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Original Research Article

Studies on the toxicokinetics of intragastrically-administered paracetamol, aminophenazone, caffeine and chlorphenamine maleate tablets in rats

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Abstract

Purpose: *To study the toxicokinetics of paracetamol (PCT), aminophenazone (ACP), caffeine (CFN) and chlorphenamine maleate CPM) tablets after a single oral gavage, and after oral gavage for 14 consecutive days in rats.*

Methods: *Eighty Sprague Dawley (SD) rats (half male, half female) were randomly divided into 4 groups with 20 rats in each group. Half of the rats were used for the toxicokinetic test after a single oral gavage of PCT, ACP, CFN and CPM tablets, while rats in the other half were used for the toxicokinetic tests after oral gavage for 14 consecutive days. The doses of the four groups were set as 0, 0.5, 1 and 2 tablets/kg body weight, respectively. Blood was taken from the rats and the plasma concentration of paracetamol was determined.*

Results: *There was a significant difference in $AUC_{0-\infty}$ between male and female rats at single oral gavage of 2 tablets/kg of each of the drugs. The exposure amount of PCT (AUC_{0-t}, $AUC_{0-\infty}$ and C_{max}) increased with increase in dose, and showed a good linear relationship after a single intragastric administration of each drug, and after 14 consecutive days of intragastric administration at low, medium and high doses.*

Conclusion: *The amount of PCT to which SD rats are exposed after a single intragastric administration of PCT, ACP, CFN and CPM tablets is lower in male than in female rats. However, no significant gender difference in exposure results when these drugs are given intragastrically for 14 consecutive days.*

Keywords: *Oral gavage, Toxicokinetics, Paracetamol, AUC*

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INTRODUCTION

Paracetamol (PCT) is an aniline antipyretic analgesic which is widely used in clinics. The adverse reactions and serious hepatotoxicity caused by excessive use and abuse of PCT have attracted much research interest [1,2]. Paracetamol (PCT), ACP, CFN and CPM tablets are often used in the treatment of colds, although studies have shown that they are associated with toxic side effects. However, not much is known on the toxicokinetics of PCT following oral administration of PCT, ACP, CFN and CPM

tablets. In the present study, the toxicokinetics of PCT after single and multiple oral intragastric administrations of PCT, ACP, CFN and CPM tablets were investigated.

EXPERIMENTAL

Equipment

The major equipment used were Waters e2695 high performance liquid chromatography, 2998 photodiode matrix detector, Empower 3 chromatographic workstation and CPA225D electronic balance.

Drugs and reagents

Paracetamol, aminophenazone, CFN and CPM tablets were purchased from Shanghai Shangyao Xinyi Pharmaceutical Factory (batch no. 15161002). The drugs contained (per tablet) 0.15 g paracetamol, 0.1 g aminopyrine, 30 mg caffeine and 2 mg chlorphenamine maleate, respectively.

The reference drug APAP was purchased from Shanghai Qiao Yu Biotechnology Co. Ltd (batch number: SJ0711GA14, purity > 98 %). Chromatographically pure methanol (HPLC grade, batch number: 1837 107 623) was purchased from Merck (Germany). Chromatographically pure formic acid was also used (batch number: J259K144. The other reagents used were analytically pure.

Animals

A total of 80 Sprague Dawley (SD) rats (SPF grade, half male and half female) were procured from Shanghai Shrek Experimental Animal Co. Ltd (certificate no. 2015000531973). The rats were aged 8 to 10 weeks, with body weight of 180 to 200 g. They were fed under SFP environment at room temperature (23 ± 1 ^{o}C) and relative humidity of 43 to 64 % in an environment with alternating 12 h light/12 h dark cycle. The use of experimental animals followed the 3R (Reduction, Replacement and Refinement) principle, and the experimental scheme was ethically reviewed and approved prior to implementation, by the Committee on management and use of experimental animals.

This research was approved by the Animal Ethical Committee of Shanghai University of Traditional Chinese Medicine (approval no. 20181022) and was performed according to the guidelines in *Principles of Laboratory Animal Care* (NIH) [3].

Mode of drug administration and doses

The rats were randomly divided into 4 groups with 20 rats in each group. Half of the animals were used for toxicokinetics test after single oral intragastric administrations of PCT, ACP, CFN and CPM tablets. The other half was used for toxicokinetics test after oral intragastric administration of PCT, ACP, CFN and CPM tablets for 14 consecutive days.

