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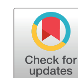
Pharmacokinetics, tissue distribution and plasma protein binding study of SM-1, a novel PAC-1 derivative



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ABSTRACT

As a PAC-1 derivative, SM-1 exhibts a promising antitumour property. To better understand the relationship between the drug concentrations and pharmacological effects, both liquid chromatography coupled with tandem mass spectrometry and high performance liquid chromatography methods were developed and validated in the work. Those methods were then applied to the pharmacokinetics (PK), tissue distribution and plasma protein binding (PPB) studies of SM-1. As a results, the proposed methods were demonstrated to be accurate, precise and stable for the analysis of the SM-1 in plasma and tissue samples. Meanwhile, the PK parameters of SM-1 showed that SM-1 had good PK properties. SM-1 had good absorption in the body, with 59.01% of the absolute bioavailability in rats and 55.63% of that in dogs. SM-1 rapidly distributed to all tissues, with the highest distribution in the lung and less in the brain and muscle. The PPB rates in rat plasma, dog plasma, and human plasma were 91.1%, 91.2%, and 90.7%, respectively. These good PK properties will contribute SM-1 to be a promising anti-tumour candidate. These results also provide insights into the further pharmacological investigation of SM-1.

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1. Introduction

Cancer is the second leading cause of death worldwide, and lung cancer was the leading cause of death in 2015 [1].Chemotherapy is currently the main treatment, but traditional chemotherapy drugs have some disadvantages, such as poor selection, easy drug resistance and ability to kill normal cells [2,3]. While the dysregulation of apoptosis often leads to the development and progression of cancer [4], the success rate of chemotherapy is very low [2]. Cancer cells are known to evade apoptosis by changing the expression of key proteins in the apoptotic cascade [5,6]; for example, cancer cell can promote p53 gene mutations [7], increase the expression of anti-apoptotic proteins in the Bcl-2 family [8], and participate in protein inactivation [9]. These change sallow cancer cells to fight apoptosis and enable uninhibited cell proliferation. The activation of damaged apoptotic cascade proteins has become a new target in the tumour cell apoptosis pathway.

In 2006, Putt was the first to perform in vitro screening of 20 thousand compounds and discovered small molecule compounds, including PAC-1, (shown in Fig. 1A), that directly activate procaspase-3 and induce cancer cell apoptosis [10]. PAC-1 displayed efficacy in mouse xenograft models and led to a Phase 1 clinical trial involving human cancer patients [11]. However, PAC-1 was found to have side effects, such as neurotoxicity and low bioavailability [12,13]. Therefore, designing new compounds that can strongly activate procaspase-3 and have high bioavailability and low neurotoxicity is urgently needed. To reduce BBB permeability, S-PAC-1, which is a PAC-1 derivative, was designed [13]. Previous studies have shown that sulfonamide successfully prevents entry into the brain, but its ability to activate procaspase-3 is only 1/10 of that of PAC-1 [14]. N'-(3-allyl-2-hydroxy-benzylidene)-2-(4-benzyl-1,4-homopiperazine-1-yl) acetohydrazide fumarate salt (Fig. 1B), which is chemically similar to SM-1, has been obtained according to

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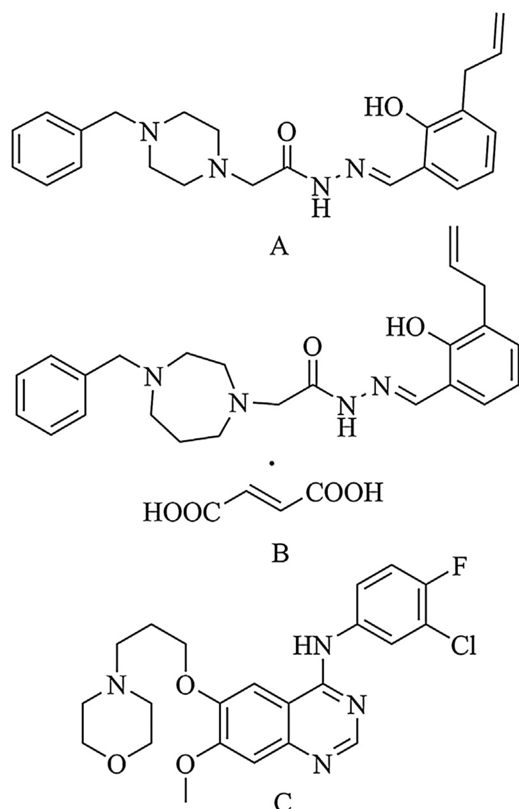

Fig. 1. Chemical structures of PAC-1 (A), SM-1 (B) and gefitinib (C).

the structure-activity relationship of PAC-1 [15]. Its mechanism is based on relieving the zinc-mediated inhibition of procaspase-3 to activate procaspase-3 and induce tumour cell apoptosis. Pharmacodynamics studies have shown that SM-1 had stronger antitumour activity in 8 human malignant cell lines and that SM-1 had higher selectivity than PAC-1 in lung, liver and uterus cancer cell lines [3]. These data suggest that SM-1 has the potential to be a new antitumour drug.

