losing weight or eating less) and/or stimulating the pancreas with one of the many medications available today for example sulphonylurea in order to produce more insulin. They usually do not require insulin injection.

The number of diabetic individuals will continue to increase worldwide. According to estimations of the World Health Organization (WHO), in 1995 there were more than 135 million adults suffering from diabetes worldwide (King *et al.*, 1995). According to WHO, it is projected that the total number of individuals with diabetes will rise to about 300 million in 2025 (King *et al.*, 1995).

1.1.2 Oral antidiabetic drugs

1.1.2.1 General aspects

The first attempts to treat human diabetes by orally-active drugs were made between 1925 and 1930 from synthalines and their derivatives. However, only in 1940's that carbutamide and tolbutamide were used for antihyperglycaemic activity (Loubatiéres, 1969). These are the first sulphonamide derivatives.

Currently, there are five groups of oral antidiabetics in clinical use

1) Sulphonylureas
2) Biguanides
3) Alpha-glucosidase inhibitor
4) Thiazolidinediones
5) Meglitinides analogue

1.1.2.2 Sulphonylureas

Oral antidiabetic drugs of the sulphonylurea family have been successfully used for the treatment of type 2 diabetes mellitus for more than four decades (Wascher & Boes, 2003). Their popularity is based on their ease of administration, reliable effectiveness and lack of symptomatic side effects other than hypoglycaemia (Groop, 1992). They are still the most favourable treatment approach. Sulphonylurea is an oral antidiabetic drug that acts mainly by increasing endogenous insulin secretion.

Sulphonylureas stimulate insulin release from β-cell of the pancreas via binding to a sulphonylurea receptor (SUR1), thus blocking K_{ATP} channel which then leads to β-cell depolarization, influx of calcium into the cells and eventual release of insulin (Ashcroft & Gribble, 1999). All sulphonylureas lower blood glucose levels in normal and in many patients with NIDDM. The two most popular sulphonylureas used to date are glibenclamide and gliclazide (Levy, 1999). According to Berger (1985), sulphonylureas are safe and effective drug for long-term treatment of diabetes.

1.1.2.3 Biguanides

The only biguanide drug currently in use in many countries is metformin (Berger, 1991).

Biguanides do not affect glycaemia in normal individuals, but they exert a reproducible hypoglycaemic effect in hyperglycaemic patients. They only give peripheral effects (i.e does not stimulate insulin release (Levy, 1999) but they increase muscle glucose uptake and metabolism and decrease hepatic glucose production (gluconeogenesis) (Schafer, 1979, Dunn & Peters, 1995, Levy, 1999). According to Dunn & Peters (1995), biguanide is not metabolised to a significant extent and is primarily excreted unaltered in the urine. Biguanides work synergistically with the sulphonylureas.

1.1.2.4 Alpha-glucosidase inhibitor

The α-glucosidase inhibitor inhibits enzymes that break down polysaccharides and sucrose in the small intestine leading to a slow down of glucose absorption from the gut (Balfour & McTavish, 1993, Levy, 1999). The first of the α-glucosidase inhibitor in clinical use was acarbose (Creuzfeldt, 1988, Balfour & McTavish, 1993). It is eliminated through two ways. The first is via intestinal digestive enzymes cleavage and the second is via biotransformation of the intestinal bacteria. According to Balfour & McTavish (1993), only trace amounts of unaltered acarbose are absorbed from the gastrointestinal tract into the systemic circulation.

Miglitol is a newer α-glucosidase inhibitor (Levy, 1999). It has a similar action as acarbose but it is almost completely absorbed from the gastrointestinal tract (GIT), so it has higher bioavailability. Miglitol is not metabolised and is excreted unaltered (Scott & Spencer, 2000).

1.1.2.5 Thiozolidinedione (TZD)

Thiozolidinedione (TZD) is peroxisome proliferator-activated receptor gamma (PPARγ) activator which was introduced in 1997. Rosiglitazone, traglitazone and pioglitazone are three examples of a TZD. They enhance the effects of insulin on cellular glucose and lipid metabolism. They are extensively metabolised in the liver (Mudaliar & Henry, 2001).

