曲马氨酚片的人体药物动力学研究

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【摘要】　**目的**　测定曲马氨酚片中曲马多和对乙酰氨基酚的药动学参数。**方法**　采用高效液相色谱法分别测定 20 名健康志愿者口服曲马氨酚片(2 个剂量组:1 片和 2 片,每组 10 名)后血清中的药物浓度,进行药动学分析。**结果**　2 组健康志愿者口服曲马氨酚片后的主要药动学参数:曲马多 $AUC_{0-24\,h}$(ng・h・mL^{-1})分别为 2 724.89±1 016.54,1 361.61±441.79;$AUC_{0-\infty}$(ng・h・mL^{-1})分别为 3 065.49±1 190.66,1 555.04±582.51;t_{max}(h)分别为 1.8±0.75,1.9±0.57;$t_{1/2}$(h)分别为 7.34±1.39,7.63±2.02;Kel(h^{-1})分别为 0.098±0.019,0.097±0.027;Clr(mL・min^{-1})分别为 31.84±13.65,30.03±9.20;MRT(h)分别为 7.62±1.07,7.77±0.75。对乙酰氨基酚的 $AUC_{0-24\,h}$(μg・h・mL^{-1})分别为 40.28±10.36,18.37±3.84;$AUC_{0-\infty}$(μg・h・mL^{-1})分别为 41.63±10.96,18.81±4.06;t_{max}(h)分别为 0.9±0.46,0.9±0.39;$t_{1/2}$(h)分别为 5.39±1.16,4.96±1.03;Kel(h^{-1})分别为 0.13±0.03,0.15±0.03;Clr(mL・min^{-1})分别为 17.17±4.57,18.42±3.89;MRT(h)分别为 4.86±0.48,4.50±0.53。**结论**　各剂量组之间的主要药动学参数(t_{max},$t_{1/2}$,Kel,Clr,V_d,MRT)无显著性差异,各剂量组 AUC_{0-t}/dose,$AUC_{0-\infty}$/dose,C_{max}/dose 比较无显著性差异;符合线性动力学特征。

【关键词】　曲马多;　对乙酰氨基酚;　药物动力学;　高效液相色谱法
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Pharmacokinetics on tramadol/acetaminophen combination tablets in Chinese healthy volunteers

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【**Abstract**】　**Objective**　To study the pharmacokinetic of tramadol and acetaminophen in healthy volunteers. **Methods**　Totally 20 healthy adult male volunteers participated in the study were randomly assigned to 2 treatment groups and were given respectively the dose of one and two pills by oral administration. Serum was separated and the concentrations of tramadol and acetaminophen in human serum were determined by HPLC using fluorescence and UV detector. The values of concentration were directly detected，and AUC was calculated by linear trapezoid method. **Results** The main pharmacokinetic parameters of tramadol and acetaminophen of 2 dosages groups were as follow：Tramadol：$AUC_{0-24\,h}$(ng・h・mL^{-1}) were 2 724.89±1 016.54 and 1 361.61±441.79; $AUC_{0-\infty}$(ng・h・mL^{-1}) were 3 065.49±1 190.66 and 1 555.04±582.51; t_{max}(h) were 1.8±0.75 and 1.9±0.57; $t_{1/2}$(h) were 7.34±1.39 and 7.63±2.02; Kel(h^{-1}) were 0.098±0.019 and 0.097±0.027; Cl$_r$(mL・min^{-1}) were 31.84±13.65 and 30.03±9.20; MRT(h) were 7.62±1.07 and 7.77±0.75. Acetaminophen：$AUC_{0-24\,h}$(μg・h・mL^{-1}) were 40.28±10.36 and 18.37±3.84; $AUC_{0-\infty}$(μg・h・mL^{-1}) were 41.63±10.96 and 18.81±4.06; t_{max}(h) were 0.9±0.46 and 0.9±0.39; $t_{1/2}$(h) were 5.39±1.16 and 4.96±1.03; Kel(h^{-1}) were 0.13±0.03 and 0.15±0.03; Clr(mL・min^{-1}) were 17.17±4.57 and 18.42±3.89; MRT(h) were 4.86±0.48 and 4.50±0.53.

