

Original Article

Pharmacokinetics of oral viaminate in healthy Chinese subjects: an open-label, sequential, single-dose, food-effect, and multiple-dose study

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Abstract: Viaminate is a retinoid derivative and possesses the therapeutic effects of retinoid but the adverse effects are less than those of other oral retinoids. However, the pharmacokinetic profiles of viaminate following single and multiple oral doses have not yet been fully characterized in human being. An open-label, dose-escalation study was performed to evaluate the pharmacokinetics of oral viaminate in 9 healthy Chinese volunteers. Pharmacokinetics parameters of viaminate were determined using serial blood samples obtained up to 15 hours after administration of single dose (50-, 100-, or 150 mg), multiple doses of 50 mg twice a day or single dose of 50 mg half an hour after a high-fat breakfast. Areas under the curve of plasma concentration vs. time (AUC) and the maximum plasma concentration (C_{max}) were found to be proportional to dose. But the elimination half-life ($t_{1/2}$) and the time reaching maximum plasma concentration (T_{max}) were not changed significantly. Food had a positive effect on the extent of absorption (AUC 57.5 [fed] vs. 8.4 ng·h/mL [fasted]), and peak concentration was significantly increased (C_{max} 18.4 [fed] vs. 1.4 ng/mL [fasted]), while rate of absorption was similarly (T_{max} 3.8 [fed] vs. 3.1 h [fasted]). Viaminate was well tolerated in the studied dose range in healthy Chinese subjects. Viaminate displayed linear pharmacokinetics in the dose range from 50 to 150 mg after a single oral dose. The ingestion of food significantly increased the extent of viaminate absorption. No marked effects of sex on the pharmacokinetic parameters of viaminate were observed.

Keywords: Viaminate, retinoids, pharmacokinetics, determination

Introduction

In the late 1970s, a comprehensive chemoprevention program was established at the Institute of Materia Medica, Chinese Academy of Medical Sciences. Since then, more than 200 retinoid compounds have been synthesized and screened for cellular differentiation and chemoprevention of cancer [1]. On the basis of screening, viaminate was selected for pharmacological [2, 3] and toxicological studies [4]. Viaminate, all-trans-N-(4-ethoxycarbophenyl) retinamide, a retinoid derivative, was prepared by all-trans retinoic acid and p-amino-bezoic acid ethyl ester. In clinic, viaminate possesses the therapeutic effects of retinoid but the adverse effects are less than those of other oral retinoids. Viaminate has been widely used in a number of skin disorders and some forms

of neoplastic disease, such as acne vulgaris, psoriasis, and seborrheic dermatitis [5-10].

Although the pharmacokinetics of viaminate have been studied in rats [11, 12], single- and multiple-dose pharmacokinetic profiles of viaminate in human being had not yet been fully characterized. In addition, dose proportionality of the plasma levels over the therapeutic range as well as the effects of food on the rate and extent of viaminate absorption had not been determined. Therefore, the purpose of this study was to evaluate the pharmacokinetics of oral viaminate after single escalating oral doses (from 50-150 mg), multiple doses (50 mg 2 times daily) and single dose of 50 mg after a high-fat meal in healthy Chinese volunteers. The effects of sex on the pharmacokinetics of viaminate were also evaluated.

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Materials and methods

Ethics approval

The study protocol was reviewed and approved by the Ethics Committee of the Hospital of Dermatology, Chinese Academy of Medical Sciences & Peking Union Medical College (Nanjing, China), and all subjects gave written informed consent form to their participation after having been fully informed by the medical supervisor about the aim, course, and possible risks of the study.

Subjects

A total of 9 healthy Chinese volunteers (4 men and 5 non-pregnant women, aged 21-37 years, BMI 19.38-23.44) were enrolled in the study. All subjects were in good health after being assessed medical history, complete physical examination, 12-lead Electrocardiograms (ECGs), standard laboratory test results (standard hematology, blood chemistry, urinalysis), and a negative pregnancy test result. Clinically significant abnormalities on physical examination, ECGs, or laboratory tests obtained during the pre-study screening were considered as exclusion criteria. Subjects were also excluded for the following reasons: a history of clinically significant cardiovascular, renal, hepatic, pulmonary, gastrointestinal, hematological, vascular diseases; a history of nervous system or a psychiatric disorder; women during breastfeeding or menstruating; a history of alcohol or drug abuse; allergy or hypersensitivity to the study drugs; a positive test result for hepatitis B virus; blood donation within the previous 3 months; participation in another investigational drug study within the previous 3 months; and treatment with any drug during the previous 2 weeks or the entry into the study.

