USM SHORT-TERM PROJECT FINAL REPORT
(GRANT A/C No. 304/PPSP/6131450)

PROJECT TITLE:

GENETIC POLYMORPHISMS OF CYP3A4 AND CYP2C8 IN HEALTHY VOLUNTEERS ADMINISTERED WITH REPAGLINIDE

PRINCIPAL INVESTIGATOR:

Dr Gan Siew Hua

CO-INVESTIGATORS:

Dr Mohd Suhaimi Ab Wahab
Pn Ruzilawati Abu Bakar

Department of Pharmacology
School of Medical Sciences
Universiti Sains Malaysia

LAPORAN AKHIR PROJEK PENYELIDIKAN

UNIVERSITI SAINS MALAYSIA

LAPORAN AKHIR PROJEK PENYELIDIKAN JANGKA *PENDEK*

FINAL REPORT OF SHORT TERM RESEARCH PROJECT

Sila kemukakan laporan akhir ini melalui Jawatankuasa Penyelidikan di Pusat Pengajian dan Dekan/Pengarah/Ketua Jabatan kepada Pejabat Pelantar Penyelidikan

1. **Nama Ketua Penyelidik:** **Dr Gan Siew Hua**
   Name of Research Leader

   ☐ Profesor Madya/ *Assoc. Prof.* ☑ Dr./ *Dr.* ☐ Encik/Puan/Cik *Mr/Mrs/Ms*

2. **Pusat Tanggungjawab (PTJ):** Jabatan Farmokologi, Pusat Pengajian Sains Perubatan
   School/Department

3. **Nama Penyelidik Bersama:** **Dr Mohd Suhaimi Ab Wahab**
   Name of Co-Researcher **Puan Ruzilawati Abu Bakar**

4. **Tajuk Projek:** Genetic polymorphisms of *CYP3A4 & CYP2C8* in healthy volunteers administered with repaglinide.
   Title of Project

5. **Ringkasan Penilaian/***Summary of Assessment*:	Tidak Mencukupi *Inadequate* 1	2	Boleh Diterima *Acceptable* 3	Sangat Baik *Very Good* 4	5
i) **Pencapaian objektif projek:** *Achievement of project objectives*	☐	☐	☐	☐	X
ii) **Kualiti output:** *Quality of outputs*	☐	☐	☐	X	☐
iii) **Kualiti impak:** *Quality of impacts*	☐	☐	☐	X	☐
iv) **Pemindahan teknologi/potensi pengkomersialan:** *Technology transfer/commercialization potential*	☐	X	☐	☐	☐
v) **Kualiti dan usahasama :** *Quality and intensity of collaboration*	☐	☐	☐	X	☐
vi) **Penilaian kepentingan secara keseluruhan:** *Overall assessment of benefits*	☐	☐	☐	☐	X

6. **Abstrak Penyelidikan**
(Perlu disediakan di antara 100 - 200 perkataan di dalam **Bahasa Malaysia dan juga Bahasa Inggeris.** Abstrak ini akan dimuatkan dalam Laporan Tahunan Bahagian Penyelidikan & Inovasi sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti & masyarakat luar).

Abstract of Research
(An abstract of between 100 and 200 words must be prepared in Bahasa Malaysia and in English). This abstract will be included in the Annual Report of the Research and Innovation Section at a later date as a means of presenting the project findings of the researcher/s to the University and the community at large)

Abstract enclosed

7. **Sila sediakan laporan teknikal lengkap yang menerangkan keseluruhan projek ini.**
[Sila gunakan kertas berasingan]
Applicant are required to prepare a Comprehensive Technical Report explaning the project. (This report must be appended separately)

Senaraikan kata kunci yang mencerminkan penyelidikan anda:
List the key words that reflects your research:

Bahasa Malaysia	*Bahasa Inggeris*
CYP2C8	*CYP2C8*
CYP3A4	CYP3A4
Repaglinide	Repaglinide

8. **Output dan Faedah Projek**
Output and Benefits of Project .

(a) * **Penerbitan Jurnal**
Publication of Journals
(Sila nyatakan jenis, tajuk, pengarang/editor, tahun terbitan dan di mana telah diterbit/diserahkan)
(State type, title, author/editor, publication year and where it has been published/submitted)

1) 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.** (2006 Impact Factor - 2.328).
- Received **Hadiah Sanjungan USM 2007** (publication category).

2) 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).

3) Ruzilawati, A. B., Mohd. Suhaimi, A. W. and Gan, S. H.
Population pharmacokinetic modeling of repaglinide in healthy subjects by using NPAG algorithm.
Journal of Clinical Pharmacy and Therapeutics (under peer-review)

(b) **Faedah-faedah lain seperti perkembangan produk, pengkomersialan produk/pendaftaran paten atau impak kepada dasar dan masyarakat.**
State other benefits such as product development, product commercialisation/patent registration or impact on source and society.

