85TH ANNUAL MEETING OF THE JAPANESE PHARMACOLOGICAL SOCIETY

KYOTO, JEPUN

14-16 MAC 2012

DR. RUZILAWATI ABU BAKAR
PUSAT PENGAJIAN SAINS PERUBATAN

O1C2-3-4 Metformin-induced lactic acidosis in Mate1 knockout mice

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Metformin is widely used for the treatment of hyperglycemia in diabetic patients. The major pharmacological action of metformin is the inhibition of gluconeogenesis in the liver. Lactic acidosis is a rare but serious adverse effect of metformin, especially in the patients with renal impairment. However, some case reports also described metformin-induced lactic acidosis in patients without any risk factors. Multidrug and toxin extrusion 1 (MATE1) is predominantly expressed in the luminal side of renal proximal tubules and bile canaliculi, and mediates the transport of metformin. In the present study, pharmacokinetics and toxicity of metformin were evaluated using Mate1 KO mice. After the single oral administration of 400 mg/kg metformin, 15-fold and 69-fold elevation in plasma and hepatic concentration of metformin was observed in Mate1 KO mice, compared with wild-type mice. In addition, higher blood lactate, lower pH and HCO_3^- levels were also observed in Mate1 KO mice. These results indicate that disruption of MATE1 led to an excess increase in the hepatic accumulation of metformin and induced lactic acidosis. Homozygous MATE1 variant could be one of the risk factors for metformin-induced lactic acidosis.

O1C2-3-5 Population pharmacokinetic modeling of repaglinide in healthy Malaysian volunteers with application of the NPAG algorithm

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A new Nonparametric Adaptive Grid (NPAG) algorithm for the estimation of population pharmacokinetic parameter values was evaluated. The algorithm, in the form of a personal computer program, was used to compute population pharmacokinetic parameter values of repaglinide (Novo Nordisk) in 121 healthy Malaysian volunteers. Each subject had received 4 mg of oral repaglinide. Six blood samples per individual were taken (0, 30, 60, 120, 180 and 240 min) for repaglinide's serum concentration determination by using high-performance liquid chromatography. The one-compartmental model pharmacokinetic parameters - elimination rate constant (k_el) and volume of distribution (V_d) - found were: mean $k_el = 0.58 \pm 0.27\ h^{-1}$ and $V_d = 23.09 \pm 9.19$ L/hr. To our knowledge, our study is the first to report specifically on the population pharmacokinetic modeling of repaglinide using NPAG algorithm.

O1C2-4-1 Angiotensin II causes impaired IRS-1/eNOS pathway via PTP1B activation in aorta from type 2 diabetic rats

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The purpose of the present study was to examine the relationship between the IRS1/eNOS pathway and endothelial function in Goto-Kakizaki (GK) type 2 diabetic rats. Endothelium-dependent relaxation was by measuring isometric force in helical strips of aortas from two groups (GK and Wistar genetic control). Protein levels and activities were assayed by Western blotting. Aortas from GK rats showed impaired endothelium-dependent relaxations in response to insulin (vs. Wistar rats), but not those to sodium nitroprusside (SNP). Moreover, the insulin-induced NOx production and IRS-1 (Tyr612)/eNOS (Ser1177) phosphorylation levels were decreased. In Wistar group, the insulin-induced relaxations were markedly inhibited by angiotensin (Ang)-II treatment, but not in GK rats. Furthermore, Membrane fraction for tyrosine phosphatase (PTP)-1B protein expression was increased by Ang II treatment in Wistar but not GK groups. In addition, co-treatment of Ang II with PTP1B inhibitor was normalized above Ang II-induced abnormalities in both groups. These results suggest that in type 2 diabetic rats, the Ang II causes impaired endothelial dysfunction via reduction of Tyr612-phosphorylated IRS-1/eNOS/NOx pathway through the PTP1B activation.

