

羟基咪达唑仑的测定采用高效液相色谱法（HPLC），参照先前方法并稍作修改。²¹ HPLC分离在 C₁₈ HPLC分析柱（4.60 × 100 mm 内径；3.5 μm 粒径）上进行，采用等度洗脱系统。流动相由10 mM醋酸钠（pH 4.0）和乙腈（55:45，v/v）组成。流速为 0.80 mL/min，于 30°C 条件下，在 220 nm 波长处监测洗脱液吸光度。

实时荧光定量RT-PCR 分析

使用TRIzol试剂（Life Sciences）按制造商说明从液氮速冻的组织样本中分离肝脏和肠道RNA。总RNA采用 ImProm-II™逆转录系统（Promega）转录为cDNA。编码 Mdr1a、Mdr1b和Slc21a5的mRNA通过TaqMan基因表达分析（Applied Biosystems, Carlsbad, CA）进行检测。目的基因表达管家基因甘油醛-3-磷酸脱氢酶基因（GAPDH）标准化后，使用StepOnePlus™ 软件通过 2^{-ΔΔCt} 计算。

药代动力学分析

辛伐他汀和辛伐他汀酸的最大血药浓度 (C_{max}) 和达峰时间 (t_{max}) 直接从浓度-时间曲线中获得。从零时到最后可定量浓度 (AUC_{0-t}) 的血浆浓度-时间曲线下面积采用梯形法则计算。辛伐他汀和辛伐他汀酸的消除速率常数 (k_{el}) 通过对浓度-时间曲线末端对数线性部分进行线性回归分析计算。AUC_{t-∞} 通过以下公式确定：
AUC_{t-∞} = C_{last} /k_{el}。消除半衰期 (t_{1/2}) 通过以下公式计算： t_{1/2} = ln 2/k_{el}.²²

统计分析

除 t_{max} 采用Wilcoxon秩检验分析外，所有药代动力学参数的统计比较均采用非配对双尾Student's t检验。mRNA 表达和CYP3a2活性分析的参数差异亦采用非配对双尾 Student's t检验。上述各项检验中，*p<0.05和**p<0.01视为差异具有显著性

结果

柚子汁中的总黄酮含量

通过显色定量分析，我们发现Thong-dee柚子汁中的总黄酮含量范围为9,169.37-12,782.62 μg/100 g柚子可食部分，基于8次重复分析。

辛伐他汀及其活性代谢物辛伐他汀酸的血药浓度-时间曲线下面积

我们随后考察了柚子汁对辛伐他汀及其主要活性代谢物辛伐他汀酸药代动力学特征的影响

大鼠单独口服辛伐他汀 (20mg/kg) 以及辛伐他汀联用柚子汁 (1 mL/kg) 后，辛伐他汀和辛伐他汀酸的平均血药浓度-时间曲线见图1，药代动力学参数见表1。如图1A所示，柚子汁显著影响了辛伐他汀的药代动力学特征。与单独给予辛伐他汀的大鼠相比，辛伐他汀联用柚子汁的大鼠中辛伐他汀的 AUC_{0-∞} 和 C_{max} 分别显著增加了4倍 (*p* < 0.01) 和3.9倍 (*p* < 0.01)。尽管 AUC_{0-∞} 和 C_{max} 发生了显著变化，柚子汁并未显著改变辛伐他汀的 t_{max}，t_{1/2} 和 k_{el}。与辛伐他汀类似，柚子汁使辛伐他汀酸的 AUC_{0-∞} 和 C_{max} 分别显著增加了 3 (*p* < 0.01) 倍和3.6倍 (*p* < 0.01)（图1B）。单独给予辛伐他汀的大鼠与辛伐他汀联用柚子汁的大鼠之间，辛伐他汀酸的 t_{max}，t_{1/2} 和 k_{el} 无差异。

