Journal of Pharmaceutical and Biomedical Analysis 83 (2013) 186–193

Contents lists available at SciVerse ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

LC–MS/MS method for the simultaneous quantitation of three active components derived from a novel prodrug against schistosome infection

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ARTICLE INFO

Article history:
Received 30 January 2013
Received in revised form 5 May 2013
Accepted 7 May 2013
Available online 16 May 2013

Keywords:
Prodrug
Artesunate
Dihydroartemisinin
10-Hydroxypraziquantel
Pharmacokinetics

ABSTRACT

Schistosomiasis is an infectious disease that has been recognized as a severe health burden for some regions of the world. While praziquantel is the drug of choice, there is an unmet medical need for novel therapies with greater efficacy and resistant profile. DW-3-15 is a novel and promising prodrug possessing both adult and juvenile schistosomes killing capability. Its proposed hydrolytic products, artesunate (ARS), dihydroartemisinin (DHA) and 10-hydroxypraziquantel (10-OHPZQ), are all active in preventing schistosomal infection in relevant disease models. To support pharmacokinetic and PK-PD studies of DW-3-15, a simple, specific and rapid liquid chromatography–tandem mass spectrometry (LC–MS/MS) method was developed and validated for the simultaneous determination of the three active components in rat plasma. Using a short C_{18} column (2.1 mm × 50 mm, 5 μm) with linear gradient, a baseline resolution of the three analytes and corresponding internal standards was achieved with a total run time of 6 min. Mass detection was carried out by electrospray ionization in positive MRM mode with ion transitions of m/z 402.2 → m/z 267.3 for ARS, m/z 302.2 → m/z 163.1 for DHA, and m/z 329.2 → m/z 219.4 for 10-OHPZQ. The method was linear over concentration ranges of 1.0–500 ng/mL for ARS, 5.0–2500 ng/mL for DHA, and 1.0–500 ng/mL for 10-OHPZQ. The accuracy was within ±10.0% for ARS, ±6.4% for DHA, and ±13.0% for 10-OHPZQ. The within-run and between-run precision of all three analytes at four concentrations tested were less than 15%, except at the LLOQ for DHA which was between 15 and 20%. The method was successfully applied to pharmacokinetic evaluation of DW-3-15 in rats following intravenous administration.

1. Introduction

Schistosomiasis is a chronic disease elicited by the infection of schistosomes. The most important types of schistosomes infecting human are *Schistosoma mansoni, Schistosoma japonicum*, and *Schistosoma haematobium* [1,2]. The infection of schistosomes can lead to disease symptoms like chronic hepatic and intestinal fibrosis, ureteric and bladder fibrosis, and calcification of the urinary tract [1,2]. According to the World Health Organization (WHO), there are more than 230 million people annually that would require treatment for schistosomiasis [3]. Therefore, schistosomiasis is still a serious disease burden for some regions in the world.

For many years, the treatment of schistosomiasis has relied on a single drug, praziquantel (PZQ), to control morbidity. PZQ is a safe and effective drug against schistosomiasis. At a single oral dose of 40–60 mg/kg, PZQ is sufficient to achieve excellent cure rates [4]. The drug is also cost-effective and its low cost makes it possible to be accessed by greater patient populations in many regions in the world. Despite all of these advantages, PZQ has been shown to be less active against immature parasites relative to the adult forms [1,2]. In addition, there are reports indicating the appearance of schistosome isolates less sensitive to the drug and the emergence of resistance to PZQ would represent a significant unmet medical need [5–7]. As such, efforts have been made to discover and develop alternative treatment strategy for schistosomiasis. Among those potential alternatives to PZQ, artemisinin analogues such as artemether and artesunate have received considerable attention [8,9]. Furthermore, the combination of artemisinin and PZQ has been shown to be effective against schistosome infection [10].

An artesunate derivative of praziquantel, DW-3-15, is a novel prodrug that is active against both juvenile and adult forms of *S. japonicum* [11]. Based on its structure (Fig. 1), DW-3-15 would release artesunate (ARS), dihydroartemisinin (DHA) and 10-hydroxypraziquantel (10-OHPZQ) upon hydrolysis, components that have been shown to be active against schistosome infection

Abbreviations: ARS, artesunate; DHA, dihydroartemisinin; ATM, artemether; PZQ, praziquantel; 10-OHPZQ, 10-hydroxypraziquantel.

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http://dx.doi.org/10.1016/j.jpba.2013.05.002