Preparation of suspensions of PCT, ACP, CFN and CPM tablets

Phenolamine tablets were ground into fine powder, and the amount to be administered and the volume of solution required were calculated according to the body weights of rats in each group. Suspensions containing no paracetamol (blank control group), aminophenazone (7.5 mg/mL), caffeine (15 mg/mL) and chlorphenamine (30 mg/mL) were prepared by evenly suspending the appropriate amounts of the tested materials in 0.5 % CMC-Na, that is, 0 paracetamol tablet/10 mL (blank control group), 0.5 aminophenazone tablet/10 mL, 1 tablet of caffeine/10 mL, and 2 tablets of chlorphenamine/10 mL.

Uniformity and concentration analyses were carried out on the day of administration of TK test. The concentration was required to be 85 to 115 % of the theoretical concentration, and the RSD of the upper, middle and lower layers of suspension was < 15 %.

Blood specimen collection

Blood was taken from the rats given a single administration of drugs on the same day, while blood was sampled from rats given multiple administrations of drugs after the last administration. Blood was taken from the control only before and 1 h after administration. In the drug treatment groups, blood samples were taken before administration (0 h), and at 10 min, 30 min, 1, 3, 6, 12, 24 and 36 h after drug administration. Blood from each rat was put in an anticoagulant container, and the resultant plasma was separated and kept at -80 ^{o}C prior to use.

Plasma sample pretreatment

Rat plasma (100 µL) was added to 300 µL of chromatographically pure methanol. After vortexing for 1 min, the mixture was centrifuged at 14000 rpm for 10 min. The supernatant (10 µL) was loaded and the content of paracetamol was determined by LC-UV method.

Determination of paracetamol in plasma

Chromatographic conditions

The chromatographic columns used were Kromasil C_{18} (250 mm × 4.6 mm, 5 μm) and Kromasil C_{18} protective column. The mobile phase was methanol: water (0.02 % formic acid) at a volume ratio of 20: 80, and a flow rate of 1.0 mL/min. The detection wavelength was 245 nm, and the column temperature was 35 ^{o}C, while the injection volume was 10 μL.

Specificity

Six different sources of blank plasma chromatogram, blank plasma plus control substance chromatogram (indicated concentration) and plasma sample chromatogram after drug administration, were investigated.

Standard curve and quantitative range

Several 90-μL portions of blank rat plasma were taken, and 10 μL of paracetamol standard solution was added to each portion, with shaking for 1 min, resulting in plasma samples with the paracetamol plasma concentrations of 1, 5, 10, 50, 100, 150 μg/mL, respectively. Then, 10 μL of the sample was applied to the column, and the chromatogram was recorded. Taking the determined concentration of paracetamol as the transverse coordinate and the determined peak area of paracetamol as the longitudinal coordinate, the linear regression equation (standard curve) was obtained using weighted (W = 1 / □^{2}) least square method.

Precision and accuracy

Plasma samples of concentrations 2.5, 30 and 120 μg/mL were prepared and treated as outlined under *Plasma Sample Pretreatment* above. Five sample analyses were carried out for each concentration, and three analytical batches were determined. From the standard curve of each batch, the concentrations of paracetamol in plasma samples were calculated. Based on the results, the intra-batch and inter-batch precision and accuracy of this method were calculated.

Stability

Plasma samples at concentrations of 2.5, 30 and 120 μg/mL were prepared. The stabilities of plasma samples kept at room temperature for 2 h, extracted samples placed in sampler at 4 ^{o}C for 12 h, and standard plasma samples frozen at 80 ^{o}C for 1 week and 2 weeks, were investigated.

Statistical analysis

The toxicokinetic parameters were calculated with non-compartment model (NCA) using DAS professional software. The areas under drug concentration - time curve (AUC_{0-t}) and $AUC_{0-\infty}$ were calculated according to the ladder method, and the peak concentration (C_{max}) and peak time (T_{max}) were calculated from the measured data. The SPSS software was used for statistical analysis. Gender differences in AUC_{0-t}, $AUC_{0-\infty}$, $T_{1/2}$ and C_{max} were tested with *t*-test, while ANOVA was used for comparison between dose groups. Non-parametric test was used for Tmax detection.

RESULTS

Plasma paracetamol levels

Specificity

As shown in Figure 1, plasma components did not interfere with the determination of paracetamol.

