THE APPLICATION OF LC-MS-MS TO STUDY THE EFFECTS OF FASTING, FOOD AND ANTACID ON THE PHARMACOKINETICS OF SIMVASTATIN IN HEALTHY MALAYSIAN SUBJECTS

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UNIVERSITI SAINS MALAYSIA
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by

KHALED MOHAMMED AHMED ALI AL-AKHALI

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<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>A</td>
<td>Antacid</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under concentration curve</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
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<tr>
<td>Cl</td>
<td>Clearance</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of variation</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>r</td>
<td>Correlation coefficient</td>
</tr>
<tr>
<td>°C</td>
<td>Degree centigrade</td>
</tr>
<tr>
<td>$K_e$</td>
<td>Elimination rate</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>Half-life</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatograph</td>
</tr>
<tr>
<td>IS</td>
<td>Internal standard</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
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<tr>
<td>LC-MS</td>
<td>Liquid chromatography mass spectrometry</td>
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<tr>
<td>LC-MS-MS</td>
<td>Liquid chromatography mass spectrometry-mass spectrometry</td>
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<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>m/z</td>
<td>Mass-charge</td>
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<tr>
<td>$C_{max}$</td>
<td>Maximum concentration</td>
</tr>
<tr>
<td>$\mu$</td>
<td>Micro</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td>µg</td>
<td>Micro-gram</td>
</tr>
<tr>
<td>µL</td>
<td>Micro-litter</td>
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<td>N-F</td>
<td>Non-fasting</td>
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<td>Polypropylene screw cap tube</td>
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<td>rpm</td>
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<td>Statistical package for social science</td>
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<tr>
<td>$T_{\text{max}}$</td>
<td>Time to reach maximum concentration</td>
</tr>
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<td>TC</td>
<td>Total cholesterol</td>
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<td>TG</td>
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Simvastatin ialah sejenis analog lovastatin dalam bentuk lakton yang digunakan untuk merawat hipekolestrolemia. Simvastatin menurunkan paras plasma kolestrol dengan merencat 3-hidroksi-3-metilglatataril-CoA reduktase.

Matlamat kajian ini adalah untuk membangun dan mengesahkan kaedah analisis yang cukup sensitif bagi mengukur simvastatin dalam plasma dan seterusnya diikuti dengan kajian farmakokinetik komparatif dos tunggal oral 40 mg simvastatin dalam keadaan berpuasa, kehadiran makanan dan keadaan diberi antasid.

Kesemua kaedah yang dibangun adalah menggunakan teknik HPLC dengan berbagai kaedah pengesanan (1) Kromatografi cecair-ultra lembayung (HPLC-UV); pengesahan kaedah HPLC-UV memberikan ketelitian dan ketepatan < 9%. Lineariti berjulat 20-1000 ng/mL dan had pengukuran dan had kuantifikasi adalah 15 ng/mL dan 20 ng/mL dicapai. (2) Kromotografi cecair-spektrometri jisim (LC-MS); pengesahan kaedah LC-MS memberikan ketelitian dan ketepatan < 10%. Lineariti berjulat 0.5-20 ng/mL dengan had pengukuran 0.4 ng/mL dan had kuantifikasi 0.5 ng/mL dicapai. (3) Kromatografi cecair-spektrometri jisim tandem (LC-MS-MS). Pengesahan kaedah LC-MS-MS memberikan ketelitian dan ketepatan < 14%. Lineariti berjulat 0.25-50 ng/mL dengan had pengukuran 0.125 ng/mL dan had kuantifikasi 0.25 ng/mL dicapai.

Kaedah HPLC-UV tidak sensitif bagi mengukur simvastatin dalam plasma selepas pengambilan secara oral. Kaedah LC-MS-MS memberikan
kespesifikasi dan sensitiviti yang lebih berbanding teknik LC-MS, seterusnya digunakan untuk menganalisis sampel plasma.

9 orang sukarelawan lelaki Malaysia yang sihat, berumur antara 22-49 tahun telah dipilih secara rawak, untuk rekabentuk kajian keratan lintang 3 kumpulan dalam 3 blok bagi kajian farmakokinetik perbandingan simvastatin. Kumpulan pertama subjek yang berpuasa diberikan dos 40 mg simvastatin. Kumpulan kedua subjek diberikan dos 40 mg simvastatin beserta antasid (100 mL). Kumpulan ketiga subjek diberikan makanan tempatan terlebih dahulu sebelum mereka diberi dos 40 mg simvastatin. Masa penyahan ialah selama seminggu.

Makanan dan antasid menghasilkan nilai AUC_{0-24}, C_{max} dan T_{max} yang lebih tinggi bagi simvastatin berbanding dengan subjek yang berpuasa. Namun nilai K_{e} dan V_{d} tidak menunjukkan sebarang perbezaan yang ketara diantara subjek yang berpuasa dengan subjek yang mengambil makanan dan subjek yang diberikan antasid. Nilai t_{1/2}, untuk subjek yang mengambil makanan adalah lebih singkat daripada yang diberi antasid dan berpuasa. Nilai CI adalah rendah sedikit dalam keadaan berantasid berbanding dengan keadaan berpuasa. Kehadiran makanan dan antasid mempunyai kesan yang sama dan tidak menunjukkan perbezaan yang signifikan keatas nilai AUC_{0-24}, C_{max}, K_{e}, T_{max}, CI dan V_{d} simvastatin. Penyingkiran simvastatin tidak dipengaruhi oleh keadaan berpuasa, makanan dan antasid. Keputusan mencadangkan makanan dan antasid meningkatkan biokeperolehan simvastatin dengan meninggikan pH salur pencernaan yang seterusnya meningkatkan kestabilan lakton simvastatin dan juga pelarutan simvastatin dengan meningkatkan masa pendudukan
gastrik. Keputusan kajian ini mencadangkan bahawa biokeperolehan simvastatin adalah bergantung kepada pH.
Simvastatin a lactone analog of lovastatin which is used in the treatment of hypercholesterolemia. Simvastatin lowers plasma cholesterol by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase.