O1C2-4-2 Enhanced prostaglandine E_2-induced contraction in superior mesenteric arteries from type 2 diabetic rats is due to altered EP3 receptor-PKCδ pathway

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Prostaglandin E_2 (PGE_2) is one of the major endothelium-derived contracting factors that were increased cardiovascular diseases such as diabetes and hypertension. However, PGE_2-mediated vasoconstrictive response and its signaling pathway in arteries from type 2 diabetes remain unclear. Here, we investigated PGE_2-mediated contraction in superior mesenteric arteries isolated from Goto-Kakizaki (GK) rats (38-42 wk old). We observed that in GK rats (vs. age-matched Wistar rats): 1) PGE_2-induced contraction was greater in the presence of L-NNA (NOS inhibitor) and endothelial denudation; 2) AH6809 (non-selective EP receptor antagonist) significantly inhibited contractions to PGE_2, whereas sc-19220 (EP1 receptor antagonist) did not affect such contraction in both groups and 3) The enhanced sulprostone (EP3 receptor agonist)- and 17-phenyl-trinor-PGE_2 (EP1 and EP3 agonist)-induced contractions were reduced by rottlerin (a selective PKCδ inhibitor). These results suggest that the enhancement of PGE_2-induced vascular smooth muscle contraction was due to EP3 receptor-mediated activation of the PKCδ pathway.

P3-13-7 Comparison of HPLC and GCMS methods for determination of morphine in human urine

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High-performance liquid chromatography (HPLC) was compared with gas chromatography-mass spectrometry (GCMS) for determination of morphine in human urine. Calibration standards were prepared in human urine. Codeine and d3-morphine were added as the internal standards for HPLC and GCMS, respectively. After solid-phase extraction, the reconstituted samples were divided into aliquots for analysis by HPLC and GCMS. The analytical performances of the two methods were compared with regard to sensitivity, precision and accuracy. Results indicated that HPLC analysis produced results at least as precise, accurate and specific as GCMS for morphine. Thus, analytical results by HPLC were comparable to GCMS. Therefore, HPLC can be useful alternative to GCMS for measuring morphine in urine.

P3-14-1 Autophagic-lysosomal pathway contributes to the sarcopenia of masseter and tongue muscles in the klotho mouse

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Klotho mutant (kl/kl) mice, a short-lived mouse model, display several aging-related phenotypes. To investigate whether the sarcopenia of skeletal muscles is induced in these mice via activation of the ubiquitin-proteasomal pathway (ubiquitin) and/or the autophagic-lysosomal pathway(autophagy) through an alteration of insulin/IGF-1 signaling, we analyzed the activity of the two pathways for protein degradation and components of the insulin/IGF signaling pathway in their skeletal muscles. The masseter, tongue, and gastrocnemius muscles in kl/kl showed marked sarcopenia compared with +/+. The autophagy in kl/kl was activated in the masseter and tongue, but not in the gastrocnemius, compared with +/+, whereas the ubiquitin in these three muscles of kl/kl were not altered. No marked difference in the phosphorylation of insulin/IGF-I signaling components in three muscles studied were found between kl/kl and +/+, but the phosphorylation of signaling component at the downstream of mTOR were suppressed in the masseter and tongue of kl/kl compared with +/+. The autophagy contributes to the sarcopenia of masseter and tongue muscles in the klotho mouse through the down-regulation of mTOR signalling pathway.

P3-14-2 The conflicting effects of eugenol on TRPV1 channel.

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Eugenol, the major component of essential oil of clove, has been used in dental practice to relieve pain. The agent is partly similar in chemical structure to capsaicin which selectively activates sensory neurons *via* a specific receptor TRP (transient receptor potential) V1. It has been reported that eugenol activates TRPV1 channel in a heterologous expression system and rat trigeminal ganglion (TG) neurons. On the other hand, it has also been reported that eugenol exert their antinociceptive effects *via* the TRPV1 located on sensory terminals in the spinal cord. In this study, to elucidate the molecular mechanisms underlying pharmacological actions of eugenol on TRPV1 channel, we investigated the channel properties of TRPV1 using mouse TRPV1 expressing HEK293 cells and mouse TG neurons. Eugenol inhibited the capsaicin-induced inward currents in a concentration-dependent manner. Moreover, eugenol (1 mM) caused small inward current in TG neurons. These results indicate that eugenol is a partial-agonist of TRPV1, and competes with capsaicin at the binding site in TRPV1. The inhibitory effect of eugenol (1 mM) was larger in the case of inward current (ca. 45%) than that of outward current (ca. 20%), suggesting the additional effect of eugenol on the moiety of the channel pore.