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Conclusions No significant difference in pharmacokinetic parameters, such as t_{max}, $t_{1/2}$, Ke, V, Cl, MRT, AUC_{0-t}/dose, $AUC_{0-\infty}$/dose and C_{max}/dose are shown between these two dose groups and a linear pharmacokinetic is featured.

【**Key words**】 tramadol; acetaminophen; pharmacokinetics; HPLC

Tramadol, (±) cis-2-[(dimethylamino)-methyl]-1-(3-methoxyphenyl) cyclohexanol[1], (Fig 1a), is a centrally acting analgesic. It is used to relieve severe pain. Acetaminophen[1], N-acetyl-p-aminophenol, (Fig 1b), is a non-opiate, non-salicylate analgesic. The U. S. Food and Drug Administration had approved Ultracet™(37.5 mg tramadol hydrochloride/325 mg acetaminophen tablets), a new centrally acting prescription pain medication in 2001. Combining two analgesic agents with complementary mechanisms of action may enhance analgesia and at the same time reduce the risk of adverse events. Furthermore, the ease of taking two analgesic agents as a fixed tablet may improve compliance[2].
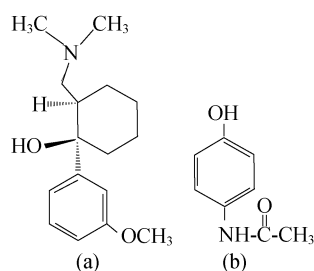
Fig 1   Structure of Tramadol (a) and Acetominophen (b)

In the last decade, several analytical methods for quantifying tramadol and acetaminophen have been reported. However, only two papers[3,4] have reported the simultaneous determination method of the tramadol/acetaminophen combination tablets, in which a liquid chromatography ion trap mass spectrum method and an HPLC-ESI-MS method instrument were employed. These methods are selective, fast and sensitive, but they are not suitable for routine clinical analysis because of their special requirement and financial reasons.

Although the pharmacokinetics and clinical pharmacology of tramadol and acetaminophen have been extensively studied, there is little data of the tramadol/acetaminophen combination tablets. Therefore, a new selective HPLC method with UV and fluorescence detection for the determination of tramadol and acetaminophen in whole blood samples after liquid-liquid extraction is developed in order to carry out a pharmacokinetic study of two oral dosages of the tramadol/acetaminophen combination tablets in healthy male Chinese volunteers.

Materials and Methods

Reagents and materials Test tramadol/acetaminophen combination tablets (batch number: 20021115, 37.5 mg tramadol/tablet, 325 mg acetaminophen/tablet) were supplied by Sida Pharmaceutical Co. Ltd. (Haikou, Hainan Province, PR China). Tramadol standard (batch number: 171242-200302) and acetaminophen standard (batch number: 100018-200107) were purchased from National Institute For the Control of Pharmaceutical and Biological Products (NICPBP) Beijing, PR China. The internal standard (IS) caffeine (>99.5%) and theophylline (>99.5%) were obtained from Shanghai Institute for Drug Control (Shanghai, PR China).

Methanol (HPLC grade reagent) and acetonitrile (HPLC grade reagent) were obtained from Merck Inc. (Darmstadt, Germany). trifluoroacetic acid(Analytical reagent, batch number: F20020913) was obtained from Sinopharm Chemical Reagent Co. Ltd (Shanghai, PR China).

Apparatus The analysis was performed on an Agilent HP 1100 HPLC system (Agilent Corp. Ltd. Palo Alto, CA, USA) equipped with binary pump, fluorescence detector, ultraviolet detector, on-line vacuum degasser, column compartment and Agilent ChemStation (Agilent Corp. Ltd. Palo Alto, CA, USA). The samples were separated on an Inertsil ODS (200 mm × 4.6 mm, 5 μm) column (GL. Sciences Inc. , Tokyo, Japan).