The aims of this study were to develop and validate sufficiently sensitive analytical methods for the determination of simvastatin in plasma. This was followed by a comparative pharmacokinetic study of a single oral dose of 40 mg simvastatin under fasting, food and antacid conditions.

The assays are HPLC methods with various detection methods: (1) High performance liquid chromatography-ultraviolet (HPLC-UV); validation of the HPLC-UV revealed precision and accuracy < 9%. Linearity was ranged from 20-1000 ng/mL with limit of detection of 15 ng/mL and the limit of quantification of 20 ng/mL was achieved. (2) Liquid chromatography mass spectrometry (LC-MS); validation of the LC-MS revealed precision and accuracy < 10%. Linearity was ranged from 0.5-20 ng/mL with limit of detection of 0.4 ng/mL and the limit of quantification of 0.5 ng/mL was achieved. (3) Liquid chromatography tandem mass spectrometry (LC-MS-MS); validation of the LC-MS-MS revealed precision and accuracy < 14%. Linearity was linear range from 0.25-50 ng/mL with limit of detection of 0.125 ng/mL and the limit of quantification of 0.25 ng/mL was achieved.

The HPLC-UV was not sensitive in measuring simvastatin in plasma after oral dosing. The LC-MS-MS method showed better specificity and sensitivity
than the LC-MS technique. Thus LC-MS-MS was used for the analysis of plasma samples.

A randomized study of 9 Malaysian healthy male volunteers aged 22-49 years old, on 3 groups crossover design in three blocks of 3 subjects was used for a comparative pharmacokinetic study of simvastatin. In first group, the fasting volunteers were given a single dose of 40 mg tablet of simvastatin. In the second group, the volunteers were given 40 mg tablet of simvastatin with liquid antacid (100 mL). In the third group, the volunteers were given local food before the administration of 40 mg simvastatin. The wash-out period between groups was one week.

Food and antacid produced higher $\text{AUC}_{0-24}$, $C_{\text{max}}$ and $T_{\text{max}}$ values of simvastatin as compared with fasting condition. The $K_{\text{e}}$, and $V_d$ did not show any significant difference between fasting, food and antacid conditions. The $t_{1/2}$ was slightly shorter in food than antacid and fasting conditions. Cl was slightly lower in antacid than fasting conditions. Food and antacid have same effect and did not show any significant difference on $\text{AUC}_{0-24}$, $C_{\text{max}}$, $K_{\text{e}}$, $T_{\text{max}}$, Cl and $V_d$ on simvastatin. The results showed that the food and antacid increased the bioavailability of simvastatin by increasing the pH of gastrointestinal tract. Consequently, that may be lead to increase the stability of lactone form of simvastatin as well as improve the dissolution of the simvastatin by increasing the gastric residence time. It was concluded that simvastatin bioavailability is pH dependent.
CHAPTER 1
INTRODUCTION

Many factors have been found to influence the rate and extent of absorption and hence the time course of a drug in the plasma and therefore at its site(s) of action. These include the food eaten by the patient, the effect of the disease state on drug absorption, the age of the patient, the site(s) of absorption of the administered drug, the co-administration of other drugs, the physical and chemical properties of the administered drug, the type of dosage form, the composition and method of manufacture of the dosage form, the size of the dose and the frequency of administration (Welling, 1977; Niazi, 1979; Welling, 1989; Gibaldi, 1991; Fleisher et al., 1999; Singh, 1999; Ashford, 2002a).

Variability in the bioavailability exhibited by a given drug from different formulations of the same type of dosage form, or from different types of dosage forms, or by different routes of administration, can cause the plasma concentration of the drug to be too high and therefore causes side effects, or it may be too low and therefore the drug will be ineffective (Fleisher et al., 1999; Ashford, 2002c).

When a drug is given intravenously it is administered directly into the blood and therefore we can be sure that the entire drug reaches the systemic circulation. The drugs are therefore said to be 100% bioavailable. However, if a drug is given by another route there is no guarantee that the whole dose will reach the systemic circulation intact. The fraction of an administered dose of the drug that reaches the systemic circulation in the unchanged form is known as
the bioavailable dose. The relative amount of an administered dose of a particular drug that reaches the systemic circulation intact and the rate at which this occurs is known as the bioavailability (Singh, 1999).

Bioavailability is pharmacokinetic term that describes the rate and extent to which the active drug ingredient is absorbed from product and becomes available at the site of drug action. The definition would not be valid in the case of prodrugs, whose therapeutic action normally depends on their conversion into a therapeutically active form prior to or on reaching the systemic circulation. It should also be noted that, in the context of bioavailability, the term systemic circulation refers primarily to venous blood (excluding the hepatic portal vein, which carries blood from the gastrointestinal tract to the liver in the absorption phase) and the arterial blood, which carries the intact blood to tissues. Therefore, for a drug which is administered orally to be 100% bioavailable, the entire dose must move from the dosage form to the systemic circulation. However, the drug must be completely released from the dosage form, fully dissolved in the gastrointestinal fluids, stable in the solution of the gastrointestinal fluids, pass through the gastrointestinal barrier into mesenteric circulation without being metabolized and pass through the liver into the systemic circulation unchanged (Gibaldi, 1991; Wilkinson, 1997; Ashford, 2002b).