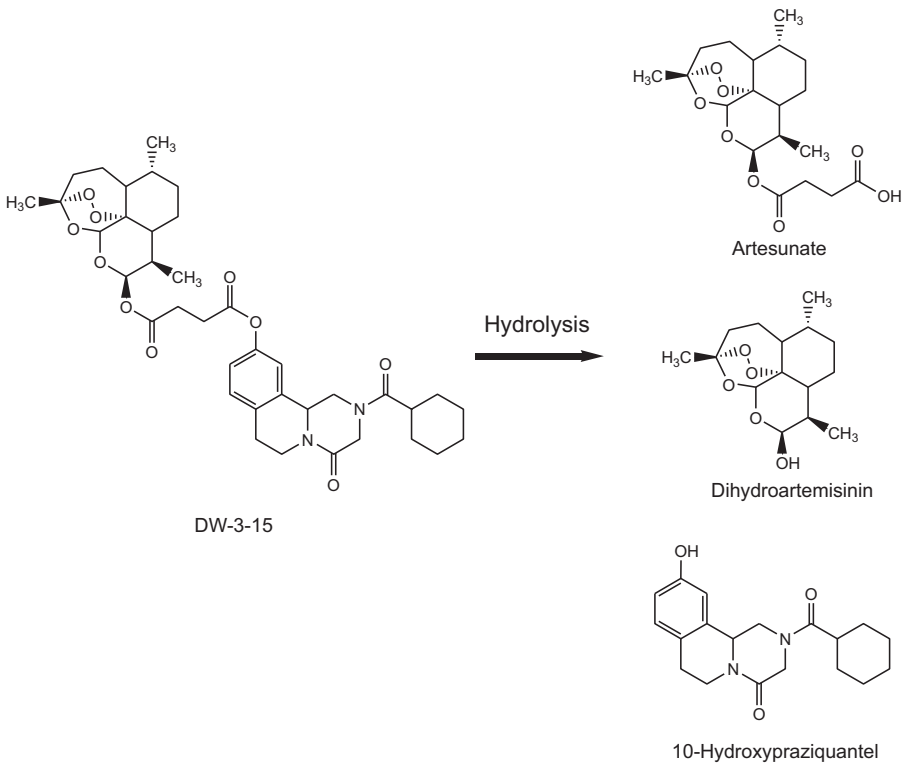

Fig. 1. Structures of DW-3-15 and its three hydrolytic products.

in vitro and in vivo [8,9,11]. As a single agent, DW-3-15 may offer advantages over different drug combinations with respect to lowering the first pass effect (known for artemisinin and its analogs, as well as praziquantel) and improving bioavailability and target distribution. To support further in vivo characterization and development of DW-3-15, a sensitive assay is needed to determine the levels of ARS, DHA and 10-OHPZQ in biological matrices. A reliable assay also would allow in-depth mechanistic investigation of DW-3-15's in vivo hydrolysis and its relationship to worm killing activity. In the present report, we describe a simple, rapid and sensitive LC–MS/MS method for the simultaneous quantitation of the above three active components in rat plasma. In addition to its excellent precision and accuracy, the method affords a good chromatographic separation of ARS, DHA and 10-OHPZQ with a short run time, making it possible to process a large number of samples and support pharmacokinetic and pharmacodynamic studies in a timely fashion.

2. Experimental

2.1. Materials and reagents

Artesunate (ARS), dihydroartemisinin (DHA) and artemether (ATM, internal standard for ARS and DHA) (all ≥99% purity) were purchased from Zhengzhou Lion Biological Co., Ltd. (Zhengzhou, China). Praziquantel (PZQ, internal standard for 10-OHPZQ) (≥99% purity) was purchased from Shanghai Nuotai Chemical Co., Ltd. (Shanghai, China). DW-3-15 and 10-hydroxypraziquantel (10-OHPZQ) were synthesized as previously described and the purity and structure were confirmed by HPLC, LC–MS and NMR [11]. Solvents and reagents such as ammonium acetate (Sinopharm, Beijing, China), acetonitrile (Merck, Darmstadt, Germany) and formic acid (Aladdin Reagent, Shanghai, China) were of HPLC grade. Ultrapurified water was obtained using the Master-S ultrapurification system (Shanghai, China).

2.2. Instrumentation

Chromatography was performed using a Shimadzu HPLC system consisting of a DGU-20A3 degasser, two LC-20AD pumps, a SIL-20A auto-sampler, a CTO-20A column temperature oven and a CBM-20A communications bus module (Shimadzu Corporation, Kyoto, Japan). Mass spectrometric detection was achieved with an AB SCIEX 4000 Qtrap system (Applied Biosystems, Foster City, CA, USA) equipped with a Turbo IonSpray (ESI) ionization source. The data acquisition and processing were performed using the Analyst® 1.5.2 software (Applied Biosystems, Foster City, CA, USA).

2.2.1. Chromatographic conditions

Chromatographic separation was achieved with an Agela Venusil C_{18} column (2.1 mm × 50 mm, 5 μm) (Tianjin, China) and the column was maintained at 40 °C. At a flow rate of 0.3 mL/min, the analytes were eluted using a stepwise gradient elution with solvent A (10 mM ammonium acetate aqueous solution containing 0.1% formic acid) and solvent B (acetonitrile). The gradient was set as the following: 0–0.5 min, 5% B; 1 min, 60% B; 3 min, 95% B; 3.0 min–4.5 min, 95% B; and 4.51 min–6.0 min, 5% B. The total run time was 6 min.

2.2.2. Mass spectrometric conditions

The mass spectrometer was operated in the positive ionization mode under ESI condition and quantitation was performed by multiple reaction monitoring (MRM). The MS/MS setting parameters were as follows: curtain gas, 20 psi; nebulizer gas (GS1), 55 psi; turbo gas (GS2), 55 psi; ion spray voltage, 5500 V; ion source temperature, 500 °C; and dwell time, 150 ms. For selected ion transitions, the declustering potential (DP) and the collision energy (CE) were optimized for the best sensitivity for each analyte (Table 1).