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Short communication

Simultaneous determination of pyrimethamine, sulfadiazine and *N*-acetyl-sulfadiazine in plasma for monitoring infants in treatment of congenital toxoplasmosis

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Abstract

A method for the simultaneous determination of pyrimethamine, sulfadiazine and its metabolite *N*-acetyl-sulfadiazine in small plasma samples from neonates in treatment for congenital toxoplasmosis has been developed. In this method only 25 μl of plasma is used and a simple sample preparation based on protein precipitation and centrifugation provides highly reliable data as the recovery is about 100% and the precision is good. The analysis is performed using high performance liquid chromatography with UV and mass spectrometric (MS) detection. Pyrimethamine was found to give a linear response using MS detection in the range 0.02–5 μg/ml. Sulfadiazine and its metabolite *N*-acetyl-sulfadiazine were preferably analysed by UV at 269 nm in the concentration ranges 0.2–200 μg/ml for sulfadiazine and 0.2–50 μg/ml for *N*-acetyl-sulfadiazine.

Keywords: Congenital toxoplasmosis; Plasma concentrations; Pyrimethamine; Sulfadiazine; *N*-Acetyl-sulfadiazine; LC-MS

1. Introduction

Infection by the protozoan parasite *Toxoplasma gondii* may be transmitted during pregnancy from mother to foetus that may result in a congenital infection causing chorioretinitis, intracranial calcifications and hydrocephalus. Later in life recurrent retinochoroiditis may occur. Congenital toxoplasmosis is treated with sulfadiazine and pyrimethamine and this treatment has not changed since the end of the fifties [1]. Dose-finding studies have never been performed, and the doses used for treatment in congenital toxoplasmosis have been estimated from animal studies [2–4]. The principles for treatment of retinochoroditis with sulfadiazine and pyrimethamine was summarized by Beverly [5] in1958 and has remained almost unchanged until recent years when the use of the long-acting sulfadoxine has gained some popularity [6], however, still without any studies in animals and humans to show it is equally effective as sulfadiazine.

January 1st 1999 a national neonatal screening programme was initiated in Denmark. The screening programme is based on detection of toxoplasmosis specific IgM and/or IgA antibodies in blood obtained shortly after birth and eluted from the blood spot on the PKU filter paper (Guthrie card).

Infants with congenital toxoplasmosis are treated for 3 months with pyrimethamine 2 mg/kg/day on day 1 and then with 1 mg/kg/day on day 2 and thereafter with a maximum of 25 mg/day and sulfadiazine 50–100 mg/kg/day, but treatment efficacy has recently been questioned [7,8]. The children are furthermore treated twice a week with 7.5 mg of folinic acid for protection of the bone marrow.

One of the reasons for poor treatment outcome may be less than optimal dosing or poor compliance. In order to verify

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patient compliance analysis of plasma samples is a natural choice. However, only few and small samples may be obtained from newborns. Therefore, very little is known about plasma concentrations of these drugs in newborns.

No methods for the simultaneous determination of pyrimethamine and sulfadiazine are presented in the literature. A few methods for the determination of pyrimethamine and other sulfa drugs in plasma or whole blood using HPLC with UV detection have been published [9–16].

In the present paper, a method for the simultaneous determination of pyrimethamine, sulfadiazine and its major metabolite *N*-acetyl-sulfadiazine is described. The method involves a simple sample preparation using only 25 μl plasma and a measurement based on high-performance liquid chromatography (HPLC) coupled to a diode array detector (DAD) and a mass spectrometer (MS). Besides high sensitivity the MS technique also provides high selectivity as single ion monitoring of the three analytes and the internal standard is used. The structures of all analytes and the internal standard are given in Fig. 1. The applicability of the method is demonstrated by monitoring the plasma concentrations of the analytes in nine infants in treatment for a period of 10 weeks.

2. Materials and methods

2.1. Chemicals

Sulfadiazine and pyrimethamine were purchased from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). *N*-Acetyl-sulfadiazine was obtained from Maybridge Plc (Tintagel, UK). The HPLC column was purchased from Phenomenex (Torrance, CA, USA). All other chemicals were of analytical reagent grade.

2.2. Instrumentation

An Agilent Technologies (Waldbronn, Germany) 1100 HPLC-system equipped with a diode array detector (operated at 269 nm) and a mass spectrometer with a single quadrupole was used.

The mass spectrometer was operated in the positive electrospray mode with single ion monitoring at 249.1 mass units (pyrimethamine), 251.1 mass units (sulfadiazine), 254.1 mass units (sulfametoxazole) and 293.1 mass units (*N*-acetyl-sulfadiazine). Further settings: fragmentor: 70; V_{cap}: 2500 V; drying gas: 10 l/min; nebulizer: 25 psi g; gas temperature: 350 °C.

2.3. Chromatography

HPLC column: Phenomenex C18, AQUA, 100 mm × 4.6 mm, 3 μm. Mobile phase A: methanol + water + conc. formic acid (50:950:1, v/v/v). Mobile phase B: methanol + water + conc. formic acid (500:500:1, v/v/v). Gradient: 0–100% B 0–3 min; 100% B 3–5 min and 100–0% B 5–6 min with a total run time of 12 min. The flow rate was 0.5 ml/min and the column temperature was set to 25 °C. The UV detection was performed at 269 nm using a diode-array detector.

2.4. Sample collection and preparation

At local hospital laboratories 100–200 μl of capillary or venous blood was collected in a micro vial or in a capillary tube and centrifuged. Each sample was send by post to the analytical laboratory where it was kept at −20 °C until analysed.

Twenty-five microliters of plasma was added 25 μl of internal standard solution (60 μg/ml of sulfamethoxazole in methanol) and 20 μl of 5% perchloric acid in a 100 μl glass insert placed in an ordinary Agilent autosampler vial and supplied with a crimp cap. The mixture was carefully mixed and then centrifuged at 4000 × *g* for 15 min. Ten microliters of the supernatant was injected directly from the vial into the HPLC without disturbing the sediment (injector draw position at 12 mm).

At too high concentrations of sulfadiazine the injection volume was reduced to 2 μl.

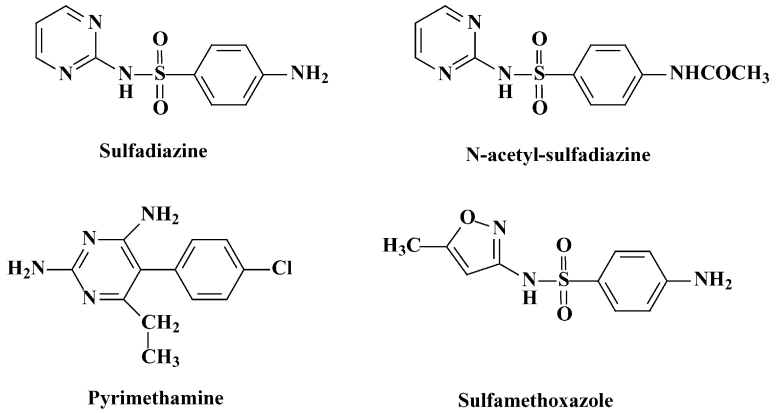

Fig. 1. Chemical structures of the analytes and the internal standard.