1.1.2.6 Meglitinide analogues

Meglitinides have a mechanism of action similar to that of the sulphonylureas, but are more rapidly absorbed and eliminated (Blickie, 2006). They are taken with meals and due to their shorter duration of action, pose a smaller risk for hypoglycaemia (Dornhorst, 2001). Therefore, compared with sulphonylureas, meglitinides have been shown to provide a better control of postprandial hyperglycaemia and better adverse effects profile with a more favourable safety profile, especially in patients with renal failure (Scheen, 2007).

Repaglinide, a benzoic acid derivative, was the first member of the meglitinide class. It was introduced in 1998. Nateglinide is a derivative of the amino acid phenylalanine and was introduced to the market in 2001 (Levine & Burns, 2001). Repaglinide can be used as monotherapy or in combination with metformin (Moses, 1999) whereas nateglinide is authorised only in combination therapy with metformin (Blickie, 2006). Both are actively metabolised in the liver and are rapidly eliminated with repaglinide having a half-life ($t_{1/2}$) of only 1 h compared to 1.7 h for nateglinide (Culy & Jarvis, 2001, McLeod, 2004).

1.2 Repaglinide

The long plasma half-lives and the long lasting effects of some sulphonylureas for example 4 h for glipizide and 16 h for gliclazide (Yang *et al.*, 2007) increase the risk of hypoglyceamia (Melander *et al.*, 1989, Hatorp *et al.*, 1999). This risk is greatest among elderly patients (Berger, 1985). In up to 20% of patients treated

for six months, mild hypoglycaemia develops (Jennings *et al.*, 1989). The incidence of severe hypoglycaemia is approximately 0.2 per 1000 patients per year (Berger, 1985). Moreover, the use of sulphonylureas can cause weight gain (Natrass, 2000).

Therefore, the usage of new agents with shorter half-lives in order to reduce the risk of hypoglycaemia is highly recommended. The usage of a new agent, repaglinide which has a half-life of 1 h with less hypoglycaemic effects, especially in elderly subjects with type 2 diabetes were recommended by the American Diabetes Association and the European Type 2 Policy Group (Alberti & Gries, 1998).

1.2.1 Therapeutic uses

Repaglinide is a novel prandial glucose regulator (PGR) for the treatment of type 2 diabetes mellitus (Marbury *et al.*, 2000, Hatorp, 2002). It reduces glucose concentrations in patients with type 2 diabetes mellitus (Natrass, 2000) and is used when diet, exercise and weight reduction have not been found to control blood glucose well enough on their own. It helps to control blood sugar by increasing the amount of insulin released by the pancreas.

1.2.2 Formulation, chemical properties and stability

Repaglinide is a new carbomoxylmethyl benzoic acid derivative chemically known as 2-ethoxy-4-[2-[[3-methyl-1-[2-(1-piperidinyl) phenyl] butyl] amino]-2-oxoethyl] (Figure 1.1). It is an (S)(+)-enantiomer.

Repaglinide is a white to off-white powder with shaped crystals. It is slightly soluble in aqueous acid, very soluble in aqueous phase and freely soluble in methanol and ethanol.

It is called Prandin in the United States, NovoNorm in Europe and Gluconorm in Canada.

1.2.3 Mechanism of action

Repaglinide is a non-sulphonylurea oral hypoglycaemic agent that is rapidly absorbed. It stimulates pancreatic insulin secretion via the sulphonylurea receptor. According to Dornhorst (2001), this receptor is a subunit of the ATP-sensitive potassium channel (K_{ATP} channel). It produces a hypoglycaemic effect by stimulating insulin secretion from the pancreatic β-cell (Henquin *et al.*, 1987, Levy, 1999). It initiates insulin secretion by closing the K_{ATP} channel (Dornhorst, 2001). When the sulphonylurea binds to the β-cell it leads to depolarisation of the β-cell with influx of calcium ions and secretion of insulin from its cell.