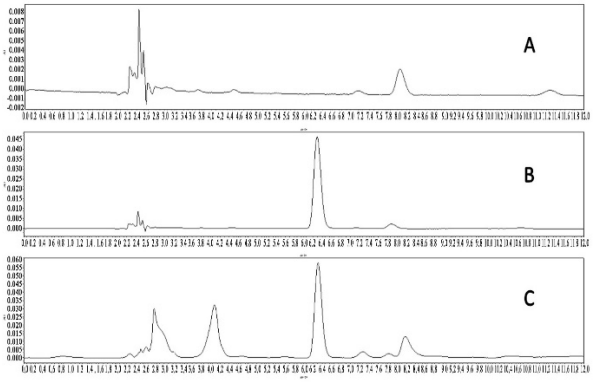

Figure 1: Chromatograms of ACP standard sample. (A): blank plasma sample (B), blank plasma sample mixed with acetaminophen standard; (C): plasma sample after administration of PCT, ACP, CFN and CPM tablets

Standard curve and quantitative range

The equation of the standard curve was:

Y = 9702.066x-1428.944, R^2 = 0.9999 (n = 5), and the linear range of standard curve was 1 to 150 μg/mL.

The results indicated that the intra-batch and inter-batch RSDs of paracetamol were less than 5 % in low, medium and high concentration samples, suggesting that the precision and accuracy of this method were high, and that the reproducibility was good. These results are shown in Table 1.

Stability

There was good stability in plasma placed at room temperature for 2 h, at -80 ^{o}C for 14 days, and samples placed at 4 ^{o}C for 12 h after pretreatment. These results are shown in Tables 2 and 3.

Toxicokinetic results

No PCT was detected in the plasma of the control group with single administration and continuous gavage before, and 1 h after administration, suggesting that there was no contamination in the experiments in this study. The concentration and uniformity of each suspension on the day of drug administration met the requirements, indicating that the doses were accurate.

Single administration toxicokinetics

The drug concentration - time curve in rats after a single intragastric administration of PCT, ACP, CFN and CPM tablets is shown in Figure 2. The statistical parameters of paracetamol toxicokinetics were calculated with non-compartment model (NCA) using the DAS professional software. The results are shown in Table 4.

Single intragastric administrations of PCT, ACP, CFN and CPM tablets to rats at the dose of 0.5 tablet/kg led to significant differences in the area under drug concentration - time curve $(AUC)_{0\text{-}36h}$ and $AUC_{0\text{-}\infty}$ between male and female rats. Moreover, single intragastric administration of PCT, ACP, CFN and CPM tablets at the dose of 1 tablet/kg produced significant differences in $AUC_{0\text{-}\infty}$ and $T_{1/2}$ between male and female rats.

When each drug was given at single intragastric dose of 2 tablets/kg, there was significant difference in $AUC_{0\text{-}\infty}$ between male and female rats. After single intragastric administration of the drugs at doses of 0.5, 1, 2 tablets/kg (equivalent to 75, 150 and 300 mg/kg paracetamol), the amount of paracetamol exposure ($AUC_{0\sim t}$, $AUC_{0\text{-}\infty}$ and C_{max}) increased with increase in dose, and showed a good linear relationship. The correlation coefficients R^2 between the administration dose and amount of drug exposure ($AUC_{0\sim t}$, $AUC_{0\text{-}\infty}$ and C_{max}) were 0.9991, 0.9826 and 0.9991 (male), and 0.9997, 0.9912 and 0.9999 (female).These values indicate that there was a linear relationship between drug dose and amount of drug exposure ($AUC_{0\sim t}$, $AUC_{0\text{-}\infty}$ and C_{max}). In addition, with increase in dose, the elimination of PCT slowed down, that is, $t_{1/2}$ was prolonged with dose.

Table 1: Intra-batch and inter-batch assay for precision and accuracy of acetaminophen at three concentrations

C (μg/mL)	Intra-day			Inter-day		
	DC (μg/mL)	P RSD/%	Accuracy %	DC (μg/mL)	P RSD/%	Accuracy %
2.5	2.510±0.080	3.19	100.40	2.566±0.085	3.33	102.64
30	30.637±0.454	1.48	102.12	30.647±0.460	1.50	102.16
120	121.528±3.428	2.82	101.27	124.519±4.376	3.51	103.77

Note: C = concentration; DC = determined concentration; P = precision

Table 2: Stability test of acetaminophen at three concentrations

Concentration (μg/mL)	Stability of sample at room temperature		Stability of sample in the injectors	
	Determined concentration (μg/mL)	*RE*	*Determined concentration (μg/mL)*	*RE*
2.5	2.569±0.047	0.72%	2.672 ±0.064	2.88%
30	29.783±0.473	0.31%	31.069± 0.320	1.4%
120	118.402±3.655	-1.52%	124.678± 1.952	1.13%