Stock solutions Stock solutions of tramadol were prepared at 100 μg · mL^{-1} in 0.1 mol · L^{-1} HCL and stored at 4 ℃ until use. The stock IS caffeine solution was prepared as methanol solution

$(100 \ \mu g \cdot mL^{-1})$. All stock solutions were proved to be stable for at least two months when stored at 4 ℃.

Stock solutions of acetaminophen were prepared at 100 $\mu g \cdot mL^{-1}$ in 0.1 mol $\cdot L^{-1}$ HCL and stored at 4 ℃ until use. The stock IS theophylline solution was prepared as methanol solution $(200 \ \mu g \cdot mL^{-1})$. All stock solutions were proved to be stable for at least two months when stored at 4 ℃.

Plasma sample preparation

Plasma sample preparation of tramadol A 0.5 mL plasma sample was extracted with 5 mL diethyl ether after addition of 50 μL IS solution $(106 \ \mu g \cdot mL^{-1})$ and 200 μL NaOH solution $(1 \ mol \cdot L^{-1})$. After vortex mixing for 2 min, the upper layer was evaporated in a water bath (40 ℃) with nitrogen steam. The residue was reconstituted in 100 μL mobile phase and 20 μL aliquot was injected into the chromatograph.

Plasma sample preparation of acetaminophen A 0.5 mL plasma sample was extracted with 5 mL acetic ether after addition of 100 μL IS solution $(20.2 \ \mu g \cdot mL^{-1})$ and 500 μL phosphate buffer solution (pH 7.2). After vortex mixing for 2 min, the upper layer was evaporated in a water bath (40 ℃) with nitrogen steam. The residue was reconstituted in 100 μL methanol and 20 μL aliquot was injected into the chromatograph.

HPLC condition

HPLC condition for tramadol HPLC separation of tramadol was performed using an Inertsil ODS (200 mm × 4.6 mm, 5 μm) reversed phase column at a column temperature of 30 ℃. Methanol $-$ 0.2% trifluoroacetic acid aqueous solution $(65/35, v/v)$ was used as mobile phase at a flow rate of 1.0 mL/min. Tramadol and the IS caffeine were detected by the fluorescence detector. The excitation and emission wavelengths were 273 and 300 nm, respectively.

HPLC condition for acetaminophen HPLC separation of acetaminophen was performed using an Inertsil ODS (200 mm × 4.6 mm, 5 μm) reversed phase column at a column temperature of 30 ℃. 0.2% trifluoroacetic acid aqueous solution-acetonitrile $(80/20, v/v)$ was used as mobile phase at a flow rate of 1.0 mL/min. Acetaminophen and the IS theophylline were detected by the UV detector, and the wavelength was set at 249 nm.

Selectivity and specificity The method selectivity was demonstrated on four blank plasma samples obtained from healthy volunteers: the chromatograms were found to be free of interfering peaks. A typical chromatogram of a plasma sample with tramadol and a chromatogram of a plasma sample with acetaminophen are shown in Fig 2.

Linearity and calibration curves Acceptable linearity of tramadol was observed over the concentration range of 5 − 500 ng $\cdot mL^{-1}$ plasma, and the typical calibration line (obtained using the least-squares method) could be expressed as Y = 0.007 9C − 0.001 5 $(r = 0.999 1)$, where Y is the peak area ratio and C is the concentration. And the linearity of acetaminophen was observed over the concentration range of 0.02 − 10 $\mu g \cdot mL^{-1}$ plasma, and the typical calibration line could be expressed as Y = 0.489 8C − 0.006 7 $(r = 0.999 9)$.

Accuracy and precision The R.S.D. of tramadol ranged from 2.44 to 6.34% for intra-day and from 5.18 to 6.79% for inter-day, respectively. And the R.S.D of acetaminophen ranged from 0.83 to 4.79% for intra-day and from 3.22 to 11.32% for inter-day, respectively.