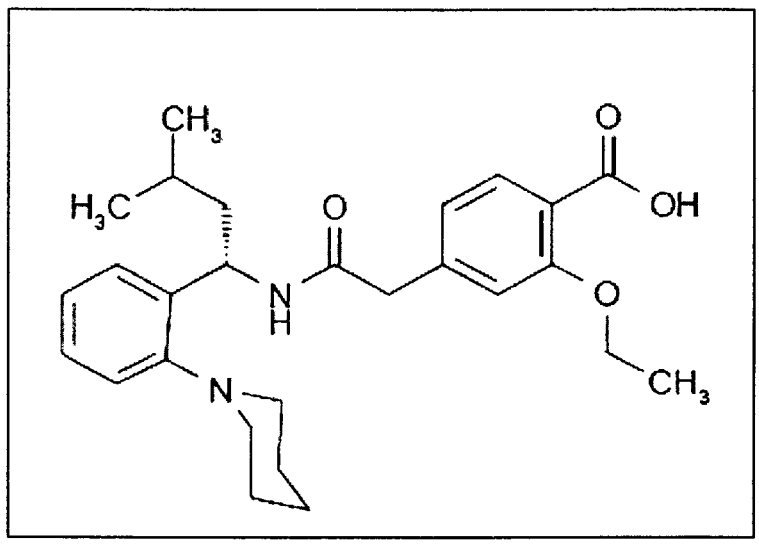

Figure 1.1 Chemical structure of repaglinide

1.2.4 Pharmacokinetics properties

Previous clinical trials have shown that repaglinide is rapidly absorbed from the gastrointestinal tract after oral administration. The peak plasma concentration is reached 30-60 min after administration (Guay, 1998). After reaching the peak, plasma levels decrease rapidly and the drug is eliminated within 4 to 6 h. Its absolute bioavailability is 63% but its absorption is not affected by food. The half-life of repaglinide is about 1 h (Hatorp *et al.*, 1998). Repaglinide has low volume of distribution (Vd) which is about 24.4 L at steady state (Hatorp, 2002). In human, it is highly bound (more than 98%) to plasma protein albumin.

Repaglinide is mainly metabolised in the liver by an oxidative biotransformation (Gaal *et al.*, 2001). Repaglinde is eliminated by metabolism to at least five different metabolites, as shown *in vivo* (van Heiningen *et al.*, 1999) and *in vitro* (Bidstrup *et al.*, 2003). Repaglinide is metabolised by formation of an aromatic amine (M1), opening of the piperadine ring to form a dicarboxylic acid (M2), hydroxylation of the piperadine ring (M4), de-ethylation (M5), formation of a tauride (M6), formation of an acylglucuronide (M7) and by N-oxidation (M12) (Bidstrup *et al.*, 2003). M1 is formed directly from repaglinide, or by oxidative N-dealkylation from M2. *In vitro*, repaglinide (M0) can also be metabolised to M0-OH (Bidstrup *et al.*, 2003) (Figure1.2).

