PHARMACOKINETICS STUDY OF AVANAFIL AND ITS ANALOGUES IN RATS

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ABSTRACT

Introduction: dysfunction (ED). The Aim of this study is to investigate the pharmacokinetics properties of avanafil and its analogues.

Materials and methods: After extraction by acetonitrile, the analytes were separated by a rapid gradient elution with acetonitrile and water as the mobile phase and detected by an mass spectrometer. Multiple reaction monitoring was performed on the ion transitions of m/z 484.2→375.2 [M-H] - (avanafil).

Results: A simple, rapid and accurate liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was developed for the quantification of avanafil and its analogues in rat plasma using Dixipan as the internal standard (IS). The plasma concentration of avanafil and its analogues in rats showed good linearity. All the analytes can maintain stable during the whole experimental process.

Discussion: The developed method was successfully applied to the pharmacokinetic study of avanafil and its analogues after oral administration to rats. The results showed that the analogues have better absorption property in vivo than avanafil.

Keywords: avanafil; analogues; chemical synthesis; pharmacokinetics; LC-MS/MS

Introduction

Erectile dysfunction (ED), which is defined as the inability to achieve or maintain an erection sufficient for satisfactory sexual performance, is a condition that has a markedly negative impact on quality of life. Treatments for erectile dysfunction include psychosexual therapy, vacuum constriction devices, vascular surgery, alprostadil dosing or prescription oral type 5 phosphodiesterase (PDE-5) inhibitors. The relatively lowcost, effectiveness and pain-free therapy offered by PDE-5 oral medication has allowed it to become a recommended first-line treatment.

The phosphodiesterase type 5 (PDE-5) inhibitors enhance the relaxant effects of nitric oxide released in response to sexual stimulation by maintaining sufficient cellular levels of cyclic guanosine monophosphate in both the corpus cavernosum and the blood vessels supplying it. Avanafil, (4-[(3-chloro-4-methoxybenzyl) amino]-2-[2-(hydroxymethyl)-1-pyrrolidinyl]-N- (2-pyrimidinylmethyl)-5-pyrimidinecarboxamide) is a selective PDE-5 inhibitor developed by Mitsubishi Tanabe Pharma Corporation (Osaka, Japan). Preclinical studies have reported that avanafil strongly inhibited PDE-5 (half maximal inhibitory concentration, 5.2 nM) in a competitive manner.

A pass/fail approach to screening products allows a reduction in time and resources used through traditional lab analysis (HPLC, GC, etc.) and speeds up turnaround times for health alerts issued by the FDA.

In the present investigation, we synthesized 16 kinds of avanafil analogues, and the 14 kinds of them have not be reported in literature.
The pharmacokinetic properties of avanafil and its analogues in rat following single doses of oral administration were assessed, and to validate rationale of the drug design with pharmacokinetic data.

Materials and methods

Chemicals and reagents
The TC compounds was synthesized by the School of Chemistry and Chemical Engineering, Henan University of Technology. Avanafil (reference) was supplied by Jinan Cheminn Chemicals Co., Ltd. Dixipan that used as internal standard (IS) was supplied by the National Institutes for food and drug Control (NIFDC, Beijing). Acetonitrile and methanol of HPLC grade were purchased from Tedia (Fairfield, OH, USA). All other chemicals were of analytical grade.

Experimental animals
The present study was approved by the Animal Ethics Committee of China Pharmaceutical University. Sprague-Dawley rats (200 ± 20 g) of both genders were purchased from Shanghai SIPPR/BK Experimental Animal Co (Shanghai, China). Animals were kept under controlled conditions with temperature maintained at 20 ± 2°C and relative humidity at 50 ± 20%, and acclimatized to the housing environment for 1 week prior to the study. The rats were fasted but provided free access to water for overnight before the administration.

Synthesis of avanafil and its analogues
In the former study, avanafil and its analogues were synthesized by cyclization, chlorination, nucleophilic, oxidation, nucleophilic replaced, hydrolysis, condensation seven steps, with S-methyl isopropyl thiourea sulfate and ethoxy methyl diethyl malonate as raw materials. In this investigation, six kinds of analogues were selected for pharmacokinetics study, named TC4, 6, 7, 9, 13 and 14, respectively.

Preparation of stock and working solutions
The stock solutions of avanafil and IS were separately prepared in DMSO at the concentration of 1 mg·mL-1 and stored in brown volumetric flasks at 4 °C before use. A series of working solutions of avanafil and IS standard solution were prepared by diluting stock solutions of the analyte and IS with methanol.

Calibration standards and quality control samples
The calibration standards of avanafil were prepared by spiking working solutions into equal volume of blank rat plasma to obtain the series concentrations of 2, 5, 10, 20, 50, 100, 200, 500 and 1000 ng·mL-1. Quality control (QC) samples were prepared in the same way as calibration standards to achieve the final concentrations of 0.5, 5 and 40 μg·mL-1. All samples were stored at 4 °C until analysis.

Preparation of dosing form
For oral administration to rats, avanafil and the analogues were prepared in the mixed suspension composed of DMSO and PEG 200, at a final concentration at 1.0 mg·mL-1. The dosing solutions were prepared freshly before use.

Pharmacokinetic study
After an oral administration of avanafil or the analogue suspensions to each rats, approximately 150 μL of blood samples were collected from the forelimb vein into heparinized tubes at the following time points: 0 h (before dosing) and 0.083, 0.25, 0.5, 0.75, 1, 1.5, 2, 4, 8, 12 h after dosing. Plasma samples were then separated by centrifugation at 8,000×g for 5 min and kept at -20 °C until analysis. For the preparation of plasma samples, a 100 μL of aliquot plasma sample was mixed with 100 μL of IS standard solution. The plasma mixture was precipitated by 600 μL of acetonitrile, vortex-mixed for 1 min and then centrifuged at 16,000×g for 10 min. Finally, 5 μL of the supernatant was directly injected onto the LC-MS/MS system for analysis.