To better understand the relationship between the drug concentrations and pharmacological effects, HPLC-UV and LC–MS/MS methods were successfully applied in our research, which focused on pharmacokinetics (PK), tissue distribution and protein binding studies. The PK study and tissue distribution rate showed that SM-1 had unique advantages. First, SM-1 displayed a better absorption ability. Second, the concentration of SM-1 was very low in the brain; thus, SM-1 dose not easily cause neurotoxicity. Moreover, SM-1 had high selectivity and strong antitumour abilities. In conclusion, SM-1 is a promising antitumour candidate.

2. Experimental

2.1. Material and reagents

SM-1 (>99% HPLC purity, Shenzhen East Sunshine Drug Discovery and Development Company, China) was synthesized by the manufacturer. Gefitinib (>98% purity, Fig. 1C, National Institutes for Food and Drug Control, China) was used as an internal standard for SM-1. Methanol (Merck, Germany) and acetonitrile (Merck, Germany) were of HPLC grade. All other chemicals were of analytical grade. Deionized water was purified through a PURELAB Classic system (ELGA Lab Water, China). An MD 3525 plasma protein binding equilibrium dialysis system (Shanghai Source Leaf Biotechnology Co., Ltd, China) was purchased to conduct the plasma protein binding study.

Considering that the concentration of SM-1 in some PK samples might be low, the LC–MS/MS method was used for the analysis of the samples collected from the PK studies in rats and dogs. The analytes detection by LC–MS/MS was performed on an Agilent 6410 triple quadrupole LC–MS/MS system (Agilent, USA) with a binary pump. A Waters XBridge C18 column (50 mm × 4.6 mm, 3.5 μm) was used to separate the analytes.

The HPLC method was used for analyzing the samples collected from the tissue distribution study and the plasma protein binding study. The analytes detection by HPLC was performed on an Agilent 1200 HPLC system comprising a G1322 A degasser, a G1311 A quat-pump, a G1329 A autosampler, a G1316 A column oven and a G1314B UV-detector (Agilent, USA). The analytes were separated by an Agilent TC-C18 column (4.6 mm × 250 mm, 5 μm, Agilent, USA).

2.2. Development and validation of the analytical method

The development and validation of the LC–MS/MS and HPLC methods were performed according to the FDA guidance for bioanalytical methods. The parameters including selectivity, linearity, accuracy and precision, recovery, matrix effects and stability were assessed in the validation of both methods.

2.2.1. LC–MS/MS

The samples collected for the PK studies from rats and dogs were detected using the LC–MS/MS. SM-1 and gefitinib were separated on an Agilent 6410 triple quadrupole LC–MS/MS system equipped with a Waters XBridge C18 column. The mobile phase consisting of A (organic phase: acetonitrile-methanol (1:1, v/v)) and B (aqueous phase: 10 mM ammonium acetate) was pumped at a flow rate of 0.6 mL/min. The gradient elution was performed according to the following protocol: 0 min, 75% A/25% B; 2.51 min, 100% A; and 5.51 min, 75% A/25% B. The mass spectrometer was operated in the positive ion electrospray ionization mode (ESI) with multiple reaction monitoring (MRM) by monitoring the transitions of *m/z* 407.3→203.4 for SM-1 and 447.3→128.3 for IS (Fig. 2). The mass parameters were set according to the following protocol: gas flow: 11 L/min, gas temp: 300 °C, nebulizer: 40 psi, capillary: 4000 V, fragment voltage: 140 V and 135 V for SM-1 and IS, respectively; and collision energy: 25 V.

The stock solutions of both SM-1 and IS were prepared in methanol at concentration level of 1 mg/mL. The concentration of the working solution of the internal standard was 1 μg/mL by diluting the stock solution with methanol. The standard solutions of SM-1 used for the preparation of the calibration curves were serially diluted with blank rat plasma to yield final concentrations of 30, 60, 120, 300, 600, 3000,5000 and 6000 ng/mL. Three levels of QC samples (80, 480, and 4800 ng/mL) were prepared similarly.

A series of calibration standard samples of SM-1 ranging from 5 ng/mL to 6000 ng/mL (5, 10, 30, 120, 5000, 600, 3000, 5000, and 6000 ng/mL) was prepared by diluting the stock solution of SM-1 with blank beagle plasma. The quality control (QC) samples were independently prepared with blank plasma and their concentrations were 12 ng/mL, 600 ng/mL and 4800 ng/mL. All SM-1 and IS solutions were stored at 4 °C.

A 50 μL aliquot of rat plasma sample (100 μL for the beagle plasma sample) was added to a 1.5 mL Ep tube, followed by the addition of 10 μL of the IS working solution (1 μg/mL), 20 μL of phosphate buffer (PBS) and 400 μL of methanol. The mixtures were vigorously vortex-mixed for 3 min and then centrifuged at 15,700×g for 10 min at 4 °C. The clear supernatants were diluted 3 times with the mobile phase before injection.

2.2.2. HPLC

The samples from the tissue distribution and binding rate of the plasma protein studies were detected with the HPLC method.

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