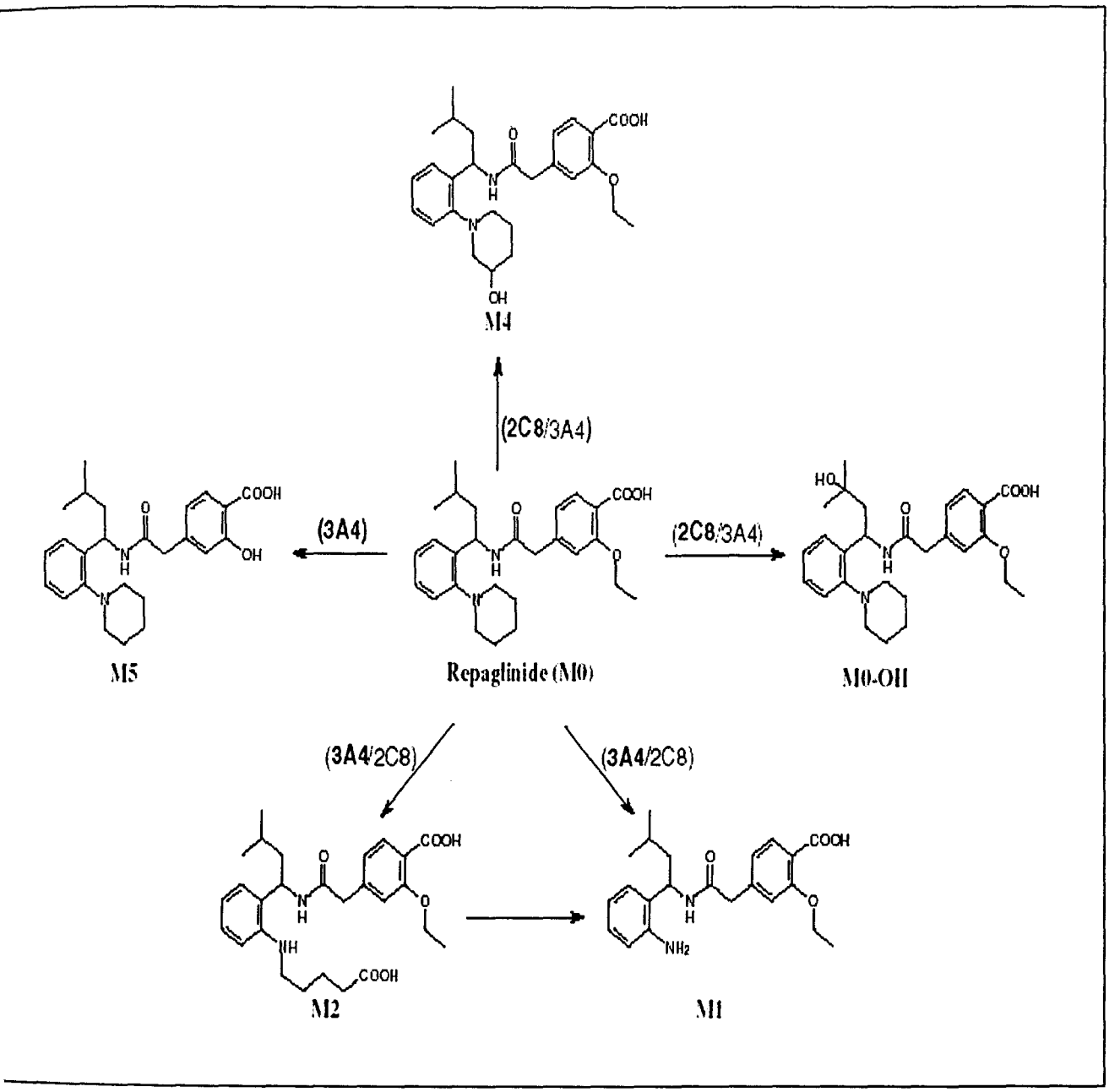

Figure 1.2 *In vitro* biotransformation pathways of repaglinide (Bidstrup *et al.*, 2003)

The most important enzymes participating in the biotransformation of repaglinide are cytochrome P450 3A4 or CYP3A4 and cytochrome P450 2C8 or CYP2C8 (Bidstrup *et al.*, 2003).

In human, the main metabolites of repaglinide are (M2) (about 66% of the dose), an aromatic amine (M1) (about 4% of the dose) and M4, while other metabolites are produced only in small amounts. However, none of its metabolites contributes to its blood-glucose-lowering activity.

In vitro, both CYP3A4 and CYP2C8 have been shown to catalyse the biotransformation of repaglinide to its major metabolites, with M1 and M2 mainly formed by CYP3A4 and M4 mainly by CYP2C8. *In vitro*, CYP3A4 and CYP2C8 contribute similarly to the metabolism of therapeutic repaglinide concentrations, with no significant biotransformation by other CYP enzymes (Kajosaari *et al.*, 2005a).