P3-14-3 Expression and role of aquaporin 5 and aquaporin 3 in various types of oral cancers

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Aquaporins (AQPs) are membrane proteins that play important roles on water and glycerol transports. Recent studies reported the expression of AQPs in several tumors such as skin, lung and prostate cancer. However, there are few reports about the expression and role of AQPs, such as AQP5 and 3, in various oral cancers. We, therefore, investigated the expression of AQP5 and 3 in human primary oral squamous cell carcinoma (SCC), adenoid cystic carcinoma (ACC) and mucoepidermoid carcinoma (MEC). Overexpressions of both AQP5 and 3 were immunohistochemically observed on tumor cells, but not non-tumor cells, in SCC. In contrast, decreased expression of AQPs was observed on tumor cells in ACC and MEC. Inhibition by antagonist and knockdown of AQPs by siRNA in SCC, cultured cell lines, showed the suppression of tumor cell growth, but not in other cells. Decreased expression of integrins and followed inhibition of mitogen-activated protein kinase pathway by the knockdown of AQPs were also observed. Our results indicate that the expression patterns and roles of AQP5 and 3 in SCC are different from those in ACC and MEC. The inhibition of AQPs might be useful for the therapy of oral cancers.

COMPARISON OF HPLC AND GCMS METHODS FOR DETERMINATION OF MORPHINE IN HUMAN URINE

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Abstract

High-performance liquid chromatography (HPLC) was compared with gas chromatography-mass spectrometry (GCMS) for determination of morphine in human urine. Calibration standards were prepared in human urine. Codeine and d3-morphine were added as the internal standards for HPLC and GCMS, respectively. After solid-phase extraction, the reconstituted samples were divided into aliquots for analysis by HPLC and GCMS. The analytical performances of the two methods were compared with regard to sensitivity, precision and accuracy. Results indicated that HPLC analysis produced results at least as precise, accurate and specific as GCMS for morphine. Thus, analytical results by HPLC were comparable to GCMS. Therefore, HPLC can be useful alternative to GCMS for measuring morphine in urine.

INTRODUCTION

- Morphine is a potent opiate analgesic psychoactive drug.
- The extensive use of morphine as analgesics together with the widespread abuse of this drug has necessitated the development of rapid and sensitive methods for the detection of this drug especially in biological samples.
- Several analytical methods are available for the determination of morphine in biological fluids, including gas chromatography mass spectrometer (GCMS) and liquid chromatography-tandem mass spectrometry (LC/MS/MS).
- However, HPLC with a UV detector is frequently used for the analysis of morphine.

Study Objective

- The objective of this study was to compare high-performance liquid chromatography (HPLC) method with gas chromatography-mass spectrometry (GCMS) method for determination of morphine in human urine

Methods

Urine hydrolysis

A 25µl β-glucuronidase reagent solution and 0.1 M pH 6.0 potassium phosphate buffer were added to 4 mL of spiked urine samples containing 1000mL of morphine (1 µg/mL)

↓

codeine solution (internal standard) were added for HPLC analysis and d3-morphine were added for GCMS analysis

↓

The samples were then mixed on a vortex mixer for 10 minutes and then tubes were incubated at 65°C for 3 hours.

↓

The samples were allowed to cool to the room temperature.

↓

The pH was verified at 5.5 – 6.5

↓

The samples were extracted by solid phase extraction method

Solid Phase Extraction

Columns were conditioned by sequential addition of 2 ml of methanol, 2 ml of deionized water and 2 ml of the 0.1 M pH 6.0 potassium phosphate buffer.

↓

Each washing were allowed for complete aspiration

↓

Minimal vacuum was used during washing and then was turned off completely when finished, to prevent the columns from drying out.