Study design

All subjects received in sequential order viaminate orally 50-, 100-, or 150 mg. All drugs were taken in the morning, in an overnight fasted state. All subjects fasted at least 10 hours before and 4 hours after drug administration. And they were given standardized meals 4 and 10 hours after drug intake. Venous blood samples (3 mL) were collected in heparinized tubes at pre-dose (0) and 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 15 hours post-dose. The plasma

samples were separated by being centrifuged at $4000 \times g$ for 3 minutes and stored at -20°C until analysis.

After the end of the single dose study, all subjects received 50 mg of viaminate twice a day for 5 consecutive days. Serial blood samples (3 mL) were collected at pre-dose (0) and 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 15 hours after the last dose on the 5th day. To establish if the steady-state condition had been achieved, additional pre-dose (trough) blood samples were collected prior to the morning dose on days 3, 4, and 5. The plasma samples were separated by being centrifuged at $4000 \times g$ for 3 minutes and stored at -20°C until analysis. The whole operation was carried out in dark room lighting with only a low-intensity red light source. In each period, the volunteers stayed in the Clinical Pharmacology Unit.

When studying the effect of food, the subjects were given a high-fat breakfast (2 eggs fried in butter, 2 pieces of bacon, 2 pieces of toast with butter, 110 g of hashed brown potatoes cooked in butter and 230 g of whole milk) half an hour before orally administration 50 mg of viaminate. The blood samples were also collected at the time points mentioned above. Every period washout interval was 1 week.

Analytical methods

Testosterone propionate (batch No: 0008-94-04), obtained from National Institutes for Food and Drug Control (Beijing, China), was used as the internal standard (I.S.). Viaminate standard (99.63% purity in HPLC) was supplied by Chongqing Huapont Pharmaceutical Co. Ltd. HPLC grade methanol was purchased from SK Chemicals (Ulsan 680-160, Korea). All other chemicals were of analytical grade and were purchased from Nanjing Chemical Reagent Co. Ltd. (Nanjing, China). Distilled de-ionized water was produced by a Mill-Q UF Plus Water Purification Unit (Millipore, USA).

Analysis was performed with LC-MS/MS system. The system consisting of a Finnigan autosampler (Thermo Electron Corporation, USA), a Finnigan LC pump, a Finnigan TSQ Quantum Ultra equipped with an electrospray ion source and operated by XCalibur software. Separations were achieved by using a VP-ODS column (250 mm \times 2.0 mm i.d., 5 μm particle size; Shimadzu,

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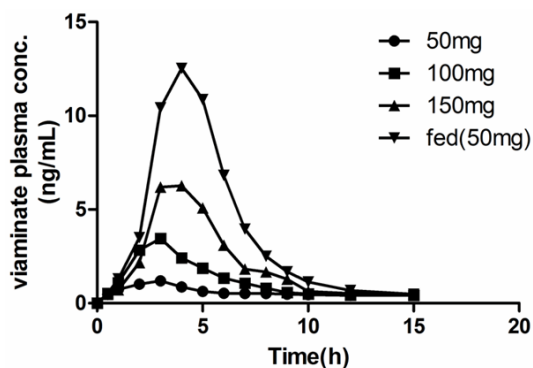


Figure 1. Mean plasma concentration of viaminat after a single oral dose.

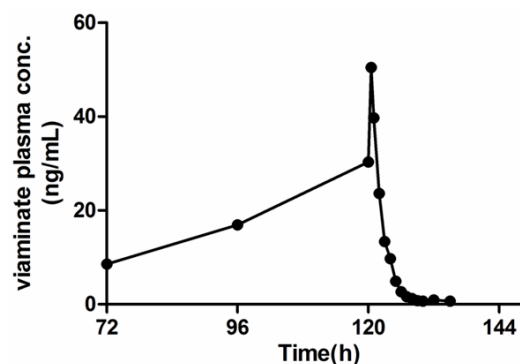


Figure 2. Mean plasma concentration following 50 mg 2 times daily oral dosing of viaminat. Last dose was given after 120 hours.

Japan) which was protected by a GVP-ODS pre-column (10 mm × 2.0 mm; Shimadzu, Japan). The mobile phase composed of methanol-0.1% acetic acid (98:2, v/v) was used at a flow rate of 0.2 mL/min. Testosterone propionate was used as internal standard (I.S.) for the quantification. The total analysis time was 12 minutes. Mass spectral analysis was performed in the negative-ion mode. The spray voltage was set at 5000 V. The sheath gas and AUX gas pressure were set at 35 and 5 psi, respectively. The collision gas (Ar) pressure was set at 1.5 mTorr. The capillary temperature was set at 270°C. Collision induced dissociation (CID) studies were performed using collision energy of 25 eV. The transitions monitored were m/z 446 (parent ion) to m/z 164 (product ion) for viaminat, and m/z 345 (parent ion) to m/z 109 (product ion) for testosterone propionate. The dwell time was 100 ms for each transition.