Repaglinide is excreted primarily through the bile (98%) (Owens, 1999). Only a very small fraction (less than 8%) of the administered dose is excreted through the urine (van Heiningen *et al.*, 1999). In the faeces, the major metabolite was M2 (66% of administered dose) (van Heiningen *et al.*, 1999).

According to Marbury *et al.* (2000), because repaglinide is mostly eliminated by non-renal routes, it may be an appropriate treatment for elderly subjects with renal impairment. Impaired renal function may result in greatly reduced excretion,

thus leading to accumulation of drug and also increasing the risk of hypoglycaemia (Marbury *et al.,* 2000). If repaglinide is used in patients with severely-impaired renal function, dose adjustments may be necessary if indicated by blood glucose levels (Schumacher *et al.*, 2003). The pharmacokinetics of repaglinide are similar in both young and elderly healthy subjects (Hatorp *et al.*, 1999). The pharmacokinetic parameters as obtained from studies done by Hatorp *et al.* (1999) and Marbury *et al.* (2000) following a 2 mg oral single-dose of repaglinide are shown in Table 1.1.

1.2.5 Dosage and administration

Repaglinide should be taken before main meals (preprandially). It is ideally consumed 10 to 15 min before meals. According to Natrass (2000), it can be taken up to 30 min before a meal. It affects around meal time glucose load which is important to the treatment of type 2 diabetes mellitus. If a meal is skipped, the dose should also be skipped. Thus, repaglinide offers a treatment which can be adjusted to suit each individual's lifestyle (Damsbo *et al.*, 1999).

For naïve patients (HbA1c < 8%), 0.5 mg of the dose should be taken before each meal whereas for patients previously treated with oral hypoglycaemic agents, the recommended dose is 2 mg before each meal (Hatorp, 2002). The recommended maximum single dose is 4 mg. The total maximum single dose per day should not exceed 16 mg (Novo Nordisk, 1998).

Table 1.1 Values for serum repaglinide pharmacokinetic variables following a 2 mg oral dose (single-dose regimen) (Hatorp *et al.*, 1999, Marbury *et al.*, 2000).

Parameters	Value	Range (min – max)
AUC (ng/ml per h)	69. 0 ± 78.0	12.0 – 302.0
C_{max} (ng/ml)	47.9 ± 32.0	18.0 - 127.0
t_{max} (h)	0.8 ± 0.6	0.5 - 2.5
$t_{1/2}$ (h)	1.0 ± 0.2	0.7 - 1.3
k_{el} (L/h)	0.37	0.18 - 0.60
Vd (L) at steady state	24.4	-
Vd (L) at elimination phase	28.9	-
CL (L/h)	33.0	-

Data are means ± standard error except where indicated

AUC - area under the serum concentration-time curve
C_{max} - peak serum concentration
t_{max} - time to reach C_{max}
$t_{1/2}$ - half-life
k_{el} - elimination rate constant
Vd - apparent volume of distribution
CL - clearance

1.2.6 Drug interactions

The effect of CYP inhibition and induction on the pharmacokinetics of repaglinide have been studied in several cross-over studies in healthy volunteers. The CYP3A4 inhibitors clarithromycin, itraconazole, and ketoconazole have all been shown to moderately increase repaglinide's plasma concentrations (Niemi *et al.*, 2001, Hatorp, *et al.*, 2003, Niemi *et al.*, 2003). Clarithromycin and itraconazole increase repaglinide's AUC by about 40% (Niemi *et al.*, 2001, Niemi *et al.*, 2003) and ketoconazole by 15% (Hatorp, *et al.*, 2003). Gemfibrozil, a CYP2C8 inhibitor, increases repaglinide's AUC by 8-fold, considerably prolonging and enhancing the blood glucose-lowering effect of repaglinide (Niemi *et al.*, 2003). Combination of gemfibrozil and itraconazole causes a nearly 20-fold increase in repaglinide's AUC, and further enhances repaglinide's blood glucose lowering effect (Niemi *et al.*, 2003). In patients taking both gemfibrozil and repaglinide, serious hypoglycaemic events have been reported (Laine *et al.*, 2003). The CYP3A4 inducer rifampicin decreases repaglinide's plasma concentrations and similar effects are expected with other CYP3A4 inducers. In one study, when repaglinide was administered 12 h after the last rifampicin pre-treatment dose, repaglinide's AUC was reduced by 57% (Niemi *et al.*, 2000) whereas in another study when repaglinide was administered simultaneously with the last rifampicin dose, a 31% reduction of repaglinide's AUC was seen (Hatorp, *et al.*, 2003). Possible drug interactions between repaglinide and a CYP inhibitor cimetidine, CYP3A4 substrates ethinyloestradiol (also a CYP2C9 and CYP2C19 inhibitor) (Laine *et al.*, 2003), simvastatin and nifedipine, and drugs with narrow therapeutic indices (digoxin,