↓

Samples were applied to columns without vacuum and then vacuum was increased to a minimal rate to provide slow passage of samples through the column (1-2 ml/ min)

↓

columns were rinsed with 2 ml of 0.1 M potassium acetate buffer, pH 4.5 and 2 ml of methanol

↓

Columns were dried for 3 min under vacuum

↓

2 ml of freshly prepared elution solution (ethyl acetate, isopropyl alcohol, NH_4OH (18:12:4 v/v/v) was added to each column

↓

extracts were evaporated to dryness with nitrogen at 40°C while under a fume hood

Samples were reconstituted in 70 ul of mobile phase before being injected into HPLC system

Samples for GCMS analysis need to be derivatized

Derivatization

The purified dried extract was added to 50 ul BSTFA agent

↓

The derivatization was performed at 40°C for 20 min in a Reacti-Vap model 18780

↓

The solution was concentrated under a stream of nitrogen

↓

20 ul of aliquots were injected into GCMS equipment

Instrumentation

GCMS

- Drug standards
  - Morphine (50, 100, 200, 300, 400, 500 and 1000 ng/mL)
  - D_3 Morphine (500 ng/mL) (as internal standard)
- Oven Temperature
  - Initial temperature at 120°C and rising at 20°C/min to 300°C, hold for 3 minutes.
  - Post run at 310°C and hold for 2 minutes
  - Injection port: 230°C
  - Interface temp: 280°C
- Carrier gas
  - helium
- Split ratio
  - splitless

HPLC

- Drug standards
  - Morphine (50, 100, 200, 300, 400, 500 and 1000 ng/mL)
  - Codeine (500 ng/mL) (as internal standard)
- The chromatographic Waters (Milford, MA, USA) was equipped with
1) Waters 2695 Separation Module (consists of a pump, degasser, autosampler, thermostat operating at 37°C)
2) Waters 2996 PDA Detector (acquire data at 285 nm)
3) Empower software (Waters) was used for data acquisition and processing
- The separations were performed on Acentis Express C18 column (150mm x 4 6mm, 2.7µm) equipped with a guard column (2.7 µm, 5 mm x 4 6 mm) was used.
- The mobile phase composed of acetonitrile and 0 01M sodium acetate buffer (10 90, v/v), pH 4 with a flow rate of 1 ml/min.

Results & discusssion

HPLC

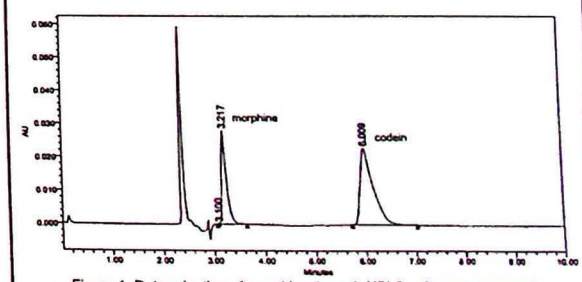

Figure 1: Determination of morphine through HPLC - chromatograms of morphine and internal standard, codeine using acetonitrile and 0.01M sodium acetate buffer, pH 4 (10% : 90%) at flow rate 1 ml/min

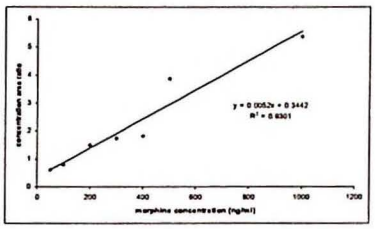

Figure 2 : Calibration curve of area ratio of morphine to internal standard, codeine versus morphine concentrations.

GCMS

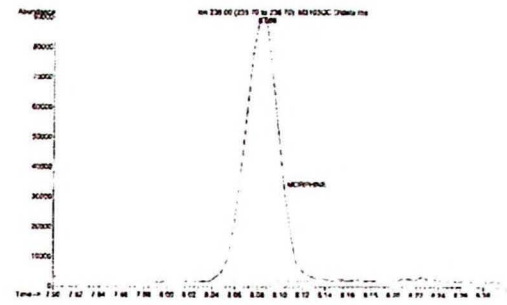

Figure 3: Determination of morphine through GCMS – chromatogram of morphine at 8.086 min.