Anything which adversely affects either the release of the drug from the dosage form, its dissolution into the gastrointestinal fluids, its permeation through and stability in the gastrointestinal barrier or its stability in the hepatic portal circulation will influence the bioavailability of that drug from the dosage form in which it is administered (Niazi, 1979; Gibaldi, 1991; Ashford, 2002a)
1.1 Background of the Study

1.1.1 The Effect of Drug Stability in Gastrointestinal Fluids on Bioavailability

Gastrointestinal (GI) fluid pH, which varies considerably along the length of the gastrointestinal tract, may have an important influence on drug absorption. A drug dosage form is initially exposed to the acidic pH in normal stomach (pH 1-3) and an abrupt increase in pH once it enters the small intestine as a result of pancreatic secretion. The pH of intestinal fluid ranges from 5-6 in the duodenum to 7-8 in the proximal jejunum and approaches a pH of about 8 in the large intestine. Among the factors affecting the pH of GI fluid are food ingestion, type of diet, stress, general health of the subject and the presence of local disease conditions along the tract (Mayersohn, 1979).

Drug stability in the GI fluids may also play a role in the efficiency of absorption of a given dose of a therapeutic agent. Drugs must remain sufficiently stable, not only during storage, but also in the gastrointestinal fluids, since reactions which result in a product that is pharmacologically inactive or less active will reduce biological availability and therapeutic effectiveness. Generally, the most important reactions that drugs undergo in the gastrointestinal tract are acid and enzymatic hydrolysis (Niazi, 1979; Gibaldi, 1991; Amidon et al., 1995).

Hydrolysis in the acidic gastric fluids frequently results in degradation of a drug to inactive compounds. Penicillin G is an example of a drug which is inactivated by hydrolysis in the stomach (Gibaldi, 1984). The stability of penicillin G in gastric fluids can be estimated from kinetic studies in vitro (Poole, 1979). The half-life of this antibiotic at pH 1 is less than 1 min, while this value is
about 9 min at pH 2. The newer semisynthetic penicillin’s are much more resistant to acid hydrolysis. For instance, ampicillin has a half-life of several hours at pH 1. This improved acid stability is partially responsible for the greater efficiency of absorption observed with these agents. Enteric coatings, properly formulated, can prevent exposure of a drug to gastric pH and enzymes and may minimize its degradation (Gibaldi, 1991; Ashford, 2002b). However, in the case of penicillin G, the use of enteric coatings have not been successful because of the intrinsically poor absorption of this antibiotic beyond the duodenum, and protective coatings generally further decrease its already relatively poor availability (Hou and Poole, 1969).

Stability studies *in vitro* can predict inefficacy of drugs due to acid hydrolysis. An example of such a study was reported for the muscle relaxant *P*-chlorobenzaldoxime (Garrett, 1962 cited by Poole, 1979). Although this compound is active parenterally, it is ineffective when administered orally. Kinetic stability studies *in vitro* demonstrated a half life of less than 20 min in the stomach and the hydrolysis product was not absorbed, accounting for its oral ineffectiveness.

For many compounds which are unstable in acidic gastric fluids, rapid dissolution can often result in decreased bioavailability (Gibaldi, 1984). Erythromycin and its esters are very unstable in gastric fluid (Rutland *et al.*, 1979; Reynolds, 1993; Drabant *et al.*, 2004). Studies on a series of erythromycin esters showed that bioavailability of these compounds are inversely proportional to their dissolution rates in 0.1 N hydrochloric acid.

In some instances, ester hydrolysis in the GIT is a prerequisite for the absorption of a parent drug (Williams, 1985). The stearate and palmitate esters
of chloramphenicol are often used, since their low solubility facilitates the flavouring of paediatric suspensions (Aguiar et al., 1967). However, the ester must be hydrolyzed in the GIT before its absorption and the hydrolysis rate of these esters is dependent on their rate of dissolution. Rate of hydrolysis can vary by a factor of 100 times when fine particles and large particles of chloramphenicol palmitate are compared (Aguiar et al., 1967).

As some drugs exhibit a chemical stability which is pH dependent, the extent of absorption will depend on their time of exposure to GI fluids will in effect represent a reduction in administered dose (Poole, 1979; Gibaldi, 1991).

Drugs such as penicillin G and erythromycin are unstable at the low pH of gastric fluid. Since the rate and extent of drug degradation will depend on the concentration of drug in solution, an attempt is often made to prepare chemical derivatives of those compounds that exhibit a limited solubility at the pH where degradation is seen (Mayersohn, 1979).

The mechanism of ester hydrolysis reaction has been extensively studied and can be applied to the hydrolysis of the lactone form (Kaufman, 1990). This mechanism involves rate determining nucleophilic attack on a protonated lactone to form an intermediate which breaks down to product. Structural features which may influence the rate of nucleophilic attack include steric and inductive effects.

Simvastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, is administered in the form of lactone prodrug. The lactone ring is hydrolyzed in vivo to produce the hydroxyl acid derivatives which are the pharmacologically active forms of this drug, and this is believed to take place
predominantly in the liver (Todd and Goa, 1990; Mauro, 1993; Plosker and McTavish, 1995).