theophylline or warfarin) have also been investigated. However, no significant pharmacokinetic changes were observed (Hatorp, *et al.*, 2000).

1.2.7 Adverse effects

Repaglinide is well tolerated when taken orally (Culy & Jarvis, 2001). A study done by Marshall *et al.* (2006) in general practice in England reported that the most frequent adverse drug reactions were diarrhoea, followed by abdominal pain nausea and vomiting. Adverse events of hypoglycaemia are similar to those caused by sulphonylurea treatment with serious hypoglycaemic effects occurring more with sulphonylureas than repaglinide.

1.2.7 Contraindications

Repaglinide is contraindicated in patients with hypersensitivity to the drug (Novo Nordisk, 1998). Patients who suffer from diabetic ketoacidosis should be treated with insulin and should not use repaglinide. The safety of repaglinide in pregnant women has not been established and therefore it is not recommended for use during pregnancy as the potential for low blood glucose in nursing infants may exist. This drug has not been studied in children and is therefore best avoided in this group of people. Patients with hepatic disorders should also not use this drug.

1.3 Pharmacokinetics, drug metabolism and pharmacogenetics

1.3.1 Pharmacokinetics

Measurement of drug concentrations in the blood, serum or plasma is the most direct approach of assessing the pharmacokinetics of drugs in the body (Shargel & Yu, 1999). Sensitive, accurate, precise analytical methods are required for the direct measurement of drugs in biologic samples. Thus, in this study, high-performance liquid chromatography (HPLC) was used for the determination of serum repaglinide's levels.

1.3.2 Drug metabolism and CYP enzyme system

All drugs are eliminated from the body by excretion and metabolism. Most drugs are lipid soluble compounds, which require biotransformation into more hydrophilic form before they can be excreted from the body (Sweeney & Bromilow, 2006). Drugs are most often metabolised in the liver by enzymes localised in the endoplasmic reticulum of hepatocytes. Drug metabolism can also occur in other tissues, for example the gastrointestinal tract, lungs and kidneys.

Drug metabolism can generally be divided into Phase I reactions (oxidation, reduction or hydrolysis) and Phase II reactions (acetylation, glucuronidation, sulphation and methylation). Phase II may precede Phase I and occurs without prior oxidation, reduction or hydrolysis if there are polar compounds (Sweeney & Bromilow, 2006). Both, most often, convert relatively lipid soluble drugs into relatively more water soluble or hydrophilic metabolites.

The cytochrome P450 (CYP) system is the most important xenobiotic metabolising system. Approximately 80-90% of human drug metabolism is CYP-mediated (Wong *et al.*, 1991, Anzenbacher & Anzenbacherova, 2001). Most of the CYP-catalysed reactions lead to detoxification of xenobiotics.

The CYP enzymes are a superfamily of heme-containing enzymes that are found on the endoplasmic reticulum. These isoenzymes are so called because they have a spectrophotometric absorption peak at or near 450 nm when bound and reduced by carbon monoxide (Nebert & Gonzalez, 1987). The most important property of all known P450s is their ability to bind and activate two atoms of oxygen in a drug molecule (Anzenbacher & Anzenbacherova, 2001). The CYPs constituting a family should have 40% similar sequences in their overlapping portions while a subfamily is created based on higher degree (55%) of sequence similarity (Anzenbacher & Anzenbacherova, 2001).