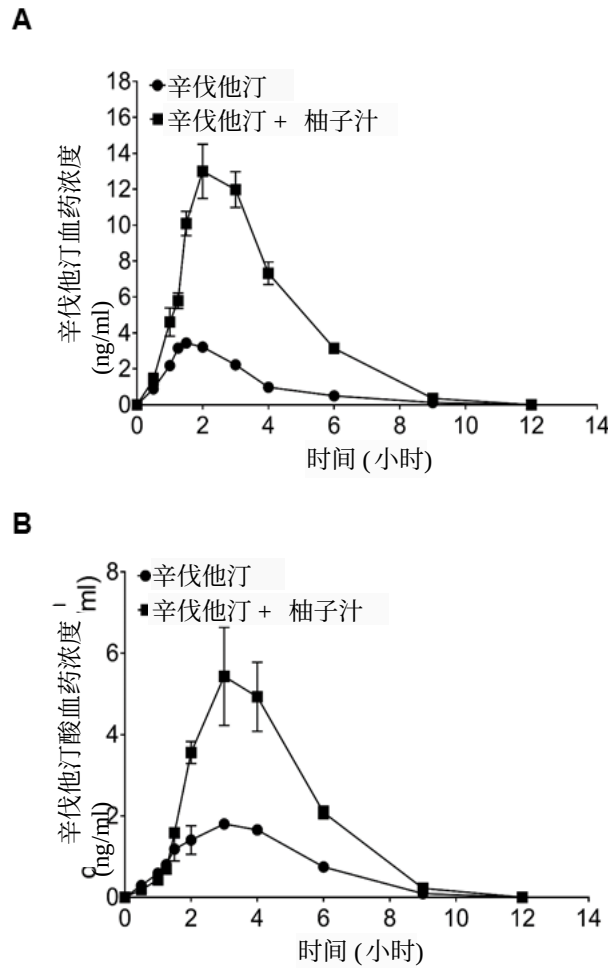


图1. 大鼠口服 20mg/kg 辛伐他汀单独给药或联用1 mg/kg柚子汁（每日两次，连续7天，并于第8天服用辛伐他汀前30分钟给予）后，(A) 辛伐他汀和 (B) 辛伐他汀酸的平均血药浓度-时间曲线。

hydroxymidazolam, was carried out using high performance liquid chromatography (HPLC) as previously described with minor modifications.²¹ HPLC separation were performed on the C₁₈ HPLC analytical column (4.60 × 100 mm i.d.; 3.5 μm particle size) with an isocratic elution system. The mobile phase consisted of 10 mM sodium acetate with pH 4.0 and acetonitrile (55:45, v/v). The flow rate was 0.80 mL/min, at 30 °C and the absorbance of eluent was monitored at 220 nm.

Real time RT-PCR analysis

Hepatic and intestinal RNA was isolated form tissue samples snap frozen in liquid nitrogen using TRIzol reagent (Life Sciences), according to the manufacturer’s instructions. Total RNA was transcribed to cDNA using ImProm-II™ Reverse Transcription System (Promega). The mRNAs encoding for *Mdr1a*, *Mdr1b* and *Slc21a5* were determined by TaqMan gene expression assays (Applied Biosystems, Carlsbad, CA). Expression of the interested genes normalized to housekeeping gene glyceraldehyde-3-phosephate dehydrogenase gene (GAPDH) was calculated by 2^{-ΔΔCt} using StepOnePlus™ software.

Pharmacokinetic analysis

The maximum blood concentration (C_{max}) and time to maximum concentration (t_{max}) of simvastatin and simvastatin acid were obtained directly from the concentration-time profiles. The area under the plasma concentration-time curve from time zero to the last quantifiable concentration (AUC_{0-t}) was calculated using the trapezoidal rule. The elimination rate constant (k_{el}) for simvastatin and simvastatin acid were calculated by linear regression analysis of the terminal log-linear part of the concentration-time profiles. The AUC_{t-∞} was determined by the equation: AUC_{t-∞} = C_{last}/k_{el}. The elimination half-life (t_{1/2}) was calculated by the equation: t_{1/2} = ln2/k_{el}.²²

Statistical analysis

An unpaired two-tailed Student’s t-test was used for statistical comparison of all pharmacokinetic parameters except t_{max} which was analyzed by the Wilcoxon rank test. Differences in parameters of mRNA expression and CYP3a2 activity analysis also employed by the unpaired two-tailed Student’s t-test. For each of these tests, differences were considered significant when **p*<0.05 and ***p* < 0.01

Results

Total flavonoid content in pomelo juice

Using chromogenic quantitative assay, we found that total flavonoid content present in the Thong-dee pomelo juice was in the range of 9,169.37-12,782.62 μg/100 g of edible portion of pomelo from 8 duplicate analyses.