Table 3: Long-term stability test of acetaminophen at three concentrations

Concentration (μg/mL)	One-week stability		Two-week stability	
	Determined concentration (μg/mL)	*RE*	*Determined concentration (μg/mL)*	*RE*
2.5	2.610± 0.102	3.98%	2.528± 0.086	0.73%
30	29.730±1.321	-2.96%	32.072±1.515	4.69%
120	121.879± 0.991	0.29%	122.200±1.966	0.55%

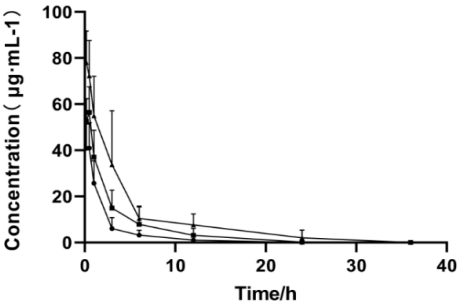

Figure 2: Mean plasma ACP concentration-time curve of single dose of PCT, ACP, CFN and CPM tablets. ***Key:*** ●*low dose;* ■*middle dose;* ▲*high dose*

Toxicokinetics of continuous administration

The drug concentration - time curve in rats after continuous administration of PCT, ACP, CFN and CPM tablets is shown in Figure 3.

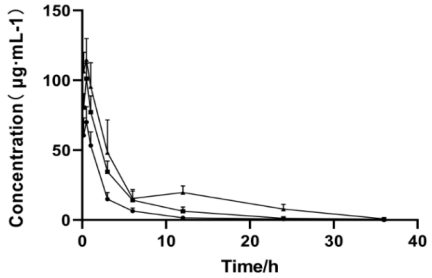

Figure 3: Mean plasma ACP concentration-time curve of continuous administration of PCT, ACP, CFN and CPM tablets. ●*low dose;* ■*middle dose;* ▲*high dose*

The statistical parameters of paracetamol toxicokinetics were calculated with non-compartment model (NCA) using the DAS professional software. The results are shown in Table 5. There were no statistically significant differences in toxicokinetic parameters between male and female rats after intragastric administration of low, medium and high dose paracetamol, aminophenazone, caffeine and chlorphenamine maleate tablets for 14 consecutive days. In addition, with increase in dose, the elimination of paracetamol was slowed down, that is, $T_{1/2}$ was prolonged with increase in dose.

DISCUSSION

The results obtained in this study showed that PCT exposure in female rats was higher than that in male rats when the doses of PCT, ACP, CFN and CPM tablets were low, medium and high. This may be due to gender differences in the enzymes involved in the absorption and metabolism of paracetamol [4,5]. *In vivo*, about 60 % of PCT is metabolized by glucuronidase (UGTs) to paracetamol glucuronic acid complex (APAP-glu), and about 30 % of PCT is metabolized by sulfate transferase (SULTs) to form paracetamol sulfuric acid complex (APAP-sulf). About 5 to 9 % of PCT is transformed and metabolized by CYP2E1, 1A2 and 3A. Due to the gender differences in glucuronidases (UGTs), the amount of enzyme in female mice is lower than that in male mice. Thus, the drug concentration in the blood is high and the half-life is long, resulting in larger drug exposure in female mice than in male mice. In addition, the higher concentration of hepatic microsomal cytochrome P450 in male rats may be one of the reasons for the higher amount of drug exposure in female rats. The gender difference in exposure in single administration rats (the exposure level of female rats was higher than that of male rats) disappeared after 14 days of continuous intragastric administration.

Table 4: Mean toxicokinetic parameters for acetaminophen in rats following a single-dose administration of PCT, ACP, CFN and CPM tablets

Parameter	Dose level	Male	Female
C_{max} (mg /L)	Low dose	35.61±15.06	53.99±8.87
	Middle dose	51.49±4.07	61.76±12.94
	High dose	79.77±16.44	77.76±19.68
AUC_{0-36h} (mg /L•h)	Low dose	58.37±21.01	98.76±15.48
	Middle dose	118.93±15.45	200.12±67.22
	High dose	323.93±49.06	417.80±110.44
$AUC_{0-\infty}$ (mg /L•h)	Low dose	61.03±19.63	102.37±16.64
	Middle dose	119.84±15.75	207.89±59.80
	High dose	340.30±53.62	529.39±130.26
$T_{1/2}$ (h)	Low dose	1.35±0.33$^{\Delta}$	1.97±0.73$^{\Delta}$
	Middle dose	1.57±0.31$^{\Delta}$	3.40±1.06$^{\Delta}$
	High dose	3.97±0.31	6.76±5.20
T_{max} (h)	Low dose	0.34±0.19	0.17±0
	Middle dose	0.42±0.17	0.17±0
	High dose	0.17±0	0.21±0.12