GCMS

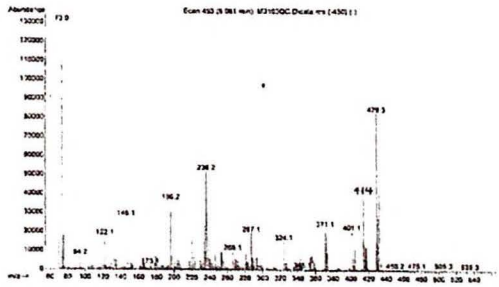

Figure 4: Full scan mass spectra of TMS-derivitized morphine shows 429, 414 and 401 ions

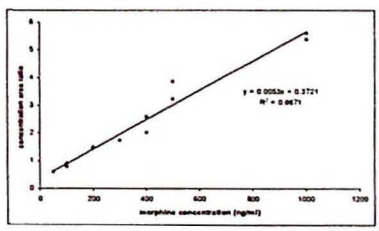

Figure 5 : Calibration curve of area ratio of morphine to d3-morphine versus morphine concentrations.

- Results indicated that HPLC analysis produced results at least as sensitive as GCMS for morphine.
- Thus, analytical results by HPLC were comparable to GCMS.
- Therefore, HPLC can be useful alternative to GCMS for measuring morphine.

References

1. Wilson, J.F., Smith, B. L., Toseland, I. D., Watson, J. Williams, Thomson, A. H., Capps, N. E., Sweeney, G. & Sandle, L. N. (2001). A survey of extraction techniques for drugs of abuse in urine. *Forensic Science International* **119,** 23 – 27
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ACKNOWLEDGEMENT

This study was financially supported by Universiti Sains Malaysia short-term grant (grant no. 304/PPSP/61310053).

Population pharmacokinetic modeling of repaglinide in healthy volunteers with application of the NPAG algorithms

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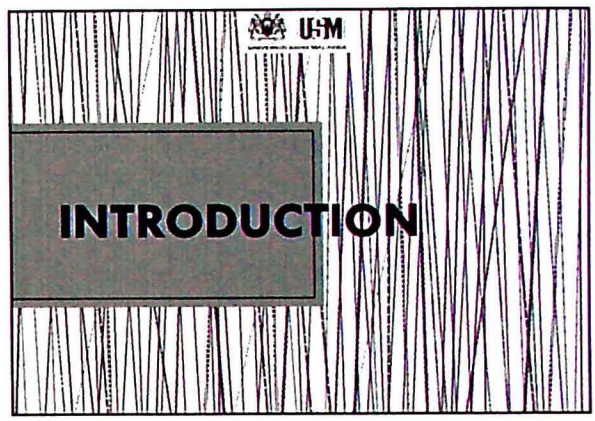
USM



Repaglinide

- Repaglinide is a novel prandial glucose regulator for the treatment of type 2 diabetes mellitus.
- It can reduce fasting glucose concentrations in patients with type 2 diabetes mellitus.
- Repaglinide is mainly metabolized in the liver by *CYP3A4* and *CYP2C8*.

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Defination of Population Pharmacokinetic

- Population pharmacokinetic is defined as the study of the variability in plasma drug concentration or pharmacological effect between individuals when standard dosage regimens are administered (Aarons, 1991, Minto & Schnider, 1998).

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Population Pharmacokinetic

- Population pharmacokinetic models - provide the means to store past experience with the behaviour of drugs and to apply it to the care of future patients (Jelliffe et al., 1993).
- The main purpose of having population pharmacokinetics is to give an appropriate dosage schedule for an individual (Samara & Granneman, 1997).

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Methods for Population Pharmacokinetic

- The current methods for population modeling can be divided into two types, the parametric and nonparametric modeling approach
- In this study, a population pharmacokinetic modeling of repaglinide using the parametric Iterative Two-Stage Bayesian Population Model (IT2B) program followed by the Nonparametric Adaptive Grid (NPAG) program was used to determine population pharmacokinetic parameter values of repaglinide.

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IT2B

- Iterative Bayesian Parametric Population Model
- The IT2B programme is a parametric (means and variances) population modeling method (Jelliffe *et al.*, 1998).

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IT2B

- IT2B can provide reasonable estimates of the ranges for the pharmacokinetic parameters that can be used by the nonparametric programmes such as NPAG.
- The advantage of IT2B is that it can handle sparse data. Therefore, it does not require so many serum concentration data points per patient (Jelliffe *et al.*, 1998).