To date, a total of 57 CYP enzymes have been identified in humans (Ingelman-Sundberg *et al.*, 2000). The families CYP1, CYP2, and CYP3 are primarily associated with the metabolism of exogenous compounds, whereas the other CYPs have mainly endogenous roles (Ingelman-Sundberg *et al.*, 2000).

The CYPs are found primarily in the liver and the gut mucosa and also can be found in the lungs, the kidney, the small intestines and brain in a small amount (Zagaria, 2004). In the liver, CYP3A4 is quantitatively the most important, with CYP2C8, CYP2C9, CYP2A6, CYP2E1 and CYP1A2 present in somewhat lower

quantities; CYP2C19 and CYP2D6 are of relatively minor quantitative importance, but their clinical importance is high (Shimada *et al.*, 1994). CYP3A4 is the major form of CYP expressed in enterocytes, and members of CYP2C subfamily are also significantly expressed (Zhang *et al.*, 1999). Table 1.2 lists some of the CYP enzymes responsible for metabolic transformation of drugs.

1.3.3 Pharmacogenetics

Drug levels in plasma may vary more than 1000-fold between two individuals having the same weight and with the same drug dosage (Ingelman-Sundberg, 2001). This interindividual variation is caused by several factors which can be divided into physiological and environmental (McKinnon & Evans, 2000, Koo & Lee, 2006). Physiological factors include age, gender, ethinicity, genetic factors and body weight while environmental factors include nutritional status, concurrent drug therapy and exposure to certain chemicals.

The term "polymorphism" is used to describe allelic variant that are detected in more than 1% of a given population (Roden, 2001). Genetic polymorphism is a common source of interindividual difference in drug metabolism (Inaba *et al.*, 1995). Much of this variation has shown to be caused by genetic polymorphisms of the CYP enzymes (Nebert & Gonzalez, 1987).

Table 1.2 Some example of human P450 enzymes (Anzenbacher & Anzenbacherova, 2001).

CYP	Localisation
CYP 1A1	lung, liver, brain, GIT, lymphocytes, heart
CYP 1A2	liver
CYP 1B1	skin, brain, heart, lung, placenta, liver, kidney, GIT, spleen
CYP 2A6	liver
CYP 2B1/2	brain
CYP 2B6	liver, heart
CYP 2C8	liver, kidney, intestines, adrenal gland, brain, mammary gland, ovary
CYP 2C9/10	liver
CYP2C19	liver, heart
CYP2D6	liver, brain, heart
CYP2E1	liver, lung, brain, endothelium, heart, bone marrow
CYP2F	lung
CYP3A4/5	liver, GIT, kidney, lung, brain, endothelium, placenta, lymphocytes
CYP3A7	fetus, placenta, liver
CYP4A9/11	kidney
CYP4B1	lung, placenta
CYP4F2/3	kidney

Depending on their metabolic status, humans are divided into poor metaboliser (PM), an intermediate metaboliser (IM), an extensive metaboliser (EM) or an ultra-rapid (ultra-extensive) metaboliser (UM). The poor metabolisers and intermediate metabolisers express dysfunctional or inactive enzymes. They possess the homozygous autosomal recessive allele (usually mutant alleles) (Ingelman-Sundberg & Evans, 2001). Extensive metabolisers have enzymes with normal activity. They have the heterozygous or homozygous dominant allele. At the extreme of the EM phenotype, gene amplification gives rise to the so-called ultra-rapid metabolisers. These individuals carry replicate functional copies of the genes (Ingelman-Sundberg & Evans, 2001).

The most common types of polymorphisms are "single nucleotide polymorphisms", known as SNPs. Polymorphisms may be present in introns or exons. When they are present in exons, they may result in an amino acid change or no change at all. When they are present in introns, they may still regulate gene expression (splicing) (Aithal *et al.*, 1999).