Since lactone hydrolysis reactions are strongly accelerated by general acid catalysis, (Serajuddin et al., 1991) it is anticipated that conversion of lactone into its hydroxyl acid may occur in the strongly acidic gastric environment. Obviously, the desirable tissue selectivity of the lactone form is not realized if hydrolytic conversion in the GIT occurs rapidly relative to lactone absorption. In addition, in vitro study showed that the lactone form in aqueous solution is susceptible to pH dependent hydrolysis at pH 2 (Kaufman, 1990). The maximum stability of lactone form is at pH 5 and no degradation of the lactone in 24 hours at pH 5 was observed (Serajuddin et al., 1991). Thus, the study presented herein is to determine the rate and extent of simvastatin lactone form prodrug absorption under the changed pH of stomach fluids in fasting condition to non-fasting by food and liquid antacid.

1.1.2 High Performance Liquid Chromatography (HPLC) Assay of the Simvastatin in Plasma

Generally, bioavailability, pharmacokinetics, pharmacokinetic interaction and bioequivalence studies require very accurate and precise assay methods that are well validated to quantify drugs in biological samples (Poon, 1997). The assay methods also have to be very selective to ensure reliable data, free from interference of endogenous compounds and possible metabolites in biological samples (Shah et al., 2000). The assay methods have to be sensitive enough to determine the biological sample concentration of the drug and/or its metabolites for a period about five elimination half-life after dosage of the drug. In addition,
methods have to be as robust and cost effective as possible, making of particular importance to pharmacokinetic studies.

In addition, the selectivity of any bioanalytical method is greatly affected by both the initial clean up sample procedures as well as the subsequent analytical process. However, a sufficiently clean sample from plasma is often fraught with difficulties because of the presence of interfering and endogenous substances. Therefore, sample clean up is the critical step in the overall analytical process. Thus method should be short and as simple as possible without compromising on the selectivity and sensitivity of the assay.

In general, solid phase extraction and liquid-liquid extraction are the two principle methods used for sample pre-treatment from plasma. Liquid-liquid extraction involves the selection of a drug from neutralized aqueous layer into a suitable organic solvent such as ethyl acetate and hexane followed by organic phase evaporation and residue reconstitution. The extraction procedure of liquid-liquid is simpler and less expensive than solid phase extraction. Solid phase is based on the principle of modern liquid chromatography (McDowall, 1989). This operation and clean up procedure prior to analysis seems to be complicated and expensive for routine measurements in the clinical laboratory.

Consequently, a selection of suitable extraction procedure, which is time economical, gives the highest possible recovery without interference at the elution time of the analyte of interest. Another important point is that the tests performed at the stage of method development should be done with the same equipment that will actually be used for subsequent routine analysis.

The criteria considered in typical method development and establishment for bioanalytical method includes determination of selectivity, accuracy,
precision, recovery, calibration curve, LOQ, LOD and stability (Green, 1996). For a method to be considered valid, specific acceptance criteria should be set in advance and achieved for accuracy and precision for validation of quality control sample (Canson, 1997).

In addition, the chromatographic methods are more widely accessible and capable of being implemented in clinical laboratories with standard high performance liquid chromatography (HPLC) instrumentation. Most HPLC methods use C_{18} silica gel reversed phase HPLC columns with isocratic elution and an acidic buffer mobile phase.

Recently, numerous methods for analysis of simvastatin and its metabolites determination by HPLC methods with difference detectors (ultraviolet, fluorescence and mass spectrometry) have been published (Stubbs et al., 1986; Carlucci et al., 1992; Ochiai et al., 1997; Wu et al., 1997; Jemal et al., 2000; Wang and Asgharnejad, 2000; Miao and Metcalfe, 2003; Yang et al., 2003; Yang et al., 2005; Barrett et al., 2006; Pasha et al., 2006). In addition, some methods of gas chromatography with mass spectrometry for determination of simvastatin and its metabolites have also been published (Takano et al., 1990; Morris et al., 1993). However, most of these methods are tedious and time consuming, which also considered costly for routine analysis work.

In this study a chromatographic methods will be developed and validated based on liquid-liquid extraction. This method will be used for quantitation of simvastatin in plasma samples for the pharmacokinetic studies.
1.1.3 Justification of the Study

To the best of the researcher’s knowledge, there is no study available to investigate the mechanism of simvastatin absorption with food and liquid antacid in healthy younger volunteers. One study has been conducted by (Schaefer et al., 2004). This study is not significant enough to confirm the pharmacokinetic of simvastatin in fasting and non-fasting. In addition, the study was done in elderly patients (mean age 62) with coronary heart disease, while the results showed higher effect in taken simvastatin with food than in fasting condition. Others studies have been published shown the drugs interactions with simvastatin (Gruer et al., 1999; Jacobson, 2004), bioequivalence (Najib et al., 2003), grapefruit juice interaction with simvastatin (Lilja et al., 1998; Lilja et al., 2000) and pharmacokinetic of simvastatin (Todd and Goa, 1990; Mauro, 1993; Plosker and McTavish, 1995).

