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Analysis of total and unbound hydromorphone in human plasma by ultrafiltration and LC–MS/MS: Application to clinical trial in patients undergoing open heart surgery

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ABSTRACT

A method for a sensitive and specific analysis of hydromorphone total and unbound drug concentrations in human plasma was developed and validated. Sample preparation was preceded with an ultrafiltration step to separate the unbound drug from the protein bound fraction of hydromorphone. Both the ultrafiltrate and plasma samples were extracted with solid-phase extraction and substituted with stable isotope-labeled hydromorphone that was used as internal standard. Chromatographic separation was performed by gradient elution with UPLC-like system and eluates were analyzed by tandem mass spectrometry equipped with an electrospray ionization source. Sample preparation was optimized for good recovery of hydromorphone and the results were consistent. Calibration curves demonstrated linearity in the concentration range of 78–5000 pg/ml for analysis of both total and unbound concentrations of hydromorphone. The limit of detection was 1 pg and the lower limit of quantification was 78 pg/ml for both total and unbound hydromorphone plasma drug concentrations. Intra- and interassay reproducibility and inaccuracy did not exceed 10%. Hydromorphone was on the average 14% bound to plasma proteins, supporting the previously published unreferenced statements that the protein binding of hydromorphone is low. Method was applied to a clinical trial in patients undergoing open heart surgery to generate a target controlled infusion model for the postoperative patient controlled analgesia with hydromorphone.

1. Introduction

Hydromorphone is a phenanthrene derivative (4,5-α-epoxy-3-hydroxy-17-methyl morphinan-6-one) which is used as an opioid analgesic for the relief of moderate to severe pain. It is related to morphine but has a greater analgesic potency. Although hydromorphone is extensively used in postoperative pain therapy, its pharmacokinetics are not thoroughly studied in this patient population. Until recently the plasma concentrations of hydromorphone were well below the limit of detection of most analytical instruments. As discussed previously [1], most of the previously reported methods for hydromorphone determination either lack the sensitivity or had methodological restrictions to be used in clinical studies. Recently Sun et al. [1] presented a sensitive LC–MS/MS based method for hydromorphone plasma concentration determination. This method could be used for clinical studies, but only total concentrations could be measured and the method is not readily available for determining unbound plasma concentrations of hydromorphone.

Drug effect is dependent on the unbound plasma drug concentrations, and alteration in protein binding may result in clinically significant changes in the pharmacokinetics [2]. Earlier studies have shown a large interindividual variability in the drug concentrations and pain ratings after hydromorphone administration [3–5]. Effects of protein binding on the concentration–time course and thus on the pharmacodynamics of hydromorphone might partly explain the finding. Previously, different findings for protein binding for hydromorphone in humans have been published. According to these reports the protein binding of hydromorphone was 7.1% [6], 19% [7] or 27% [8]. However, only one of these reports [7] is an original study using equilibrium dialysis to measure protein binding, and after extensive literature search we could not trace original studies published in peer reviewed journals confirming the other two statements. These findings might not only reflect the differences in the plasma protein binding, but also in the protein content of plasma or in the effect of different pathophysiological states on the pharmacokinetics of hydromorphone.

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Previous data regarding the influence of drug binding to plasma proteins on pharmacodynamic parameters are scarce, and it is recommended to use the unbound instead of the total concentration whenever possible in pharmacokinetic–pharmacodynamic (PK–PD) modeling for drugs that are binding plasma proteins [9,10].

Target controlled infusion (TCI)-systems have been introduced to calculate instantaneously the infusion rate needed to achieve and maintain a given therapeutic drug plasma concentration based on population pharmacokinetic parameters [11,12]. Recent studies demonstrate that TCI systems provide more stable analgesia and better hemodynamic control when compared to bolus dosing [13,14]. Also, smaller amounts of opioids are administered with TCI [15]. TCI combined with patient controlled analgesia is of increased interest for postoperative analgesia [16,17], and we have recently shown that intraoperatively administered sufentanil affects the postoperative patient-controlled analgesia with respect to postoperative opioid consumption and pain in patients undergoing cardiac surgery [18]. Currently we are investigating the pharmacokinetic and pharmacodynamic interactions of hydromorphone and sufentanil and we recently developed a method to measure unbound sufentanil concentration [19]. A method for determining the unbound hydromorphone drug concentrations had also to be established, since we are not aware of any published reports describing the analysis of both total and unbound hydromorphone concentrations in human plasma.

A method to determine the plasma levels of total and unbound hydromorphone in patients undergoing cardiac surgery with cardiopulmonary bypass was developed. Samples were ultrafiltrated preceding the preparation of human plasma samples to facilitate the analysis of unbound hydromorphone. To increase the efficiency and sensitivity of the analysis, an UPLC-like system with tandem mass spectrometry is utilized [20]. Finally, a deuterated hydromorphone was used as an internal standard to improve the precision and accuracy of the method as well as the robustness of the quantification against matrix effects.