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NPAG

- Nonparametric Adaptive Grid Population Model
- The NPAG program can then be used to further define the entire parameter distributions.
- NPAG is reported to provide accurate computations, consistent behavior and good statistical efficiency to yield precise parameter estimation and good convergence

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IT2B & NPAG

- Both parametric (IT2B) and nonparametric approaches (NPAG) were used in this study to take advantage of each of their individual strengths
- There are not many available population pharmacokinetic data on repaglinide, thus it is useful to develop such a model.

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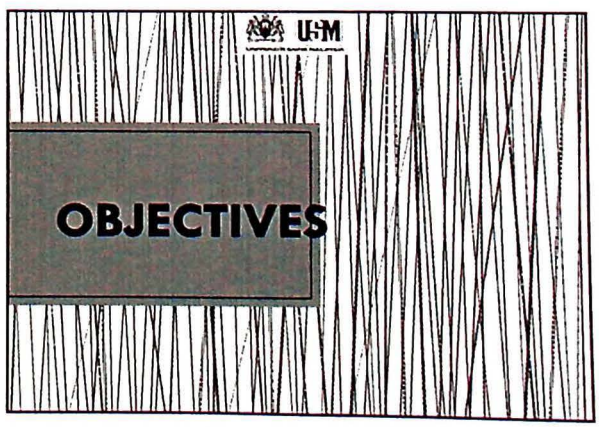

The objectives of the study

- to determine the population pharmacokinetics of repaglinide in healthy Malaysian subjects by using the IT2B (parametric programme) and NPAG (nonparametric programme)
- to estimate repaglinide's population pharmacokinetic parameter values in a group of healthy Malaysian subjects.

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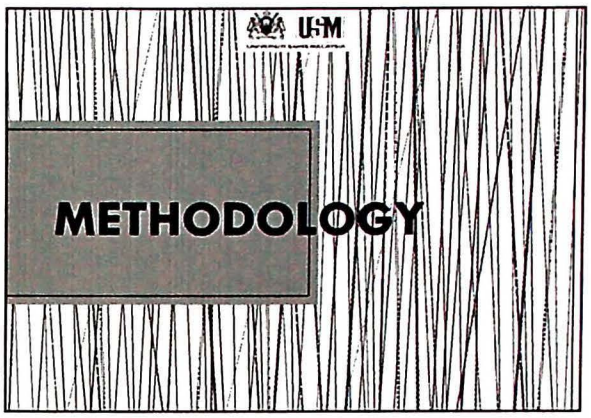

121 healthy students and staff at USM, Kelantan participated in the study after giving written informed consent.

(The study protocol was approved by our local Research and Ethics Committee, School of Medical Sciences, Universiti Sains Malaysia.)

↓

Subjects received oral administration of repaglinide (4 mg)

↓

Subjects were given a standardized breakfast precisely 10 min after repaglinide administration and consumed within 10 min of serving.

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↓

Six blood samples per individual were taken (0 min, 30 min, 60 min, 120 min, 180 min and 240 min)

↓

Serum repaglinide was extracted by liquid-liquid extraction and measured by HPLC

↓

IT2B programme followed by NPAG programme was used to determine population pharmacokinetic parameter values of repaglinide

↓

Data were analysed using the SPSS package (ver. 11, SPSS, Chicago, IL). Differences were considered statistically significant at $p<0.05$.

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Population PK modeling

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Model Parameterization

USC*PACK® - collection of PC programmes, Laboratory of Applied Pharmacokinetics, University of Southern California School of Medicine (BigWinPop programme)

PASTRX

↓

BOXES model — BOXES programme allows boxes and arrows to be created and converts them into their corresponding differential equations.

↓

IT2B — Iterative Bayesian Parametric Population Model (IT2B) The ITB of the USC*PACK® is the first part of the population modeling programme.

↓

NPAG — Nonparametric Adaptive Grid Population Model (NPAG) The NPAG of the USC*PACK® was run next using the parameter ranges determined earlier by the IT2B programme.

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PASTRX

- Individual subject's data including age, height, weight, sex, repaglinide's dosage and concentrations-time data were entered into "PASTRX" module of the USC*PACK® (version 12.0).
- A total of 564 repaglinide concentrations were obtained from 121 subjects.