Given the above, the current study aims to investigate the effect of local food (Malaysian food) and liquid antacid on the pharmacokinetic profiles of simvastatin in healthy Malaysian volunteers with enough randomization to obtain significant findings. In addition, the study will also investigate the stability of lactone form by increasing the gastrointestinal fluid pH with local food and liquid antacid. As well as to compare the absorption of lactone form enters the general circulation when simvastatin is taken together with local food and liquid antacid than when simvastatin is taken in the fasting state. Therefore, the present study is considered original and has not been carried out and reported in Malaysia and elsewhere.
1.2 Aims of the Present Study

In this study, the work was conducted to study the rate and extent of absorption of current dosage of 40 mg of simvastatin by neutralizing gastric contents and elevating pH of gastric contents by Malaysian food ingredient (fat, carbohydrate and protein) and liquid antacid. The aims of this study were:

1. To develop and validate a sufficiently sensitive analytical method for the determination of simvastatin quantities in biological fluids.

2. To compare the rate and extent of absorption of single oral dose of 40 mg simvastatin (Zocor®, MSD) as follows.

   Group I. To study the effect of fasting state on the pharmacokinetic of single dose of 40 mg simvastatin in healthy adult male Malaysian volunteers.

   Group II. To study the effect of multi-dose suspension of magnesium and aluminium hydroxide mixture on the pharmacokinetic of single dose of 40 mg simvastatin in healthy adult male Malaysian volunteers.

   Group III. To study the effect of local food on the pharmacokinetic of single dose 40 mg simvastatin in healthy adult male Malaysian volunteers.

1.3 Contribution of the Study

The contribution of this study will be reflected in many aspects which have the potential to be a useful tool to gain many benefits in the pharmacy practice on the impact of research finding on the simvastatin administration and uses. In addition, it might provide benefits to the scientific knowledge as it contributes to know the stability of lactone form (simvastatin) with food and liquid antacid. Furthermore, the output findings from these works will effectively
lead to build up strong bases of knowledge for researches on lactone form (simvastatin) absorption in future.

1.4 Organization of the Study

Chapter 1: Background, justification, objectives, contribution and organization of study.

Chapter 2: Literature review of the study.


Chapter 4: Methods validation for measuring simvastatin in human plasma using liquid chromatography mass spectrometry (LC-MS) and liquid chromatography tandem mass spectrometry (LC-MS-MS).

Chapter 5: Clinical pharmacokinetic study of simvastatin.

Chapter 6: General conclusion and recommendation.
2.1 Introduction

This chapter gives an overview of the key concepts of the study. It deals with 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors. Then it deals with simvastatin one of the major four most important drugs used today to treat hyperlipidaemia. Then the chapter goes on to review of pharmacokinetic and metabolism. It touches upon lipophilicities, solubilities and partition coefficient of HMG-CoA reductase inhibitors, pravastatin, lovastatin, mevastatin and simvastatin. The chapter touches upon lactone hydrolysis of HMG-CoA reductase inhibitors (lovastatin and simvastatin). The chapter touches briefly on the effects of food and pH on drug absorption. Finally, determination of simvastatin in human plasma by using HPLC methods concludes the chapter.

2.2 3-Hydroxy-3-Methylglutaryl-Coenzyme A Reductase Inhibitors

2.2.1 Chemical Structures

Mevastatin, the first representative of this new class of statin compounds, is derived from a strain of *Penicillin citrinum*. Lovastatin is a natural product, simvastatin and pravastatin are derived from natural products and fluvastatin is totally synthetic racemic mixture (Illingworth and Tobert, 1994).

Lovastatin, simvastatin and pravastatin are derived from fungi. Simvastatin is chemically modified 2,2-dimethylbutyrate analogue of lovastatin (Hoffman *et al.*, 1986). Pravastatin is a purified active metabolite of mevastatin with an open hydroxyl acid instead of a lactone ring (Lennernas and Fager,
The fungal products lovastatin, pravastatin and simvastatin are structurally related since they have a hydronaphthalene in common and differ only at a few sites in the molecule (Figure 2.1).

Furthermore, the major difference between the open form of the substituent R3 and HMG-CoA is the presence of methyl group on R3 (Figure 2.1 and 2.2). The inhibitors with a lactone substituent R3 must tautomerise to the open form \textit{in vivo} to become active.

In addition, lovastatin and simvastatin are orally administered as inactive prodrugs in the lactone form, whereas pravastatin is given in the active open hydroxyl acid forms. Fluvastatin is purely synthetic and is given orally in its active hydroxyl acid form (Lennernas and Fager, 1997).

\subsection*{2.2.2 Pharmacological Actions and Therapeutic Used}

The drugs which are able of lowering circulating blood lipid level was discovered in the early 1950s, before epidemiological studies showing a direct correlation between cholesterol blood levels and coronary risk (Kannel \textit{et al.}, 1961 cited by Desager and Horsmans, 1996). However, before 1970, lipid lowering therapy was not considered satisfactory because of its adverse effects. Additionally, improvement in the knowledge of cholesterol biosynthesis and catabolism provide an opportunity for researchers to design and synthesize new drugs (Grundy, 1969 cited by Desager and Horsmans, 1996).

Moreover, the second generation fibrates (e.g. fenofibrate), that act mainly by breaking down cholesterol (or cholesterol containing particles), opened a new era. At the same time, Endo (1992) began an intense period of research into therapeutic inhibition of cholesterol biosynthesis.
The four 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors used today are lovastatin, simvastatin, pravastatin and fluvastatin (Desager and Horsmans, 1996). The HMG-CoA reductase is the key enzyme of cholesterol synthesis. HMG-CoA reductase inhibitors are potent reversible inhibitors of this enzyme, which act by competing for the substrate HMG-CoA (Desager and Horsmans, 1996).