Pharmacogenetics is best defined as the study of genetic variations that cause variation in drug response and includes the genetic polymorphism of drug transporter, drug metabolising enzymes and drug receptors (Ingelman-Sundberg, 2001). The aim of pharmacogenetics is to aid physicians in prescribing the appropriate dose of the right medicine to a person in an attempt to obtain maximum efficacy and minimum toxicity based on genetic tests (Koo & Lee, 2006). Individuals who inherit the deficiency in the enzyme may benefit from appropriately

adjusted doses of the affected drugs based on genetic tests. Pharmacogenetics is a growing discipline with great potential of improving human health-care, in terms of understanding individual drug responses, adverse drug reactions associated with genetics so that medicine could be tailored accordingly to prevent side effects and thus reducing cost of therapy. It becomes more important when the prescribed drugs have narrow therapeutic indexes and are metabolised by polymorphic enzymes as with repaglinide.

1.3.3.1 Genetic polymorphisms of *CYP2C8*

There are four known human CYP2C enzymes: CYP2C8, CYP2C9, CYP2C18 and CYP2C19. These enzymes are responsible for the metabolism of about 20% of clinically described drugs (Evans & Relling, 2004).

CYP2C8 is the major human hepatic P450, constituting about 12% of total microsomal CYP content in the liver (Rendic & Carlo, 1997) in which it conducts oxidative metabolism of at least 5% of drugs cleared by phase I metabolism. It can also be found in other tissues including the kidney, intestines, adrenal gland, brain, mammary gland, ovary and heart as well as in breast cancer tumours (Klose *et al.*, 1999, Nishimura *et al.*, 2003, Knupfer *et al.*, 2004).

Drugs for which CYP2C8 contributes significantly to their biotransformation include the anticancer drug paclitaxel (Sonnichsen *et al.*, 1995, Dai *et al.*, 2001), the antidiabetic drug rosiglitazone and troglitazone (Yamazaki *et al.*, 1999) and

repaglinide (Bidstrup *et al.*, 2003), the antimalarial amodiaquine (Dai *et al.*, 2001) and the hydroxymethylglutaryl co-enzyme A reductase inhibitors such as cerivastatin and fluvastatin (Muck, 2000, Wang *et al.*, 2002). Table 1.3 lists examples of some substrates, inhibitors and inducers of CYP2C8.

CYP2C8 is located on chromosome 10q24.1 and consists of nine exons (Dai *et al.*, 2001). The CYP2C enzymes are all genetically polymorphic with 20 SNPs identified thus far (Totah & Rettie, 2005). An updated list of allelic variant is available at (www.imm.ki.se/CYPalleles).

The most common variant alleles are *CYP2C8*2* and *CYP2C8*3*. *CYP2C8*2* has an Ile269Phe substitution in exon 5. *CYP2C8*2* is expressed in black populations with an allele frequency of 18% but is very rare among white subjects (Dai *et al.*, 2001). *CYP2C8*3* includes both Arg139Lys and Lys399Arg amino acid substitutions in exons 3 and 8 and is expressed most commonly in white subjects (allele frequency, 23%). It is quite rare in black subjects (allele frequency, 2%) and appears to be absent in Japanese subjects (Dai *et al.*, 2001). *CYP2C8*4* represents a mutation that causes an amino acid change Ile264Met in exon 5 and has a frequency of 8% in white subjects (Bahadur *et al.*, 2002). The *CYP2C8*5* variant has a base deletion at position 475 resulting in a frameshift at

Table 1.3 Examples of some substrates, inhibitors and inducers of CYP2C8

Substrates	Inhibitors	Inducers
Amodiaquine	Gemfibrozil	Rifampin
Cerivastatin	Glitazones	Phenobarbitol
Montelukast	Quercetin	
Paclitaxel	Trimethoprim	
Repaglinide		
Rosiglitazone		
Troglitazone		