Journal of Pharmaceutical and Biomedical Analysis 107 (2015) 196–203

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

A novel, simple and inexpensive procedure for the simultaneous determination of iopamidol and *p*-aminohippuric acid for renal function assessment from plasma samples in awake rats

Violeta Rodríguez-Romero[a,b], Karla I. González-Villalva[a,c], José L. Reyes[d], Rebecca E. Franco-Bourland[a,e], Gabriel Guízar-Sahagún[a,f], Gilberto Castañeda-Hernández[a,b], Leticia Cruz-Antonio[a,c,*]

[a] *Proyecto Camina A.C., Calz. Tlalpan 4430, 14050 Mexico City, Mexico*
[b] *Departamento de Farmacología, Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional, Av. Instituto Politécnico Nacional 2508, 07360 Mexico City, Mexico*
[c] *Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de Mexico, Av. Guelatao 66, 09230 Mexico City, Mexico*
[d] *Departamento de Fisiología Biofísica y Neurociencias, Centro de Investigación y de Estudios Avanzados, Instituto Politécnico Nacional, Av. Instituto Politécnico Nacional 2508, 07360 Mexico City, Mexico*
[e] *Servicio de Bioquímica, Instituto Nacional de Rehabilitación, Calz. Mexico Xochimilco 289, 14389 Mexico City, Mexico*
[f] *Unidad de Investigación en Enfermedades Neurológicas, Instituto Mexicano del Seguro Social, Av. Cuauhtémoc 330, 06720 Mexico City, Mexico*

ARTICLE INFO

Article history:
Received 22 August 2014
Received in revised form 5 December 2014
Accepted 9 December 2014
Available online 3 January 2015

Keywords:
Analytical method
High performance liquid chromatographic
Glomerular filtration rate
Tubular secretion
Renal dysfunction

ABSTRACT

The purpose of the current study was to design, validate and implement a novel analytical method for the simultaneous plasma measurement of iopamidol and *p*-aminohippuric acid (PAH) to estimate renal function in awake rats. A reverse-phase high performance liquid chromatographic (RP-HPLC) method for the simultaneous measurement of iopamidol (for glomerular filtration rate estimation, GFR) and PAH (for tubular secretion determination, TS) was designed and validated using a C-18 column, 0.1 M acetic acid–10% acetonitrile (90:10, v/v) as mobile phase, at a flow rate of 0.3 ml/min, and UV detection at 270 nm. Iopamidol (244.8 mg/kg) was administered intravenously followed immediately by sodium PAH (100 mg/kg) to healthy female Sprague-Dawley rats. Plasma samples obtained at 2.5, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min after drug administration were deproteinized with 2.5% trichloroacetic acid containing *p*-aminobenzoic acid as internal standard, and separated by the validated RP-HPLC method described above. The iopamidol and PAH chromatographic data were analyzed using a non-compartmental model. The results demonstrated that the RP-HPLC method was linear in ranges between 15–120 μg/ml and 2.5–120 μg/ml for iopamidol and PAH, respectively. Precision and accuracy were within 15% for both drugs. Recovery of iopamidol and PAH was 92% and 100%, respectively. Plasma iopamidol and PAH clearances in awake rats, estimates for GFR and TS, respectively, were 1.49 ± 0.20 ml/min and 3.73 ± 0.38 ml/min. In conclusion, the method here described is a simple and reliable procedure, for the simultaneous and time-saving determination of GFR and TS from plasma samples in the conscious rat.

1. Introduction

Methods described to date for the assessment of renal function are hampered by all sorts of inconveniences, creating the need for an improved method for its simple and reliable estimation.

The kidney plays an important role in elimination from the body of a wide variety of drugs. Pharmacokinetic behavior and consequent renal clearance is used to determine the specific mechanism of renal elimination of a drug by glomerular and tubular function [1].

Glomerular filtration rate (GFR) describes the flow rate of filtered fluid through glomerular capillaries into the Bowman's capsule, and can be defined as the volume of plasma cleared per unit time (usually expressed as ml/min) of an ideal nontoxic substance (such as inulin) that is freely filtered, not bound to plasma proteins, and is neither secreted nor reabsorbed by renal tubules [2].

* Corresponding author at: Facultad de Estudios Superiores Zaragoza, Universidad Nacional Autónoma de México, Av. Guelatao 66, 09230 Mexico City, Mexico. Tel.: +52 55 55730029; fax: +52 55 56230749.
E-mail address: letycruza@yahoo.com.mx (L. Cruz-Antonio).

http://dx.doi.org/10.1016/j.jpba.2014.12.009
0731-7085/

Inulin's rate of excretion is directly proportional to the rate of filtration of water and solutes across the glomerular capillaries, and is therefore used experimentally as reference measurement for GFR [3]. In clinical practice, creatinine clearance is the test of choice although it is considered to be a poor GFR marker because it is influenced by a variety of drugs, individual gender, and physical conditions that lead to overestimate the true value of this parameter of renal function, not to mention the inconvenience for the requirement of a 24 h urine collection [4]. Radiolabeling has also been used, with the disadvantage of exposure to radioactivity during the test, as well as other drawbacks that include the need for storage of radioactive material and waste disposal [4,5].

Non-ionic monomers, such as iohexol, iopamidol, and iopromide that are freely filtered by the glomeruli, have been proposed as alternative GFR markers [6–9], with the added advantage of their lower hypertonicity as compared to conventional ionic agents [10].

Iopamidol was chosen here as a GFR marker for its properties as a non-ionic water-soluble radiographic contrast medium with low osmolarity, and low chemical toxicity [9].

