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Short communication

A rapid liquid chromatography tandem mass spectrometry-based method for measuring propranolol on dried blood spots

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ABSTRACT

Propranolol, a non-selective beta blocker drug, is used in young infants and newborns for treating several heart diseases; its pharmacokinetics has been extensively evaluated in adult patients using extrapolation to treat pediatric population.

The purpose of the present study was to develop and validate a method to measure propranolol levels in dried blood spots. The analysis was performed by using liquid chromatography/tandem mass spectrometry operating in multiple reaction monitoring mode. The calibration curve in matrix was linear in the concentration range of 2.5–200 μg/L with correlation coefficient $r = 0.9996$. Intra-day and inter-day precisions and biases were less than 8.0% ($n = 10$) and 11.5% ($n = 10$) respectively. The recoveries ranged from 94 to 100% and the matrix effect did not result in a severe signal suppression.

Propranolol on dried blood spot showed a good stability at three different temperatures for one month.

This paper describes a micromethod for measuring propranolol levels on dried blood spot, which determines a great advantage in neonates or young infants during pharmacokinetic studies because of less invasive sampling and small blood volume required.

1. Introduction

Propranolol has been used for several decades in young infants and newborns for a variety of indications, such as congestive heart failure in infants with congenital heart disease [1], hypertrophic obstructive cardiomyopathy [2], supraventricular tachycardia [3], thyrotoxicosis in term and preterm infants [4,5], in the management of hypercyanotic spells in Fallot's tetralogy [6], and in the control of dangerous hypertension [7].

In recent years, propranolol, has been reported as an effective drug in reducing the growth of infantile hemangiomas the most common tumor of infancy [8,9].

In a mouse model of oxygen-induced retinopathy, a well-established model of retinopathy of prematurity (ROP), we have recently demonstrated that the pharmacological blockade of beta-adrenoreceptors (β-ARs) with propranolol improves retinal neovascularization and blood retinal barrier breakdown consequent to hypoxia [10], and subsequently we have demonstrated that these effect are mediated mainly by the blockade of β_2-AR [11]. These findings suggest a role of the adrenergic system in the pathogenesis of proliferative retinopathies and open the perspective of a possible therapeutic use of β-AR blockers to counteract retinal neovascularization in ROP. As a result of these discoveries, a pilot randomized trial has been planned to evaluate the safety and the efficacy of oral propranolol administration in preterm

Abbreviations: DBS, dried blood spot; LC–MS/MS, liquid chromatography/tandem mass spectrometry; MRM, multiple reaction monitoring; ROP, retinopathy of prematurity; β-ARs, pharmacological blockade of beta-adrenoreceptors; HPLC, high-performance liquid chromatography; MS, mass spectrometry; GC/MS, gas chromatography coupled with mass spectrometry; LLOD, lower limit of detection; LLOQ, lower limit of quantitation; Hct, hematocrit.

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newborns suffering from a precocious phase of ROP to counteract its progression [12].

It is therefore likely that hereafter, an increasing number of newborns, especially preterm infants, could be treated with propranolol.

While propranolol pharmacokinetics has been studied extensively in adults, no pharmacokinetic study has ever been performed in neonates and infants. The knowledge of propranolol pharmacokinetics in this age group may be helpful to assess proper dosage and its frequency of daily administration.

Several methods have been proposed for the determination of propranolol in human plasma and other biological fluids by high-performance liquid chromatography (HPLC) coupled to fluorescence detector [13] or mass spectrometry (MS) [14–17], and gas chromatography coupled to mass spectrometry (GC/MS) [18]. Most sample preparation techniques are laborious, time consuming and require a large volume of plasma (>100 μL), therefore less suitable to the pediatric population especially preterm one.

Our proposed method is based on the analysis of propranolol from small size blood samples, easily obtained by heel or finger pricks and spotted onto filter paper.

This method also allows to collect several samples from newborns, including preterm infants, in which little is known concerning drug pharmacokinetics and for which rarely it is possible to extrapolate drug efficacy from studies in adults [19].

2. Materials and methods

2.1. Standards

Chemical standard of propranolol hydrochloride (purity >99%) was purchased by Sigma–Aldrich (Steinheim, Germany); the internal standard, propranolol-d_7 (97.8% D purity) was from CDN Isotopes (Quebec, Canada). Stock solutions, prepared in methanol and kept in the freezer (−20 °C), were 16.2 mmol/L and 11.3 mmol/L respectively. Successive dilutions were made using HPLC grade water. Analytical grade methanol, acetonitrile and water were purchased from Panreac (Barcelona, Spain).