Extraction recovery The extraction recovery of tramadol was determined at concentrations of 20, 100 and 320 ng $\cdot mL^{-1}$. And the extraction recovery of acetaminophen was determined at concentrations of 0.1, 0.5 and 5.0 $\mu g \cdot mL^{-1}$, respectively. Recovery was calculated by comparison of the peak areas of tramadol/acetaminophen extracted from plasma samples with those of standards diluted in the mobile phase. And the recoveries were 86.94, 83.91 and 77.84% in 5, 20, 100 ng $\cdot mL^{-1}$ plasma tramadol standard, and they were 69.84, 87.35, 83.14% in 0.1, 0.5, 5 $\mu g/mL$ plasma acetaminophen standard respectively.

Stability The stability was also assessed after storage at − 20 ℃ for one month of tramadol and acetaminophen plasma samples spiked at 350 ng $\cdot mL^{-1}$ and 2 $\mu g \cdot mL^{-1}$, respectively. Tramadol and acetaminophen were determined at the 1st,

10th and 30th day （$n = 3$）. The results obtained were between 95 and 105% of the initial value. Freeze and thaw stability were evaluated by examining both the plasma samples of tramadol （350 ng · mL^{-1}） and the ones of acetaminophen （2 μg · mL^{-1}） after three freeze-thaw cycles. No significant degradation of both tramadol and acetaminophen was observed.
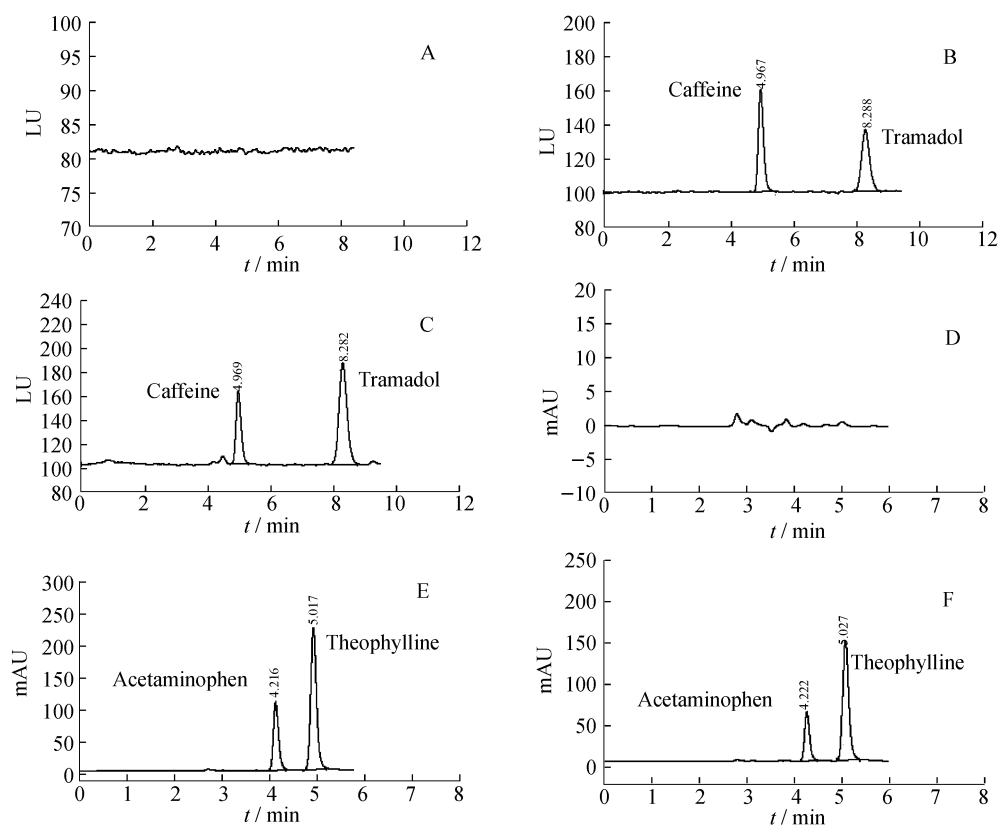
Fig 2   Chromatograms of different groups