The statins are reversible inhibitors of the microsomal enzyme HMG-CoA to mevalonate. This is an early rate limiting step in cholesterol biosynthesis (Lennernas and Fager, 1997). The rate limiting enzyme in cholesterol synthesis is 3-hydroxy-3-methylglutaryl-coenzyme A reductase. This enzymatic activity takes place during the early steps that lead from acetate to cholesterol skeleton (Desager and Horsmans, 1996). Figure 2.2 shows the mevalonate pathway and cholesterol synthesis. Inhibition of HMG-CoA reductase by statins decreases intracellular cholesterol biosynthesis. The liver is the target organ for the statins, since it is the major site of cholesterol biosynthesis, lipoprotein production and LDL catabolism (Lennernas and Fager, 1997).

The hypocholesterolemic effects of HMG-CoA reductase inhibitors are based on the ability of these drugs to partially inhibit hepatic HMG-CoA reductase, thereby causing a depletion of cellular pool of cholesterol, which, in turn, leads to an increase in the expression of high affinity receptors for low density lipoprotein (LDL) on hepatocyte membranes and concurrently, a reduction in the synthesis of very low density lipoprotein and LDL (Illingworth and Tobert, 1994).

HMG-CoA reductase inhibitors are now widely used and account for the majority of prescriptions for lipid lowering drugs in many countries. HMG-CoA
reductase inhibitors are the most effective agents developed to date for the treatment of patients with primary and secondary hypercholesterolaemia associated with increased levels of LDL cholesterol (Illingworth and Tobert, 1994; Lennernas and Fager, 1997).

Lovastatin, simvastatin, pravastatin and fluvastatin have similar pharmacodynamic properties. All can reduce LDL-cholesterol by 20 to 35%, a reduction which has been shown to achieve decreases of 30 to 35% in major cardiovascular outcomes. Simvastatin has this effect at doses of about half those of other 3 statins (Lennernas and Fager, 1997).

Finally, statins can exert a direct antiatherosclerotic effect on the arterial wall, beyond their lipid lowering properties, which could translate into a more significant prevention of heart disease (Corsini et al., 1999).
Figure 2.1 Chemical structures of the main 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (Adopted from Desager and Horsmans, 1996)
Figure 2.2 The mevalonate pathway and cholesterol synthesis
(Adopted from Desager and Horsmans, 1996)
2.2.3 Overview of Pharmacokinetics Properties of 3-Hydroxy-3-
Methylglutaryl-Coenzyme A Reductase Inhibitors

The pharmacokinetics of HMG-CoA reductase inhibitors have been
summarized in several reviews (Slater and MacDonald, 1988; Henwood and
Heel, 1988; Pan et al., 1990; Todd and Goa, 1990; Pentikainen et al., 1992;
Jungnickel et al., 1992; McTavish and Sorkin, 1992; Mauro, 1993; Quion and
Jones, 1994; Illingworth and Tobert, 1994; Deslypere, 1995; Plosker and
McTavish, 1995; Plosker and Wagstaff, 1996; Haria and McTavish, 1997; Lea
and McTavish, 1997; Kong et al., 1997; Paoletti et al., 2002).

Six reviews have specifically focused on the similarities and differences
of HMG-CoA reductase inhibitors (Desager and Horsmans, 1996; Lennernas
and Fager, 1997; Christians et al., 1998; Corsini et al., 1999; Davidson and
Toth, 2004; Shitara and Sugiyama, 2006), and others have been devoted to
specific aspects, such as tissue distribution (Sirtori, 1993) and drug interactions
(Christians et al., 1998; Jacobson, 2004; Shitara and Sugiyama, 2006). The
pharmacokinetics of HMG-CoA reductase inhibitors are compared in Table 2.1.

In general, statins absorption from the small and large intestine is
affected by dose/dissolution ratio, dissolution rate, degradation/metabolism in
the lumen, complex binding in the lumen, intestinal transit and effective
permeability across the intestinal mucosa (Lennernas and Fager, 1997).

Lovastatin and simvastatin are prodrugs and are converted by hydrolytic
enzymes in the plasma and liver to the active acid form (Tang and Kalow,
1995). All other HMG-CoA reductase inhibitors are given in their active form.

Moreover, lovastatin and simvastatin in their lactone form, but not in their
acid form, cross the blood brain barrier (Tsuji et al., 1993; Saheki et al., 1994;
Lennernas and Fager, 1997). More hydrophilic HMG-CoA reductase inhibitors, such as pravastatin and fluvastatin do not reach the central nervous system (CNS) in measurable concentrations (Tsuji et al., 1993; Saheki et al., 1994; Christians et al., 1998). Distribution into the CNS is dependent on lipophilicity (Sirtori, 1993) and affinity to \( p \)-glycoproteins, which constitute an important efflux mechanism for lipophilic drugs as part of the blood-brain barrier (Tsuji et al., 1993; Saheki et al., 1994; Shitara and Sugiyama, 2006). In addition, immunosuppressants such as cyclosporine, tacrolimus, and sirolimus (Christians et al., 1996, Corsini et al., 1999) and several HMG-CoA reductase inhibitors are \( p \)-glycoprotein substrates. This constitutes a potential drug interaction locus which results in enhanced access of HMG-CoA reductase inhibitors and/or immunosuppressants into the CNS.