LC-MS/MS Assay
The avanafil and its analogues were analyzed by a validated LC-MS/MS method. The LC-MS/MS system consisted of an API4000 and an HPLC system. The analytical column was Shimadzu Shim-pack VP-ODS (150×2.0mm), coupled with a Security Guard C18 guard column (4×3.0 mm, Phenomenex, Torrance, CA, USA). The mobile phase containing A (0.1% formic acid diluted in distilled deionized water) and B (acetonitrile) was pumped at a flow rate of 1.0 mL·min-1. The temperature of the column was set at 40 °C.

The mass spectrometer was operated in negative ion mode with multiple reaction monitoring (MRM) at unit resolution for the detection of m/z 484.2→375.2 [M-H] - (avanafil) and m/z
285.3→154.2 [M-H] - (IS). The peak area ratio of drug to internal standard, and the concentrations were calculated by Analyst software (version 1.5.1).

Data analysis

A non-compartmental model was used to analyze individual time-concentration profiles of avanafil and its analogues. The pharmacokinetic parameters were calculated by WinNonlin computer program (Version 4.0; Pharsight Corp., Mountain View, CA).

Results

Method validation

A full method validation was performed according to the US FDA guidelines.

Linearity and sensitivity

An nine-point calibration curve was established with the linearity range from 2 to 1000 ng·mL⁻¹ for avanafil and its analogues. The calibration curves were constructed by plotting the peak area ratios between the analytes with weighted (1/x²) least squares linear regression. The deviation between the nominal concentration and measured concentration was generally less than 15%. This method was sensitive enough for the determination of acetazolamide in beagle plasma.

Specificity and selectivity

The specificity was assessed by analyzing six different batches of blank rat plasma. The results suggested that there was no significant interference on the determination of avanafil or the analogues and IS from rat plasma, and this method showed good specificity and selectivity for the following pharmacokinetic study. Under the conditions of this experiment, avanafil or the analogues and IS can be well separated.

Matrix effect

To investigate the matrix effect, peak area of post-preparative blank plasma sample spiked with avanafil or the analogues at three QC concentration levels were compared to that of standard solutions which were directly diluted by mobile phase to the same concentrations. The peak area ratios were used to evaluate matrix effect. Results suggested that there were no interferences from plasma matrix on the detection of both analytes.

Stability

The stability of avanafil or the analogues in plasma under various conditions was evaluated. Three-level QC samples were stored at room temperature for 4 h before preparation and kept at 4 °C for 24 h after preparation. The QC samples also underwent three freeze (-75 °C) -thaw cycles and long-term freezing (-75 °C) condition for 30 days. The results showed that plasma samples were stable under all storage conditions described.

Pharmacokinetic study

In pharmacokinetic study, twenty-one healthy rats were randomly divided into one groups, the drug concentration versus time profile was determined after oral administration of avanafil or the analogue suspensions. The results showed that the developed LC-MS/MS method can be successfully applied to the pharmacokinetic study of avanafil and its analogues in rats. The mean plasma concentration-time profiles of analogues was shown in Fig 1. The main pharmacokinetic parameters were calculated with non-compartment model and were listed in Table 1.

Fig 1. Mean plasma concentration-time curves over 12 h of analogues of avanafil following a single oral dose of formulation to rats (Each point represents the mean ± SD, n=3).

From the above results we can conclude that the T1/2 of TC9 and TC14 was higher than avanafil, while that of the rest was lower.
It should be noted that AUC(0-t) and AUC(0-∞) of almost all the analogues were higher than avanafil, except the AUC(0-t) of TC9. Most notable was that TC4 showed the largest AUC in these seven compounds, about 40 folds as much as avanafil for both AUC(0-t) and AUC(0-∞), indicating the best extent of absorption in vivo. In summary, these analogues could be better absorpt than their parent compound. The comparisons of AUCs for avanafil and its analogues were shown in Fig 2 and Fig 3.

**Discussion**

In this study, dixipan was selected as the IS in this method mainly because of its high ionization efficiency under ESI source and similar chromatographic separation properties with avanafil, so that the optimization process could be unified and efficient. Moreover, dixipan can maintain its stability during sample preparation and the whole determination process.

In pharmacokinetic studies, sample pretreatment method is crucial since errors caused by operation and low recovery usually can be introduced in this section\(^{(11)}\). The most commonly used sample preparation method was liquid-liquid extraction (LLE), but the developed sample pretreatment methods were not only complex, but also time-consuming. While in this study, sample pretreatment could be simplified due to the improved sensitivity and selectivity of our current established LC-MS/MS conditions under which the protein precipitation method could also meet the requirement for avanafil determination and obtain higher absolute recovery. As a result, protein precipitation adopted in the current study was not only rapid and convenient but also had good reproducibility, thus simplifying and speeding up the entire pharmacokinetic study\(^{(12, 13)}\).

A simple, practical and accurate LC-MS/MS method for the determination of plasma avanafil and its analogues has been established and validated. And the pharmacokinetic behavior of avanafil and its five analogues after oral administration to rats has been successfully analyzed by using the developed method. As discussed above, the sample pretreatment process has been greatly simplified owing to the optimized LC-MS/MS conditions in current study, thus saving time and money. In summary, this method showed its highly convenience and reliability for the rapid determination of avanafil in high-throughput pharmacokinetic studies.
References


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