-

* Sila berikan salinan/*Kindly provide copies*

(c) **Latihan Sumber Manusia**
Training in Human Resources

i) Pelajar Sarjana:
Graduates Students
(Perincikan nama, ijazah dan status)
(Provide names, degrees and status)

Puan Ruzilawati Abu Bakar

PhD student - waiting for viva

ii) Lain-lain:
Others

9. **Peralatan yang Telah Dibeli:**
Equipment that has been purchased

-

29/6/08

Tarikh
Date

Komen Jawatankuasa Penyelidikan Pusat Pengajian/Pusat
Comments by the Research Committees of Schools/Centres

Projek diselesaikan dgn jaya
bersertz penerbitan

29/7/08

Tarikh
Date

BORANG LAPORAN HASIL PENYELIDIKAN
PPSP

Tajuk geran: **Genetic polymorphisms of *CYP3A4* and *CYP2C8* in healthy volunteers administered with repaglinide**
Penyelidik: **DR GAN SIEW HUA**
Jenis geran: **SHORT-TERM**
Tempoh geran: **2 TAHUN**

Jenis laporan: Laporan Kemajuan [ ] Alatan di beli [ ] Ya : nyatakan.....

Laporan Akhir*: / / Tidak

OBJEKTIF SPESIFIK KAJIAN (sama spt dalam proposal asal)	**SECARA RINGKAS TERANGKAN PENCAPAIAN/HASIL**	**OBJEKTIF TERCAPAI ATAU TIDAK**
1. To optimize PCR-RFLP & multiplex PCR techniques in order to detect *CYP3A4* & *CYP2C8* genetic polymorphisms	Telah berjaya dibangun dan dioptimumkan	Tercapai
2. To use the optimized method (obj. 1) in the study of *CYP3A4* & *CYP2C8* genetic polymorphisms among heathy volunteers administered with repaglinide	Telah berjaya dijalankan dan keputusan kajian telah diperolehi	Tercapai
3. To investigate the clinical relevance of *CYP3A4* & *CYP2C8* genetic polymorphisms in the use of repaglinide among healthy volunteers	Telah berjaya dijalankan	Tercapai
4.		

- *Laporan Akhir perlu disertakan salinan manuskrip dan surat yang dihantar kepada mana-mana jurnal untuk penerbitan.*

UNIVERSITI SAINS MALAYSIA
JABATAN BENDAHARI
KUMPULAN WANG PENYELIDIKAN GERAN USM(304)
PENYATA PERBELANJAAN SEHINGGA 31 MEI 2008

Jumlah Geran:	RM	19,947.00	Ketua Projek:	DR. GAN SIEW HUA
Peruntukan 2006 (Tahun 1)	RM	9,973.00	Tajuk Projek:	Genetic Polymorphisms of CYP3A4 & CYP2C8 in Healthy Volunteers Administered with Repaglinide
Peruntukan 2007 (Tahun 2)	RM	9,974.00		
Peruntukan 2008 (Tahun 3)	RM	0.00	Tempoh:	15 Julai 06- 14 Julai 08
			No.Akaun:	304/PPSP/6131450

Kwg	Akaun	PTJ	Projek	Donor	Peruntukan Projek	Perbelanjaan T'kumpul Hingga Tahun Lalu	Peruntukan Semasa	Tanggungan Semasa	Bayaran Tahun Semasa	Belanja Tahun Semasa	Baki Projek
304	11000	PPSP	6131450		-	-	-	-	-	-	-
304	14000	PPSP	6131450		-	-	-	-	-	-	-
304	15000	PPSP	6131450		-	-	-	-	-	-	-
304	21000	PPSP	6131450		300.00	103.10	196.90	-	-	-	196.90
304	22000	PPSP	6131450		-	-	-	-	-	-	-
304	23000	PPSP	6131450		-	88.28	(88.28)	-	-	-	(88.28)
304	24000	PPSP	6131450		-	-	-	-	-	-	-
304	25000	PPSP	6131450		-	-	-	-	-	-	-
304	26000	PPSP	6131450		-	-	-	-	-	-	-
304	27000	PPSP	6131450		13,974.00	8,905.70	5,068.30	589.90	-	589.90	4,478.40
304	28000	PPSP	6131450		-	-	-	-	-	-	-
304	29000	PPSP	6131450		5,673.00	8,362.80	(2,689.80)	-	-	-	(2,689.80)
304	32000	PPSP	6131450		-	-	-	-	-	-	-
304	35000	PPSP	6131450		-	-	-	-	-	-	-
304	A11102	PPSP	6131450		-	-	-	400.00	-	400.00	(400.00)
					19,947.00	17,459.88	2,487.12	989.90	-	989.90	1,497.22

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 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. Therefore, subjects having *CYP3A4*1/*18* may need to receive lower doses of repaglinide.

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 genetik polimorfik *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. Subjek yang mempunyai genotip *CYP3A4*1/*18* berkemungkinan memerlukan dos yang lebih rendah.