Area under the plasma drug concentration time curve of simvastatin and simvastatin acid

We then investigated the effect of pomelo juice on pharmacokinetic profiles of simvastatin and simvastatin

acid, its primary active metabolite. The mean plasma concentration-time profiles of simvastatin and simvastatin acid after oral administration of simvastatin alone (20 mg/kg) and simvastatin in concomitant with pomelo juice (1 mL/kg) are shown in Figure 1 and the pharmacokinetics parameters of simvastatin and simvastatin acid are illustrated in Table 1. As shown in Figure 1A, pomelo juice significantly affected pharmacokinetic profile of simvastatin. AUC_{0-∞} and C_{max} of simvastatin in rats receiving simvastatin combined with pomelo juice were significantly increased by 4-fold (*p*<0.01) and 3.9-fold (*p*<0.01), respectively, when compared with those in rats receiving simvastatin alone. Despite notable changes in AUC_{0-∞} and C_{max}, pomelo juice did not significantly alter t_{max}, t_{1/2} and k_{el} of simvastatin. In a manner similar to simvastatin, pomelo juice significantly increased the AUC_{0-∞} and C_{max} of simvastatin acid by a factor of 3 (*p*<0.01) and 3.6 (*p*<0.01), respectively (Figure 1B). There were no differences in t_{max}, t_{1/2} and k_{el} of simvastatin acid between rats treated with simvastatin alone and rats treated simvastatin combined with pomelo juice.

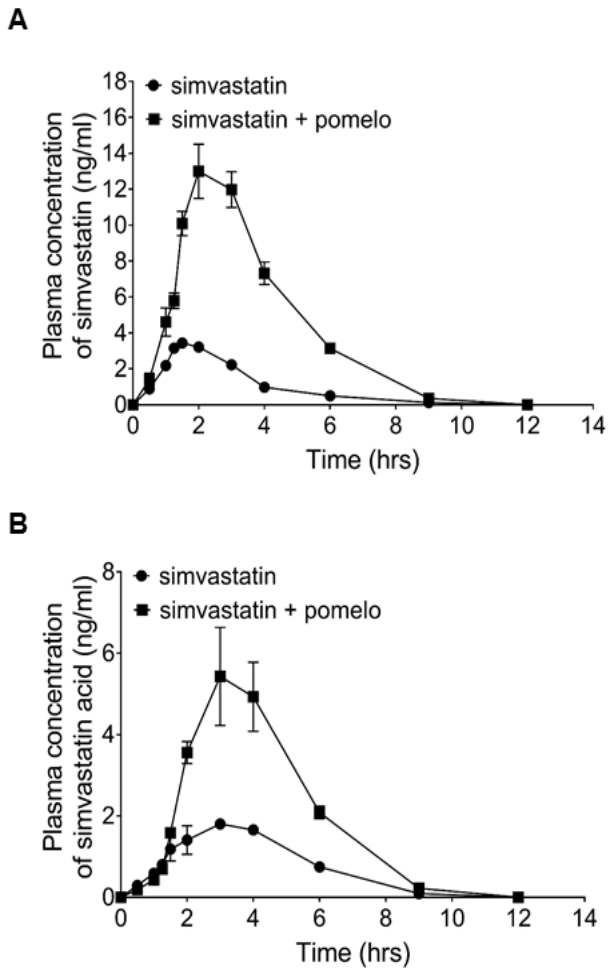


Figure 1. Mean plasma concentration-time profiles of (A) simvastatin and (B) simvastatin acid in rats after oral administration of 20 mg/kg simvastatin alone or concomitant with 1 mg/kg pomelo juice twice daily for 7 days and 30 minutes before simvastatin ingestion at day 8.