A:Blank plasma of tramadol; B:Blank plasma spiked with 100 ng/mL tramadol and 10 μg/mL caffeine (internal standard); C:Plasma sample from a healthy volunteer 3 h after oral administration 37.5 mg of tramadol; D:Blank plasma of acetaminophen; E:Blank plasma spiked with 5 μg/mL acetaminophen and 20 μg/mL theophyline (internal standard); F:Plasma sample from a healthy volunteer 3 h after oral administration 325 mg of acetaminophen

Pharmacokinetics   The present HPLC method was employed for pharmacokinetic study on two doses of tramadol/acetaminophen combination tablets. Twenty healthy male volunteers were randomly divided into 2 groups，each 10 participants. Group A （mean age 22.5 years，mean body mass 65.6 kg） received a single dose of one tramadol/acetaminophen combination tablet and group B （mean age 20.6 years，mean body mass 62.7 kg） received a single dose of 2 pills. The volunteers fasted overnight before the administration of drug and 4 h post-dosing. Any medication，cigarette，wine and drinks containing caffeine were not allowed at least two weeks prior to and during the periods of the test. Blood samples （3 mL） were drawn and transferred into heparinized tubes before dosing and 0.5，1，1.5，2，3，4，5，6，8，12，24 h post-dosing. The plasma samples were separated and frozen at -20 ℃ before analysis. The clinical pharmacokinetic study was approved by Shanghai Jing-an District Centre Hospital. All volunteers gave written informed consent to participate in the study according to the principles of the Declaration of Helsinki.

Results and Discussions

The mean （10 volunteers） plasma concentration-time curves of 2 oral dosages of tramadol （37.5 mg and 75 mg） are shown in （Fig 3）. The mean （10 volunteers） plasma concentration-time curves of 2 oral

dose of acetominophen (325 mg and 650 mg) are shown in (Fig 4). The main pharmacokinetic parameters (C_{max}, t_{max}, $AUC_{0-24 h}$, $AUC_{0-\infty}$, $t_{1/2}$) of tramadol are shown in Table 1, and the main pharmacokinetic parameters (C_{max}, t_{max}, $AUC_{0-24 h}$, $AUC_{0-\infty}$, $t_{1/2}$) of acetaminophen are shown in Table 2.

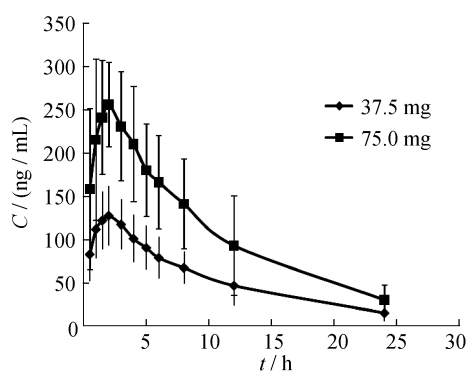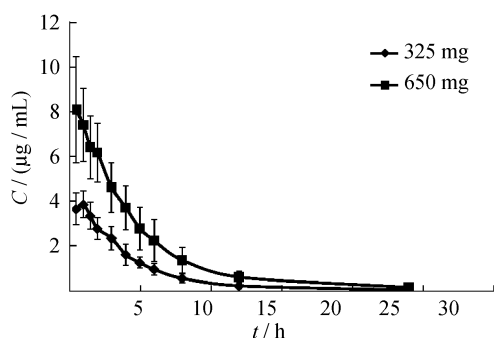**Fig 3　The mean plasma concentration curves after oral administration of 37.5 mg and 75 mg tramadol ($n=20$)**

Fig 4　The mean plasma concentration curves after oral administration of 325 mg and 650 mg acetaminophen ($n=20$)

Tab 1　Pharmacokinetic parameters of tramadol after oral administration of two doses (75 mg and 37.5 mg) to 20 subjects　($\bar{x} \pm s$)

