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Determination of hydromorphone in human plasma by a sensitive RP-HPLC–ESI-MS method and its application to a clinical pharmacokinetic study in postoperative patients after low dose intravenous administration with infusion pump

Luning Sun[a,b], Yinbin Pan[c,1], Li Ding[a,*], Xuemei Luo[a], Zhengyu Yan[b,**,2], Cunming Liu[c], Yanning Qian[c], Yan Chu[a]

[a] Department of Pharmaceutical Analysis, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[b] Department of Analytical Chemistry, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China
[c] Department of Anesthesiology, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China

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ABSTRACT

A sensitive reverse phase high performance liquid chromatography–electrospray ionization-mass spectrometry (RP-HPLC–ESI-MS) method has been developed and validated for the determination of hydromorphone in human plasma using naloxone as the internal standard (IS). After alkalization with saturated sodium bicarbonate, the plasma samples were extracted with ethyl acetate. Chromatographic separation was performed on a C18 column with the column temperature of 50 °C and a mobile phase of 5 mM ammonium acetate buffer containing 1% formic acid–methanol (88:12, v/v). Hydromorphone and the IS were detected by selected ion monitoring using the protonated molecules at *m/z* 286.2 for hydromorphone and *m/z* 328.2 for the IS. Calibration curve was linear over the range of 0.01–50 ng/mL. The lower limit of quantification was 0.01 ng/mL. The method was successfully applied to the pharmacokinetic study in postoperative patients after intravenous infusion of 1.5 mg hydromorphone hydrochloride. The obtained main pharmacokinetic parameters of hydromorphone in postoperative patients were as follows: the maximum hydromorphone plasma concentration (C_{max}) was (24.15 ± 12.51) ng/mL, the time to the C_{max} was (10.0 ± 0.0) min, and the elimination half-life was (2.7 ± 0.8) h.

1. Introduction

Hydromorphone (see Fig. 1), a semi-synthetic derivative of morphine, has been used extensively for the management of postoperative pain and morphine-resistant cancer-related pain [1], and has been considered as an alternative to morphine [2]. Although the pharmacokinetic characters of hydromorphone in healthy subjects have been studied [3–5], no data is available regarding this drug in postoperative patients or healthy Chinese subjects. Moreover, Chinese subjects have a higher clearance of morphine analogs compared with European subjects due to ethnical differences [6]. Therefore, in order to accurately elucidate the pharmacokinetics of hydromorphone hydrochloride injection in Chinese postoperative patients, it was necessary to develop a sensitive method for the determination of hydromorphone in human plasma. Various methods have been reported for the determination of hydromorphone in human plasma, which involved radioimmunoassay (RIA) [7], high performance liquid chromatography using electrochemical detector (HPLC–ECD) [8,9] and high performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS) [10–15]. The radioimmunoassay lacked of selectivity, while the HPLC–ECD methods [8,9] were not sensitive enough and required large plasma sample volume for assay. Among the six reported HPLC–MS/MS methods, four [10–13] of those methods focused on doping control or therapeutic drug monitoring of hydromorphone using the long HPLC–MS/MS run time, and were not sensitive enough for human pharmacokinetic study. The other two methods [14,15] were both normal phase high performance liquid chromatography–electrospray ionization-mass spectrometry (NP-HPLC–MS/MS) methods with the LLOQ of 0.1 ng/mL [14] and 0.05 ng/mL [15], respectively. But the need of automated SPE equipments [14] was not available in most laboratories. In these

* Corresponding author. Tel.: +86 25 83271289; fax: +86 25 83271289.
** Corresponding author.
E-mail addresses: dinglidl@hotmail.com (L. Ding), yanzhengyujiang@hotmail.com (Z. Yan).
[1] Co-first author.
[2] Co-corresponding author.

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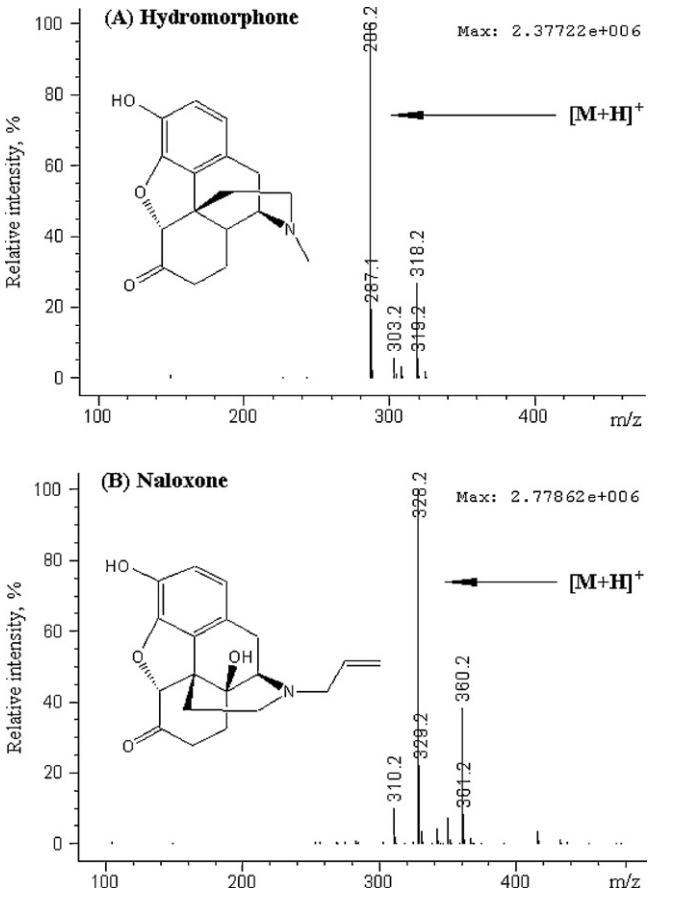