Briefly, the 50 μ L working I.S. solution was spiked to 1 mL of plasma, and then 1 mL ethanol followed by 4 mL cyclohexane was added and vortexed. After centrifugation, the organic layer was transferred to another centrifuge tube and evaporated to dryness under a stream of nitrogen in a water bath at 37°C. The residue was re-dissolved in methanol, and then vortexed and centrifuged. Finally, an aliquot of 10 μ L was injected into the LC-MS/MS system. The whole operation was carried out in dark room lighting with only a low-intensity red light source as viaminat is light sensitive.

The method was validated and found to be linear over the concentration range of 0.10 to 200.0 ng/mL ($r = 0.9995$). Using $(1/C^2)$ weighted least squares regression, the lower limit of

quantitation (LLOQ) was 0.10 ng/mL. The intra-assay and inter-assay coefficients of variation (CV%) for the 3 quality control standards (1.0, 10.0, and 100.0 ng/mL) were $\leq 7.7\%$. The mean absolute recovery varied from 88.02% to 104.0%, whereas accuracy ranged between 93.08% and 106.0% for the plasma samples. A stability study showed that viaminat and the I.S. were stable in plasma at room temperature for at least 24 hours, as well as for 35 days at -20°C after 5 freeze-thaw cycles.

Statistical methods

Single- and multiple-dose pharmacokinetic parameters were calculated from plasma concentration-time data by noncompartmental methods. The maximum plasma concentration (C_{max}) and the time reaching maximum plasma concentration (T_{max}) were obtained from observed data. The area under the plasma concentration vs. time curve (AUC) from 0 to the last measurable concentration (C_t), AUC_{0-t} , was calculated with the linear trapezoidal rule. The terminal elimination half-life ($t_{1/2}$) was calculated as $(\ln 2)/\lambda_z$. Mean steady-state concentration (C_{ss}) was calculated using $AUC_{ss(0-\tau)}/\tau$, where τ is the dosing interval.

Results

Pharmacokinetics

Mean concentration-time profiles after single and multiple doses of viaminat are shown in **Figures 1** and **2**. The mean values of the pharmacokinetic parameters are presented in **Tables 1** and **2**, respectively. Over the dose range

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Table 1. Main pharmacokinetic parameters (mean \pm SD) following a single oral dose of viaminate ($n = 9$)

| Parameter | 50 mg | 100 mg | 150 mg | 50 mg (fed) |
|--------------------------|---------------|----------------|----------------|-----------------|
| $t_{1/2}$ (h) | 4.5 \pm 0.8 | 3.3 \pm 0.8 | 4.6 \pm 4.3 | 2.6 \pm 0.7 |
| C_{max} (ng/mL) | 1.4 \pm 0.7 | 4.3 \pm 1.2 | 7.7 \pm 1.7 | 18.4 \pm 6.7 |
| T_{max} (h) | 3.1 \pm 1.2 | 3.1 \pm 0.9 | 3.8 \pm 0.8 | 3.8 \pm 0.8 |
| $AUC_{(0-15)}$ (ng·h/mL) | 8.4 \pm 2.4 | 17.2 \pm 5.8 | 30.9 \pm 8.7 | 57.5 \pm 22.8 |

Table 2. Main pharmacokinetic parameters (mean \pm SD) following multiple oral doses of viaminate 50 mg 2 times daily ($n = 9$)

| Parameter | |
|-----------------------------|------------------|
| C_{ss_max} (ng/mL) | 59.3 \pm 39.2 |
| C_{ss_min} (ng/mL) | 28.4 \pm 26.0 |
| AUC_{ss} (ng·h/mL) | 123.2 \pm 65.3 |
| C_{ss_av} (ng/mL) | 8.2 \pm 4.4 |
| T_{max} (h) | 0.6 \pm 0.3 |
| $t_{1/2}$ (h) | 1.5 \pm 0.4 |
| $AUC_{(120-135)}$ (ng·h/mL) | 123.2 \pm 65.3 |