Tubular excretion is an efficient mechanism for extracting compounds from the circulation and excreting them into the urinary compartment, and can have an important impact on the drug's pharmacokinetic behavior [11]. PAH is a non-toxic molecule that is neither bound to proteins in plasma, nor is permeable to erythrocyte membranes [12]. It is a commonly used marker for renal plasma flow [3], and has been used as a measure for tubular function [1].

Described here is an improved procedure that has been designed for the simultaneous cost-effective determination of GFR and tubular secretion (TS) from rat plasma by measuring iopamidol and PAH in the same biological sample. It is a simple, sensitive, and reproducible reversed-phase high performance liquid chromatographic (RP-HPLC) method with UV detection that has been validated for the simultaneous determination of iopamidol and PAH clearance in rat plasma as estimates for GFR and TS, respectively.

2. Materials and methods

2.1. Study design

This study was performed in two phases. In the first phase a RP-HPLC method for iopamidol and PAH measurement in rat plasma was designed and validated using *p*-aminobenzoic acid (PABA) as internal standard. In the second phase this procedure was implemented in a pharmacokinetic study in rats for iopamidol and PAH clearance estimation as indices of GFR and TS, respectively.

Full validation of the analytical method was conducted in accordance with official Mexican guidelines (NOM-177-SSA1-1998), which are basically in accordance with ICH Q2R1 international guidelines for the following validated parameters: selectivity, linearity, precision and accuracy, recovery and stability.

GFR and TS measurements were performed in healthy Sprague-Dawley female rats weighing 230–250 g body weight. Rats were randomly assigned to three groups of six animals each for the administration of one of the following formulations: iopamidol, iopamidol/PAH, and probenecid/iopamidol. Probenecid was used as an inhibitor of a possible secretory component in iopamidol's kinetics.

The local institutional Animal Care Committee approved this study which was carried out in accordance with the Ethical Guidelines for Investigation of Experimental Pain in Conscious Animals [13]. Rats were housed in a temperature-controlled environment and a 12 h/12 h light/dark regimen. They were kept on Purina rat chow and water ad libitum.

2.1.1. Test substances

A commercially available iopamidol formulation (Solucont 300®, Bonaplast SA de CV, México) was used. Just before administration, a PAH (Sigma, México) solution was prepared by dissolving it in physiological isotonic saline to a final concentration of 100 mg/ml. Probenecid was dissolved in a diluted alkaline solution (1% NaOH) which was adjusted to pH 7.4 with 1% HCl.

2.2. Analytical method validation

2.2.1. Iopamidol and PAH extraction from plasma and RP-HPLC analysis

Sample preparation and RP-HPLC analysis described here, were modified from a previous method for dog plasma [14].

Iopamidol and PAH plasma were extracted from 100 μl plasma with 850 μl 2.5% trichloroacetic acid (TCA), in the presence of 50 μl of PABA (100 μg/ml), as internal standard. This mixture was shaken in a vortex for 5 min.

After centrifugation at 16 000 × *g*, acid supernatant aliquots (60 μl each) were analyzed by RP-HPLC at room temperature using a Symmetry C18 column (5 μm, 150 mm × 4.6 mm i.d.) (Waters Corp., Milford, MA, USA) equipped with a guard column Zorbax SB-C18 (5 μm, 12.5 mm × 4.6 mm i.d.). The mobile phase consisted of a solution of acetic acid (0.1 M)–acetonitrile (90:10, v/v) (pH* 3–4), run at a constant flow rate of 0.3 ml/min; the effluent was monitored by UV detection at 270 nm. Peak area ratios were determined using a Beckman 427 integrator set at a chart speed of 0.25 cm/min.

2.2.2. Solutions for RP-HPLC analysis

Iopamidol stock solution was 20.4 mg iopamidol/ml (equivalent to 10 mg I_2/ml). This solution was prepared by diluting 1 ml Solucont 300® (612 mg iopamidol, equivalent to 300 mg I_2) in 30 ml of distilled deionized water. Chromatographic reference standards were obtained by further diluting this solution.

PAH stock solution was 1 mg PAH/ml. It was prepared by dissolving 250 mg PAH in 25 ml distilled deionized water. PABA, used as internal standard, was prepared as a 100 μg/ml solution in distilled deionized water.

Standard curves and quality control (QC) samples were prepared by combining various volumes of iopamidol and PAH stock solutions (40–120 μl and 50–150 μl, respectively), 100 μl blank rat plasma, 50 μl PABA stock solution, and a minimum protein precipitating volume of 2.5% TCA (610 μl) which was also used to bring final volume of the mixture to 1 ml. Iopamidol and PAH UV standard curves were created using the following concentrations: 15, 30, 60, 90, and 120 μg/ml iopamidol, and 2.5, 5, 15, 45, 80, and 120 μg/ml PAH. QC concentrations for iopamidol were set at 17, 50, and 100 μg/ml and 3.75, 22, and 90 μg/ml for PAH for low, medium, and high concentration levels, respectively.

2.2.3. RP-HPLC data analysis

All RP-HPLC data were obtained from chromatograms. Selectivity was evaluated by comparing chromatograms of six different blank rat plasma samples and the same number of rat plasma samples with added drugs (diclofenac, naproxen sodium, salicylic acid, pentoxifylline, ciprofloxacin, and enrofloxacin) to ensure that there were no significant interfering UV-absorbing substances coeluting with the analytes.

Linearity was evaluated by linear regressions of peak area ratios for iopamidol and PAH relative to PABA (y) versus the actual analyte concentrations (x), obtained from six calibration curves, which ultimately resulted in linear average standard curves represented by the following equation: $y = mx + b$, where m and b are the slope and intercept of the straight line, respectively.

Precision and accuracy of the assay were determined by the repeatability (intra-day analysis) and reproducibility (inter-day