$^{\Delta}P < 0.05$, compared to high dose

Table 5: Mean toxicokinetics parameters of acetaminophen for rats with continuous administration of PCT, ACP, CFN and CPM tablets

Parameter	Dose	Male	Female
C_{max} (mg/L)	Low dose	46.19±14.71	47.08±4.03
	Middle dose	60.79±9.44	67.00±10.51
	High dose	77.29±9.62	90.34±4.25
AUC_{0-36h} (mg /L•h)	Low dose	83.22±44.35	117.98±37.48
	Middle dose	145.16±58.39	230.89±115.69
	High dose	282.46±121.91	268.36±121.29
$AUC_{0-\infty}$ (mg /L•h)	Low dose	84.63±46.98	120.40±39.59
	Middle dose	163.01±87.20	235.41±117.64
	High dose	301.19±126.71	272.40±121.02
$T_{1/2}$ (h)	Low dose	0.87±0.32$^{\triangle}$	2.23±1.42
	Middle dose	2.19±1.73	2.96±1.66
	High dose	2.84±1.09	3.56±1.10
T_{max} (h)	Low dose	0.5±0	0.34±0.19
	Middle dose	0.50±0	0.42±0.17
	High dose	1.04±1.31	0.34±0.19

$^{\triangle}p < 0.05$, compared to high dose

This may be due to the induction of PCT metabolism in rats and increases in activities of the metabolizing enzymes. Thus, there was acceleration of drug metabolism and elimination after 14 days of oral intragastric administration of PCT, ACP, CFN and CPM tablets. Probably, the enzyme induction had a stronger effect in female mice, leading to the disappearance of the initial gender difference.

Since the paracetamol, aminophenazone, caffeine and chlorphenamine maleate tablets were compound preparations which contained caffeine, aminopyrine and other components, the enzyme induction may be either due to paracetamol itself or due to other components. Similar to literature reports [6], in this study, the metabolism and elimination of paracetamol was dose-dependent [4]. In other words, the elimination half-life ($t_{1/2}$) of paracetamol in rats differed with dose, i.e., the $t_{1/2}$ of paracetamol in female and male rats increased with increase of dose.

CONCLUSION

Gender differences and toxic reactions caused by drug metabolism and elimination should be fully considered in the clinical applications of drugs.

DECLARATIONS

Acknowledgement

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Shenghua Gu designed the study and interpreted the results. Wei Sun, Shenghua Gu, Xin Zhang, Jinyao Lu, Yingmin Gu, Yiwen Huang, Guifeng Xu, Jiajun Xie collected data and drafted the manuscript. Wei Sun and Shenghua Gu performed the experiments. Wei Sun and Shenghua Gu contributed equally to this work and should be considered as co-first authors.

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REFERENCES

1. *Ni HM, Bockus A, Boggess N, Jaeschke H, Ding WX. Activation of autophagy protects against acetaminophen-induced hepatotoxicity. Hepatol 2012; 55(1): 222-232.*

2. Larson AM, Polson J, Fontana RJ, Davern TJ, Lalani E, Hynan LS, Reisch JS, Schiødt FV, Ostapowicz G, Shakil AO, et al. Acetaminophen-induced acute liver failure: results of a United States multicenter, prospective study. Hepatol 2005; 42(6): 1364-1372.
3. World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56.
4. Gochfeld M. Sex Differences in Human and Animal Toxicology: Toxicokinetics. Toxicol Pathol 2017; 45(1): 172-189.
5. Court MH, Zhu Z, Masse G, Duan SX, James LP, Harmatz JS, Greenblatt DJ. Race, Gender, and Genetic Polymorphism Contribute to Variability in Acetaminophen Pharmacokinetics, Metabolism, and Protein-Adduct Concentrations in Healthy African-American and European-American Volunteers. J Pharmacol Exp Ther 2017; 362: 431-440.
6. Hirate J, Zhu CY, Horikoshi I, Bhargava VO. First-pass metabolism of acetaminophen in rats after low and high doses. Biopharm Drug Dispos 1990; 11(3): 245-252.