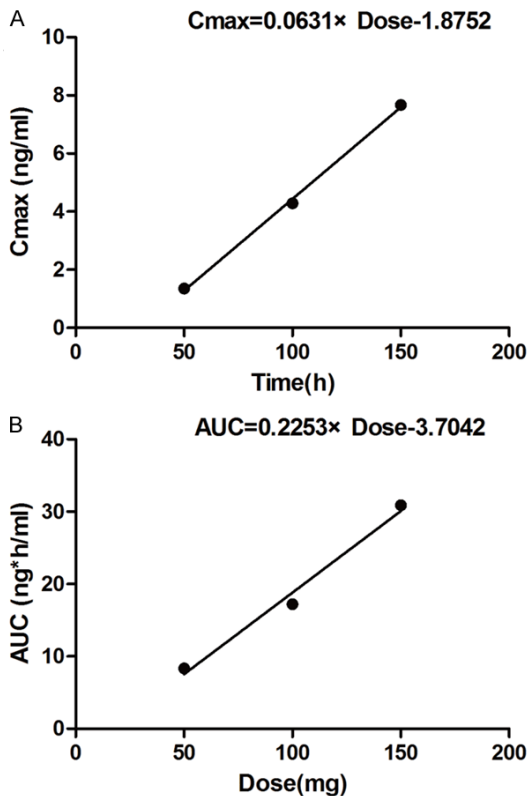


Figure 3. Linear correlation between C_{max} and increasing dose of viaminate (A) and linear correlation between AUC_{0-15} and increasing dose of viaminate (B).

studied, C_{max} and $AUC_{(0-15)}$ increased in proportion to the doses ($r = 0.9991$ and 0.9926 , respectively) (Figure 3), while the T_{max} and $t_{1/2}$ were not affected by dose.

Food had a significant effect ($P < 0.01$) on the extent of absorption (AUC_{0-15} is 57.5 ± 22.8 ng·h/mL vs. 8.4 ± 2.4 ng·h/mL for the fed and

the fasted conditions, respectively), and peak plasma concentration was significantly ($P < 0.01$) increased (C_{max} 18.4 ± 6.7 ng/mL [fed] vs. 1.4 ± 0.7 ng/mL [fasted]), while rate of absorption was similarly (T_{max} 3.8 ± 0.8 [fed] vs. 3.1 ± 1.2 hours [fasted]). Not all trough plasma concentrations between days 3, 4, and 5 achieved the steady-state conditions after multiple doses of viaminate 50 mg 2 times daily. Single and multiple oral dosing data (AUC , C_{max} , T_{max} and $t_{1/2}$) from all subjects were combined to examine the effects of sex on the pharmacokinetics of viaminate, and there were no significant ($P > 0.05$) differences in the pharmacokinetic parameters between male and female subjects.

Safety

Single and multiple doses of viaminate were well tolerated, and there were no withdrawals and no serious adverse events reported in this study.

Discussion

The pharmacokinetic characteristics and dose proportionality of viaminate were investigated in healthy Chinese volunteers following single oral doses of 50-, 100- or 150 mg, multiple doses of viaminate 50 mg 2 times daily and single dose of 50 mg after a high-fat breakfast in this phase I clinical trial. Under fasted conditions, C_{max} levels and AUC values of viaminate increased in a linear and proportional manner with increasing oral doses, while the T_{max} and $t_{1/2}$ were not affected by dose. Not all trough plasma concentrations between days 3, 4, and 5 achieved the steady-state conditions after multiple doses of viaminate 50 mg 2 times daily, which meant that absorption of viaminate might be affected by time and food.

Food effects on drug absorption can be described by 5 categories: decreased or increased,

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delayed or accelerated, or unaffected absorption [13]. The effects of food on the pharmacokinetics of viaminate were determined following a single oral dose of viaminate 50 mg under fed conditions (high-fat breakfast) in 9 healthy volunteers. Concomitant food intake significantly ($P < 0.01$) altered the bioavailability of viaminate, as indicated by increasing AUC, C_{max} , while rate of absorption (T_{max}) was similarly. Most interesting thing was that the plasma concentration of 50 mg viaminate after food was even higher than that of 150 mg, which suggested that viaminate probably was a kind of fat soluble drug. Probably manufacturer should apply soft capsule into producing this drug. The results also suggested that low-dose viaminate should be taken with food to increase the drug effect, while high-dose viaminate should not be taken with food to decrease the adverse effect.

As another main objective of the study, these data were used to examine the effects of sex on the pharmacokinetics of viaminate. In whole study parts, there were no significant differences in AUC, T_{max} , $t_{1/2}$ between male and female subjects. Hence, no adjustment of dosage on the basis of sex was needed.