Fig. 1. Structures and mass spectra of hydromorphone (A) and naloxone (B).

two methods, to have to adopt the NP-HPLC system instead of the common and widely applied RP-HPLC system was mainly owing to the poor MS sensitivity and peak shape deterioration. However, these problems could also be solved by optimizing the RP-HPLC chromatographic and mass spectrometric conditions under the RP-HPLC system. The pilot study in our laboratory showed that an LLOQ of 0.01 ng/mL was necessary for our pharmacokinetic study. Therefore, the goal of this work was to develop a more sensitive RP-HPLC–MS method to determinate the hydromorphone in human plasma and illustrate the pharmacokinetics of hydromorphone in Chinese postoperative patients after 1.5 mg intravenous infusion administration of hydromorphone hydrochloride.

2. Experimental

2.1. Chemicals and reagents

The reference standard of hydromorphone hydrochloride (purity > 99.0%) was provided by Yichang Humanwell Pharmaceutical Co., Ltd. (Yichang, China). The reference standard of naloxone (see Fig. 1, purity > 99.0%) was purchased from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Methanol of HPLC grade was purchased from Merck KGaA (Darmstadt, German). Sodium bicarbonate, ethyl acetate, ammonium acetate and formic acid were of analytical grade purity and purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Distilled water was used throughout the study. Blank plasma was supplied by the Nanjing Branch of the Red Cross Society of China.

2.2. Instrumentation

The HPLC–ESI-MS methods were performed using an Agilent Technologies Series 1100 LC/MSD SL system (Agilent Technologies, Palo Alto, CA, USA), which included an Agilent 1100 G1312A binary pump, vacuum degasser (model G1322A), G1316A injection temperature controlled column compartment, Agilent 1100 autosampler (model G1313A), and an Agilent 1100 MSD single quadrupole mass spectrometer equipped with an electrospray source (model G1956B). The signal acquisition, peak integration and concentration determination were performed using the ChemStation software (10.02 A) supplied by Agilent Technologies.

2.3. LC–MS conditions

A Hedera ODS-2 column (5 μm, 150 mm × 2.1 mm i.d., Hanbon Science and Technology, Huaian, China) with a security Guard-C_{18} (5 μm, 4 mm × 2.0 mm, Phenomenex, Torrance, CA, USA) was used for the analytes separation. Isocratic elution employed a mobile phase of 5 mM ammonium acetate buffer solution containing 1% formic acid (pH 2.5)–methanol (88:12, v/v) at a flow rate of 0.20 mL/min. The column temperature was maintained at 50 °C. The autosampler was maintained at 8 °C.

The HPLC–ESI-MS method was carried out using nitrogen to assist nebulization. The quadrupole mass spectrometer equipped with an ESI source was set with the drying gas (N_2) flow of 10 L/min, the nebulizer pressure of 30 psig, the drying gas temperature of 350 °C, the capillary voltage of 4.0 kV and the positive ion mode. The fragmentor voltage was set at 160 V. The ESI-MS was performed in the selected ion monitoring (SIM) mode using the target ions $[M+H]^+$ at *m/z* 286.2 for hydromorphone and *m/z* 328.2 for the IS. Fig. 1 shows typical full-scan ESI mass spectra of hydromorphone and the IS.

2.4. Preparation of standard solutions

The stock solution of hydromorphone with a concentration of 1.0 mg/mL was prepared by dissolving hydromorphone in methanol. A series of standard working solutions with concentrations in the range of 0.5–10 μg/mL for hydromorphone were obtained by further dilution of the stock solution with methanol. The IS working solution (538 ng/mL) was prepared by diluting the stock solution of naloxone with methanol. All the solutions were stored at −20 °C and were brought to room temperature before use.

2.5. Plasma sample preparation

A plasma sample (1 mL) and 30 μL IS (538 ng/mL) were placed into a tube in turn. After vortex-mixing for 10 s, 1 mL saturated sodium bicarbonate was added and vortex-mixed for 30 s to alkalize the plasma. Then, the alkalized plasma sample was extracted with two 4 mL ethyl acetate by vortex for 5 min, and centrifuged at 4000 rpm for 8 min at room temperature. The supernatant was separated and evaporated to dryness under a gentle stream of nitrogen in a water bath of 40 °C. The residue was reconstituted with 120 μL mobile phase, and a 20 μL aliquot of the reconstituted solution was injected into the RP-HPLC–ESI-MS for analysis.

2.6. Preparation of calibration curves and quality control samples

The calibration standards of hydromorphone were prepared by spiking appropriate amount of hydromorphone working solutions into 1.0 mL blank plasma from healthy subjects. The standard curve was prepared for hydromorphone at concentration levels of 0.01024, 0.03072, 0.1024, 0.3072, 1.024, 3.072, 10.24, 25.60, 51.20 ng/mL. And those standard plasma samples were prepared according to the plasma sample preparation. The calibration curve