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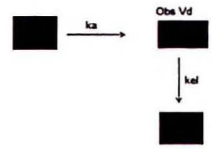
BOXES MODEL

This programme allows the user to draw boxes and arrows with their corresponding differential equations.



Box 1 - the gut for the parent drug
Box 2 - the serum compartment for the parent drug
Box 3 - the metabolic and excretory sink for the parent drug

ka - absorption constant
kel - elimination constant
Obs Vd – observed volume of distribution

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IT2B

- The IT2B program has the assumption that parameter distribution was normal.
- The IT2B program was begun by setting the initial estimates for kel, Vd, Cl, ka and other PK parameters.
- Ten thousand iterations were initially chosen and the convergence criterion and tolerance were set at 0.001.
- Convergence was reached on cycle 39 with a convergence index of 0.06 for IT2B program. A converge index below 1.0 indicates that the model has converged

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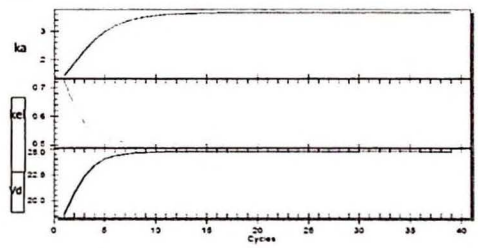

Convergence plots of the normalised mean values of the IT2B programme for ka, kel and Vd indicating that the model was stable by the time convergence was achieved after 39 cycles.

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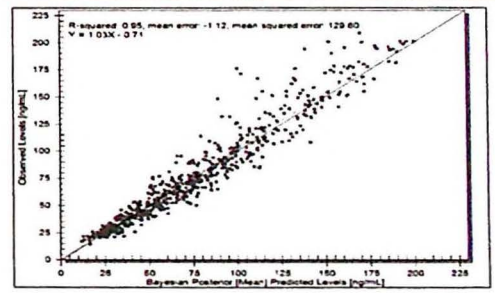

Predicted versus observed concentrations found with the IT2B programme using Bayesian prediction showing a good fit for the model (r^2 = 0.95).

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NPAG

- The NPAG of the USC*PACK® was run next using the parameter ranges determined earlier by the IT2B program.
- The maximum number of grid points of 80021 was used.
- It was run using a maximum of 10000 iterations and the convergence criterion was set at 0.001.
- Convergence was reached on cycle 500 with a convergence index of 0.26 for NPAG program.

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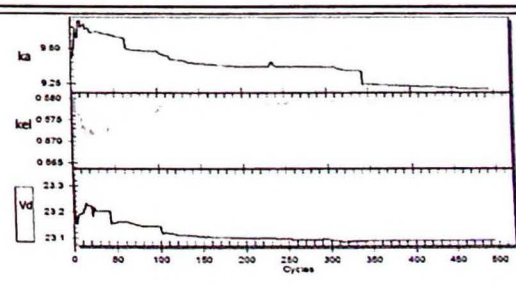

Convergence plots of the normalised mean values of the NPAG programme for ka, kel and Vd indicating that the model was stable by the time convergence was achieved after 500 cycles.

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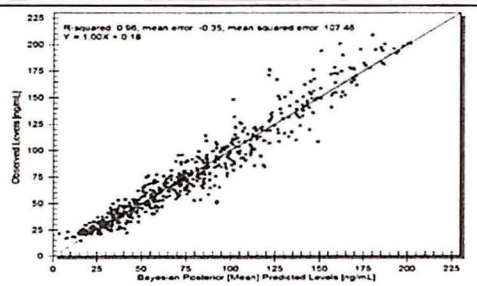

Predicted versus observed concentrations found with the NPAG programme using Bayesian prediction showing a good fit for the model (r^2 = 0.96).