Pravastatin is the only HMG-CoA reductase inhibitor that is mainly eliminated unchanged (Quion and Jones, 1994). Its main metabolite is inactive and it has a terminal plasma half-life slightly shorter than that of pravastatin (Corsini et al., 1999; Davidson and Toth, 2004; Shitara and Sugiyama, 2006). All other HMG-CoA reductase inhibitors are eliminated mostly as metabolites and with the exception of fluvastatin, have active metabolites that significantly contribute to their lipid-lowering effect. There is indirect evidence that active metabolites of lovastatin, simvastatin, atorvastatin and cerivastatin with longer terminal half-life than the parent compound exist, but none of these has been well characterized (Corsini et al., 1999).

Pharmacokinetic studies using non-specific analytical methods yielded markedly longer terminal plasma half-life for lovastatin and simvastatin than studies using specific methods (Lennernas and Fager, 1997). Long terminal
half-life of the parent drug or its metabolites was led to accumulation in plasma and tissues, which can facilitate toxic consequences. Since drug interactions with the elimination of HMG-CoA reductase inhibitors complicate lipid-lowering therapy in cyclosporine treated transplant patients, an HMG-CoA reductase inhibitor with short terminal half-life for parent drug and metabolites, such as pravastatin or fluvastatin, might be a safer choice in this specific group of patients. Among the HMG-CoA reductase inhibitors, pravastatin exhibits the highest clearance by the kidney. It is eliminated both by the kidneys (average, 47%) and the liver (53.00%) to a similar extent (Singhvi et al., 1990; Quion and Jones, 1994). Pravastatin pharmacokinetics were not significantly changed in patients with mild, moderate, and severe kidney dysfunction in comparison with healthy controls (Halstenson et al., 1992), implying that impaired renal clearance was compensated for by an increase in liver clearance.

The plasma concentrations of total HMG-CoA reductase inhibitors after a single dose of lovastatin were 2-fold higher in patients with renal insufficiency (creatinine clearance < 30 mL.min⁻¹) (Christians et al., 1998; Corsini et al., 1999; Davidson and Toth, 2004), as compared with patients with normal renal function, leading to the recommendation that lovastatin doses > 20 mg. day⁻¹ should be implemented with caution in these patients. The extent of dosage adjustment applies to transplant patients, for whom low lovastatin doses of < 20 mg are recommended, but who are at a higher risk than non transplant patients due to drug interactions with cyclosporine, is unknown. There are no significant changes in the area under the curve (AUC) values of atorvastatin and cerivastatin (Christians et al., 1998) found in patients with renal impairment.
Data about the influence of impaired kidney function on simvastatin and fluvastatin pharmacokinetics are not available. But since about 90% of these drugs are eliminated by the liver, little impact would be anticipated (Christians et al., 1998).

Compared with healthy subjects, pravastatin AUC values were increased by only 34% in patients with liver cirrhosis (Christians et al., 1998, Davidson and Toth, 2004; Shitara and Sugiyama, 2006). Fluvastatin AUC and $C_{\text{max}}$ values were 2.5 fold higher in patients with liver cirrhosis than in healthy subjects. Atorvastatin AUC values and $C_{\text{max}}$ were 7 and 5 fold higher, respectively, in patients with impaired liver function than in healthy controls and the increase was dependent on the severity of liver disease: the AUC values were 4 fold higher in patients with Childs-Pugh A and 12 fold higher in patients with Childs-Pugh B liver impairment. The elimination half-life and $T_{\text{max}}$ were not changed, but the higher AUC values did result in a more efficient lipid-lowering effect. On the basis of these pharmacokinetic changes, atorvastatin is not recommended for treatment of patients with liver dysfunction (Christians et al., 1998).

Additionally, the effect of impaired liver function on the plasma concentrations of lovastatin, simvastatin (Christians et al., 1998), and cerivastatin has not been reported yet, but considering the important role of the liver in their elimination, a significant effect can be expected. In addition, in transplant patients with cholestasis, such as liver graft patients (Christians et al., 1991) and bone marrow graft patients with graft versus host disease of the liver (Christians et al., 1996; Christians et al., 1998), cyclosporine metabolites accumulate and potentially enhance the cyclosporine drug interaction with the elimination of HMG-CoA reductase inhibitors. At the moment, pravastatin is the
only HMG-CoA reductase inhibitor studied in liver graft patients, and it was found to be safe (Christians et al., 1998; Corsini et al., 1999; Davidson and Toth, 2004; Shitara and Sugiyama, 2006).

Fresh or frozen grapefruit inhibits intestinal CYP3A4 but appears to have minimal effects on hepatic CYP3A4. Therefore, grapefruit juice (at least 200 mL) can increase serum concentrations of numerous CYP3A4 substrate drugs that undergo intestinal first-pass metabolism by this enzyme. This effect has been reported by Kupferschmidt et al (1993); Hollander et al (1995); Ameer and Weintraub (1997); Lilja et al (1998); Gruer et al (1999).

Grapefruit juice was reported to significantly increase serum concentrations of the calcium channel blocker felodipine and simvastatin. Pravastatin pharmacokinetics is not affected by grapefruit juice (Ameer and Weintraub, 1997; Lilja et al., 1999; Shitara and Sugiyama, 2006). The primary substance responsible for inhibition was identified in vitro to be a furanocoumarin compound which is widely found in nature, 6,7-dihydroxybergamottin. This inhibitory substance is less potent than known CYP3A4 inhibitors such as ketoconazole, and itraconazole (Neuvonen et al., 1997). There is a lack of published reports of myopathy caused by grapefruit juice and CYP3A4 statins are lacking.