The large inter-subject variability in the plasma concentration-time profiles observed in this study was not unusual for retinoids after oral administration. It was assumed that this variability was mainly due to differences in rate and extent of disposition among individuals rather than that in absorption, as the T_{max} and C_{max} showed smaller inter-subject variability than $t_{1/2}$ did.

This study showed that viaminate was well tolerated after given single 50-, 100-, or 150 mg dose and multiple doses of 50 mg and no adverse events were reported after single administration of 50 mg with high-fat food. No clinically relevant laboratory abnormalities or clinically significant changes in vital signs or electrocardiogram recording were observed.

Viaminate was rapidly absorbed following single oral dose, with maximum plasma concentrations occurring about 3 hours after administration, which supported the use of viaminat for a 2 times daily dose regimen.

Conclusion

In conclusion, the results of the present study indicate that viaminat has predictable phar-

macokinetics. These pharmacokinetics parameters should facilitate the definition of dose regimens. Since viaminat was a retinoid derivative, these conclusions also suggest that other retinoids might also have similar pharmacokinetics and high-fat solubility.

Acknowledgements

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Disclosure of conflict of interest

None.

Abbreviations

I.S., internal standard; LLOQ, the lower limit of quantitation; CV%, interassay coefficients of variation; C_{max} , the maximum plasma concentration; T_{max} , the time reaching maximum plasma concentration; AUC, the area under the plasma concentration vs. time curve; C_t , from 0 to the last measurable concentration; $t_{1/2}$, the terminal elimination half-life; C_{ss} , mean steady-state concentration.

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References

- [1] Xu SP, Guo ZR, Yuan ZL, Li LM and Huang L. [Studies on compounds of tumor prevention-synthesis of derivatives of retinoic acid]. Yao Xue Xue Bao 1981; 16: 678-686.
- [2] Cai HY, Zhang JS and Cui XX. [Inhibitory effect of some new retinoids on carcinogenesis]. Yao Xue Xue Bao 1981; 16: 648-653.
- [3] Song ZP, Liu YH and Han R. [Differentiation of human promyelocytic leukemia (HL-60) cells induced by new synthetic retinoids 4-(ethoxycarbophenyl) retinamide and 4-(hydroxycarbophenyl) retinamide]. Yao Xue Xue Bao 1984; 19: 576-581.
- [4] Du CZ, Su XL and Xu YT. [A comparison of the toxicities of 4-(ethoxycarbophenyl) retinamide and some other retinoids]. Yao Xue Xue Bao 1982; 17: 331-337.
- [5] Li YT, Chen H, Li SY, Chen HQ, Huang WN, Wang MJ, Su XY, Yang SL and Ou FX. [Clinical

Pharmacokinetics of oral viaminate in healthy Chinese subjects

- and in vitro study of viaminate in acne]. *Chin J Dermatol* 2003; 36: 33-35.
- [6] Ni WQ. [Treatment of acne vulgaris with two therapeutical project of viamnate in 44 patients]. *Chin J New Drugs Clin Rem* 2004; 23: 545-547.
- [7] Zhao SJ and Yu AY. [The application of viaminate in skin disease]. *J Dermatology and Venereology* 2005; 27: 9-11.
- [8] Liu T, Mou YZ, Liu Y and Wang L. [Effects of tripterygium wilfordii combined with viaminate capsules on psoriasis patients' immune indexes, endothelium, inflammatory reaction, sI-CAM-1 and NF- κ B]. *Journal of Hainan Medical University* 2018; 24: 1615-1618.
- [9] Zhang L, Zhao HL and Wang N. [Clinical efficacy of viaminate capsules combined with tacrolimus ointment in the treatment of seborrheic dermatitis]. *Chin J Nosocomiol* 2018; 28: 551-554.
- [10] Lang J. [Determination of viaminate and related substances in viaminate capsules by HPLC]. *Anhui Medical and Pharmaceutical Journal* 2012; 16: 42-44.
- [11] Cheng Q, Gu QM and Han R. [Pharmacokinetics of N-(4-carboxyphenyl) retinamide in rats]. *Chinese Journal of Pharmacology and Toxicology* 1994; 8: 143-146.
- [12] Cao L, Zheng F, Ma P, Liu W, Sun D, Chen X, Lai Y and Gou M. LC-APCI-MS-MS method for the tissue distribution of viaminate after oral administrations to rats. *J Chromatogr Sci* 2008; 46: 701-706.
- [13] Singh BN. Effects of food on clinical pharmacokinetics. *Clin Pharmacokinet* 1999; 37: 213-255.