2.2. Sample collection

26 whole blood samples were collected from 7 pediatric patients with confirmed ROP to whom propranolol was administered. Both plasma and dried blood spots (DBS) specimens were obtained from each sample.

DBS was prepared by spotting whole blood onto filter paper cards (903®, Whatman GmbH, Dassel, Germany), let to dry and stored at 4 °C in a sealed plastic bag containing desiccant until analysis. The remaining whole blood was centrifuged (2200 × *g*, 10 min); plasma was collected and stored at −20 °C.

The study was approved by the Human Ethics Committee of the Meyer University Children's Hospital (reference 277/2010).

2.3. Sample preparation

Each DBS sample was punched obtaining a 3.2 mm diameter disk (containing about 3.3–3.4 μL of blood) into a vial and extracted with 200 μL methanol/water 95:5 (V/V) + 0.1% formic acid solution containing 1 μg/L of propranolol-d_7. The extraction was performed using an orbital shaker for 25 min at 37 °C, and then the solution was transferred into new vial and analyzed by LC–MS/MS.

For plasma sample 20 μl of each was added to 200 μL methanol/water 95:5 (V/V) + 0.1% formic acid solution containing 1 μg/L of propranolol-d_7 and shaken for 10 s by vortex. The clot was removed by centrifugation at 10,000 × *g* in a microfuge for 3 min and the resulting supernatant was transferred into a new vial and analyzed by LC–MS/MS.

2.4. Validation procedures

In order to set up and validate the method, whole blood from healthy adult donors was spiked with propranolol at different concentration; a 20 μl volume of each fortified blood was spotted on filter paper and used as calibrators.

Calibration curve was prepared by spotting on filter paper human blood spiked with aliquots of a stock solution of propranolol to obtain concentrations at 2.5, 5, 10, 20, 50, 100 and 200 μg/L. To determine intra-day precision of the assay, replicate (n = 10) of blood spot calibrators of propranolol at four different concentration (2.5, 50, 100 and 200 μg/L) were analyzed. Each calibrator was tested in triplicate over ten days to obtain inter-day precision and accuracy data.

To calculate the linear regression, the peak areas ratios (propranolol/propranolol-d_7) were plotted against the drug concentrations in micrograms per liter.

Recovery was evaluated by comparing the extraction yield of pre- and post-spiked DBS at different concentration (2.5, 50, 100 and 200 μg/L). In order to evaluate matrix effect, calibrator signals were compared to the equivalent concentrations prepared matrix free.

Stability testing on DBS samples was evaluated for all calibrators up to one month after storage at −20 °C, +4 °C, and RT.

2.5. Mass spectrometry

The samples were measured using analytical HPLC coupled to a QTRAP 5500 (AB SCIEX, Toronto, Canada) equipped with the TurboIonSpray source operating in positive ion mode. The capillary voltage was set to 5500 V and heated turbo gas (air) with a flow rate of 10.0 L/min was used at 450 °C. The following transitions were monitored in Multiple Reaction Monitoring (MRM) mode: m/z 260.1 > 116.1 (quantifier) and m/z 260.1 > 183.1 (qualifier) for propranolol and m/z 267.1 > 116.1 (quantifier) and m/z 267.1 > 189.1 (qualifier) for propranolol-d_7. Optimal CXP (Collision Cell Exit Potential) was found to be 7 V for quantifiers and 11 V for qualifiers respectively. The Declustering Potential (DP) applied was 60 V and Collision Energy (CE) was 25 V.

Quantitation experiments were performed by using a Series 1290 Infinity LC System (Agilent Technologies, Waldbronn, Germany) HPLC Capillary Pump coupled to an Agilent Micro ALS autosampler. The chromatographic column was a Zorbax SB-C18 Rapid Resolution HT, 2.1 mm × 50 mm, 1.8 μm (Agilent Technologies, Waldbronn, Germany), operating at 0.2 mL/min for a total running time of 4 min. Gradient elution was achieved using a program with mobile phase A (water + 0.1% formic acid) and mobile phase B (acetonitrile + 0.1% formic acid) as follows: 5% B to 95% B in 1 min and maintained for 1.5 min, then back to 5% B in 0.1 min and re-equilibration for 1.4 min. The propranolol retention time was fixed to 1.76 min. The eluent from the column was directed to the TurboIonSpray probe without split ratio. Three microliters of the extracted sample was injected for the LC–MS/MS experiments. System control and data acquisition were performed with Analyst 1.5.1 software including the "Explore" option (for chromatographic and spectral interpretation) and the "Quantitate" option (for quantitative information generation). Calibration curves were constructed with the Analyst Quantitation program using a linear least-square regression non-weighted.