Parameters	Tramadol	
	75 mg	37.5 mg
$AUC_{0-24 h}(\text{ng} \cdot \text{h} \cdot \text{mL}^{-1})$	2 724.89 ± 1 016.54	1 361.61 ± 441.79
$AUC_{0-\infty}(\text{ng} \cdot \text{h} \cdot \text{mL}^{-1})$	3 065.49 ± 1 190.66	1 555.04 ± 582.51
$C_{max}(\text{ng} \cdot \text{mL}^{-1})$	268.14 ± 56.03	134.81 ± 33.96
$t_{max}(\text{h})$	1.8 ± 0.75	1.9 ± 0.57
$t_{1/2}(\text{h})$	7.34 ± 1.39	7.63 ± 2.02
$\text{Kel}(\text{h}^{-1})$	0.098 ± 0.019	0.097 ± 0.027
$V_d(\text{L} \cdot \text{kg}^{-1})$	5.66 ± 2.86	5.67 ± 2.59
$Cl_r(\text{mL} \cdot \text{min}^{-1})$	31.84 ± 13.65	30.03 ± 9.20
$\text{MRT}(\text{h})$	7.62 ± 1.07	7.77 ± 0.75

Tab 2　Pharmacokinetic parameters of acetaminophen after oral administration of two doses (650 mg and 325 mg) to 20 subjects　($\bar{x} \pm s$)

Parameters	Acetaminophen	
	650 mg	325 mg
$AUC_{0-24 h}(\mu\text{g} \cdot \text{h} \cdot \text{mL}^{-1})$	40.28 ± 10.36	18.37 ± 3.84
$AUC_{0-\infty}(\mu\text{g} \cdot \text{h} \cdot \text{mL}^{-1})$	41.63 ± 10.96	18.81 ± 4.06
$C_{max}(\mu\text{g} \cdot \text{mL}^{-1})$	8.48 ± 1.85	4.09 ± 0.60
$t_{max}(\text{h})$	0.90 ± 0.46	0.90 ± 0.39
$t_{1/2}(\text{h})$	5.39 ± 1.16	4.96 ± 1.03
$\text{Kel}(\text{h}^{-1})$	0.13 ± 0.03	0.15 ± 0.03
$V_d(\text{L} \cdot \text{kg}^{-1})$	2.20 ± 0.62	2.19 ± 0.59
$Cl_r(\text{mL} \cdot \text{min}^{-1})$	17.17 ± 4.57	18.42 ± 3.89
$\text{MRT}(\text{h})$	4.86 ± 0.48	4.50 ± 0.53

A simple, specific and accurate HPLC method for determination of tramadol and acetaminophen in human plasma was established. This method is suitable for the pharmacokinetic studies and therapeutic drug monitoring of tramadol and acetaminophen in human subjects. The major pharmacokinetic parameters of tramadol/acetaminophen combination tablets obtained in Chinese volunteers are very important for its optimal clinical usage. Comparing the pharmacokinetic parameters of tramadol and acetaminophen between two dosage group (see Table 1 and Table 2), it was found that there were no significant differences in t_{max}, $t_{1/2}$, Ke, V_d, Cl, MRT, AUC_{0-t}/dose, $AUC_{0-\infty}/\text{dose}$ and C_{max}/dose ($P>0.01$). It means that different dosages of tramadol and acetaminophen did not affect or change the absorption, distribution and elimination in the human body. There are several studies on the pharmacokinetics of some conventional tablets which contain either tramadol or acetaminophen been reported[5-9]. Comparing with the reported ones, no significant differences in t_{max}, $t_{1/2}$, MRT, AUC_{0-t}/dose, $AUC_{0-\infty}/\text{dose}$ and C_{max}/dose were observed. For example, the average $t_{1/2}$ of tramadol and acetaminophen reported are usually 5.33 - 6.30 h and 2.71 - 3.17 h, the average $AUC_{0-\infty}$ of tramadol(dose of 75 mg) and acetaminophen(dose of 650 mg) reported are usually 2 878 - 3 340 ng h · mL^{-1} and 43.08 - 74.21 μg h · mL^{-1}. It suggests that the combination of tramadol and acetaminophen would not change their pharmacokinetics in the human body.