Nevertheless, it is probably advisable to separate by 2 hours the dosing of CYP3A4 statins with grapefruit juice (Lilja et al., 2000). Orange juice, which lacks 6,7-dihydroxybergamottin, does not inhibit CYP3A4. Little is known about the effects of other citrus fruits on CYP enzymes (Fuhr and Frummert 1995; Ameer and Weintraub, 1997).
Table 2.1 Comparison of HMG-CoA Reductase inhibitors (Adopted from Christians et al., 1998)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Lovastatin</th>
<th>Simvastatin</th>
<th>Pravastatin</th>
<th>Fluvastatin</th>
<th>Atorvastatin</th>
<th>Cerivastatin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prodrug</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Crosses blood brain barrier</td>
<td>Lactone</td>
<td>Lactone</td>
<td>No</td>
<td>No</td>
<td>N.A</td>
<td>N.A.</td>
</tr>
<tr>
<td>Lipophilicity</td>
<td>Lipophilic</td>
<td>Lipophilic</td>
<td>Hydrophilic</td>
<td>Hydrophilic</td>
<td>Lipophilic</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

**Oral pharmacokinetics**

- **Dose (mg/day)**
  - Lovastatin: 20-8
  - Simvastatin: 10-40
  - Pravastatin: 20-4
  - Fluvastatin: 20-80
  - Atorvastatin: 2.5-80
  - Cerivastatin: 0.1-0.3

- **Absorption (%)**
  - Lovastatin: 30
  - Simvastatin: 60-85
  - Pravastatin: 35
  - Fluvastatin: 98
  - Atorvastatin: N.A.
  - Cerivastatin: 12

- **Bioavailability (%)**
  - Lovastatin: < 5
  - Simvastatin: < 5
  - Pravastatin: 10
  - Fluvastatin: 10-35
  - Atorvastatin: 12
  - Cerivastatin: 60

- **Effect of food**
  - Lovastatin: ↑ 50%
  - Simvastatin: No
  - Pravastatin: ↓ 30%
  - Fluvastatin: ↓ 15-25%
  - Atorvastatin: ↓ 13%
  - Cerivastatin: ↓ 23%

- **T<sub>max</sub>**
  - Lovastatin: 2-6
  - Simvastatin: 1.3-2.4
  - Pravastatin: 0.9-1.6
  - Fluvastatin: 0.5-1.5
  - Atorvastatin: 2-4
  - Cerivastatin: 0.5-4

- **Terminal half-life (hr)**
  - Lovastatin: 2.5-15
  - Simvastatin: 1.9-15.6
  - Pravastatin: 1.3-2.6
  - Fluvastatin: 0.5-3.1
  - Atorvastatin: 14
  - Cerivastatin: 1.7-2.7

- **Hepatic extraction (%)**
  - Lovastatin: 62-69
  - Simvastatin: > 78
  - Pravastatin: 46
  - Fluvastatin: 68
  - Atorvastatin: N.A.
  - Cerivastatin: N.A.

- **Renal elimination (%)**
  - Lovastatin: > 90
  - Simvastatin: > 90
  - Pravastatin: 43-48
  - Fluvastatin: 95-98
  - Atorvastatin: > 95
  - Cerivastatin: N.A.

- **Protein binding (%)**
  - Lovastatin: > 90
  - Simvastatin: Yes
  - Pravastatin: Yes
  - Fluvastatin: Yes
  - Atorvastatin: Yes
  - Cerivastatin: Yes

- **p-Glycoprotein substrate**
  - Lovastatin: Yes
  - Simvastatin: N.A.
  - Pravastatin: Yes
  - Fluvastatin: Yes
  - Atorvastatin: N.A.
  - Cerivastatin: N.A.

- **CYP substrate**
  - Lovastatin: CYP3A
  - Simvastatin: CYP3A
  - Pravastatin: No
  - Fluvastatin: CYP2C9
  - Atorvastatin: CYP3A
  - Cerivastatin: CYP3A

- **Metabolites effect**
  - Lovastatin: Yes
  - Simvastatin: Yes
  - Pravastatin: No
  - Fluvastatin: No
  - Atorvastatin: Yes
  - Cerivastatin: Yes

- **Mostly eliminated as**
  - Lovastatin: Metabolites
  - Simvastatin: Metabolites
  - Pravastatin: Unchanged
  - Fluvastatin: Metabolites
  - Atorvastatin: N.A.
  - Cerivastatin: Metabolites

N.A., not available
2.3 Simvastatin

2.3.1 Chemical Structure

Simvastatin is a methyl analogue of lovastatin and is synthesized from a fermentation product of *Aspergillus terreus* (Hoffman et al., 1986). Simvastatin is a nonhygroscopic white crystalline powder, insoluble in water but quite soluble in chloroform, methanol and alcohol (Mauro, 1993) with pK$_a$ of 4.68 (Corsini et al., 1999). The molecular weight of this compound C$_{25}$H$_{38}$O$_5$ is 418.57. Simvastatin is the pharmacologically inactive lactone form of simvastatin acid, butanoic acid, 2,2-dimethyl-1,2,3,7,8,8a-hexahydro-3,7-dimethyl-8-[2-(tetrahydro-4-hydroxy-6-oxo-2H-pyran-2-yl) ethyl]-1-naphthalenyl ester. Simvastatin is a lactone which needs the opening of the ring for it to become active. Figure 2.3 shows the chemical structure of simvastatin (Mauro, 1993).