2. Materials and methods

2.1. Drugs and chemicals

Hydromorphone (1 mg/ml in methanol) and internal standard (IS) hydromorphone-D3 were from LGC-Standards (Wesel, Germany). Acetonitrile, methanol (HPLC Gradient Grade), formic acid and ammonia (30%) were purchased from Roth (Karlsruhe, Germany). Deionized water was prepared with a Simplicity system from Millipore (Schwalbach, Germany). All eluents were degased in an ultrasonic bath and perfused with helium gas. Drug free human plasma was obtained from Recipe Chemicals (München, Germany).

2.2. Standard solution and calibration standards

Stock solutions of hydromorphone and hydromorphone-D3 (20 ng/ml) were prepared by dissolving with water and stored at −20 °C. Standard working solutions for hydromorphone were obtained by further dilution of the stock solution with water. Quality control (QC) samples were prepared to drug free plasma and blank study plasma samples (zero samples) by adding 50 μl hydromorphone standards to 450 μl drug free plasma. The calibration samples for the determination of total and unbound hydromorphone (5000, 2500, 1250, 625, 312.5, 156.3 and 78.15 pg/ml) were prepared in the same way using drug free plasma. All standards were prepared for the LC–MS/MS analysis according to the plasma sample preparation.

2.3. Sample preparation

Before further preparation, plasma samples to be analyzed were thawed and centrifuged for 5 min at 2000 × *g*.

2.3.1. Unbound hydromorphone

For the estimation of the unbound concentrations of hydromorphone, plasma proteins with protein bound hydromorphone had to be separated. An aliquot (500 μl) of plasma was supplied with IS (50 μl of 10 ng/ml hydromorphone-D3) and transferred to YM-30 Centrifree centrifuge tubes (Merck Millipore Ltd., Carrigtwohill, Ireland). Tubes were centrifuged for 20 min at a relative centrifugal force of 5000 × *g* (Beckmann Coulter Avanti – J-E centrifuge with JA-20.1 rotor) at +15 °C. The ultrafiltrate was supplied to OASIS HLB solid phase extraction cartridges (Waters, Eschborn, Germany), which had been conditioned with 2 ml of methanol followed by 2 ml water. The cartridges were rinsed twice with 1 ml water. For descaling the residual water, the cartridges were centrifuged for 3 min at 2000 × *g*. The analytes were eluted with 0.9 ml acetonitrile, containing 5% ammonia under a slight vacuum and evaporated to dryness under nitrogen. The analytes were then reconstituted with 100 μl 0.02% formic acid.

2.3.2. Total hydromorphone

Total hydromorphone was estimated out of an aliquot of 500 μl plasma without ultrafiltration. The extraction procedure was the same as described above.

2.4. Equipment

Chromatographic separation was carried out with the Waters Alliance HPLC system with autosampler. The Alliance system was upgraded and modified by Fischer Analytics (Bingen, Germany) to allow UPLC-like pressures of more than 400 bar. The analytes were detected with Waters Quattro Micro tandem mass spectrometer equipped with an electrospray ionization interface (ESI). Data were collected and analyzed with MassLynx™ V4.0 software.

2.5. HPLC conditions

To separate the analytes, a Kinetex (C18 Core-Shell HPLC, 150 mm × 2.1 mm, 2.6 μm, 100 Å) analytical column (Phenomenex, Aschaffenburg, Germany) protected by a HPLC Guard Cartridge system (C18 Security Guard, Phenomenex) was used. Gradient elution was used for chromatographic separation with mobile phase consisting of 0.02% formic acid in water and acetonitrile (95:5, v:v). The amount of acetonitrile was increased after 0.5 min in the mobile phase (45:55, v:v). After separation the column was reequilibrated with starting conditions for the next 4 min and the total chromatographic time was 8 min. The mobile phase flow was kept at 500 μl/min and 10 μl of the extracted sample were injected. With a column temperature of +24 °C, the retention time for hydromorphone and hydromorphone-D3 was 2 min.

2.6. Mass spectrometry conditions

ESI-MS was accomplished in positive electrospray mode with multiple-reactions monitoring (MRM). Nitrogen was used as the desolvation and cone gas and argon was used as the collision gas. The optimal settings for mass spectrometry were found to be: ion source temperature at 140 °C, the capillary and cone voltages 3.5 kV and 40 V, respectively and the cone gas flow at 50 l/min. Drying gas temperature was 500 °C and the flow was set 900 l/min. The effluent was directed with an automatic divert valve to a waste container for 0.7 min, to avoid soiling the mass spectrometer with the matrix. The valve was then switched to the mass spectrometer