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Statistical analysis

- The mean pharmacokinetic parameter values for all the subjects (n=121) was performed with SPSS program version 11 (SPSS Inc., Chicago, IL)

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RESULTS & DISCUSSION

Pharmacokinetic parameters

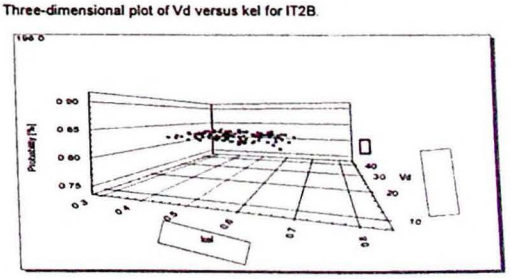
Mean pharmacokinetic parameters of a single oral dose of repaglinide (4 mg) administered to healthy subjects (n = 121).

Variables	Mean ± SD	Min	Max
Mean kel (min^{-1})	9.2066 ± 9.2563	0.7325	27.0598
Mean Vd (L)	22.9541 ± 9.2796	1.2547	47.2196
Mean Kel (h^{-1})	0.5794 ± 0.2662	0.2483	1.5936
Mean CL (L/h)	31.7908 ± 3.9588	12.1884	22.6404
Mean $t_{1/2}$ (h)	1.4111 ± 0.5329	0.4350	2.7915
Mean Cmax (ng/ml)	83.7887 ± 27.9238	42.2667	157.7726
Mean tmax (h)	0.6210 ± 0.3810	0.1484	1.3791
Mean AUC (ng/ml per h)	341.662 ± 114.581	115.7472	611.9342

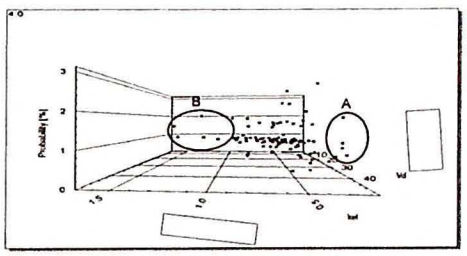
Pharmacokinetic parameters

Pharmacokinetic parameters	Our population	Others
Mean Vd (L)	22.95 L	24 L (Hatorp, 2002)
Mean kel (L/h)	0.58 L/h	0.37 L/h (Marbury et al., 2000)
Mean $t_{1/2}$ (h)	1.41 h	1.0 h (Hatorp, 2002)
Mean CL (L/h)	31.79 L/h	33 L/h (Hatorp, 2002)
Mean AUC (ng/ml per h)	341.662 ng/ml per h	69 ng/ml per h (2 mg dose) (Hatorp, 2002)
Mean C_{max} (ng/ml)	83.7887 ng/ml	47.9 ng/ml(2 mg dose) (Hatorp, 2002)
Mean t_{max} (h)	0.621 h	0.75 h (Marbury et al., 2000)

Three-dimensional plot of Vd versus kel for IT2B.



IT2B did not find the small clusters of kel which may represent the subpopulations detected by NPAG.



Three-dimensional plot of Vd versus kel for NPAG showing a cluster of main population and the possible presence of two subpopulations having lower (A) and higher kel (B) values respectively.

NPAG discovered more diversity in the population than did IT2B

- Using the USC*PACK population modeling program, one can determine the total overall intraindividual variability by a factor called gamma.
- In this study, for IT2B and NPAG, gamma was 1.5727 and 1.6855 respectively, indicating that the intra-individuality among subjects was low.

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- Intraindividual variability can be due to the errors in recording when the doses were administered or when the various serum samples were obtained and any unsuspected changes in parameter values that have taken place during the analytical period

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CONCLUSION

- A population pharmacokinetic model for repaglinide has been developed using the parametric Iterative Bayesian Two-Stage method (IT2B) and the Nonparametric Adaptive Grid method (NPAG).
- This model can be used to estimate important population pharmacokinetic parameter values for repaglinide such as kel, Vd and CL.
- To our knowledge, the present study is the first to report specifically on the population PK modeling of repaglinide using the new NPAG algorithms.

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Acknowledgements

- This study was financially supported by Science Fund (grant no. 305/PPSP/6113206) and Universiti Sains Malaysia short-term grant (grant no. 304/PPSP/6131450).
- Our gratitude to Staff Nurse Maria Yoharnis (HUSM) for their help in conducting the clinical studies.
- We are also grateful to Prof Roger Jelliffe (University of Southern California, USA) for his invaluable advice with regards to pharmacokinetic modeling using NPAG algorithm.

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Thank you

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