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Technical note

Simple, rapid and sensitive method for the determination of indomethacin in plasma by high-performance liquid chromatography with ultraviolet detection

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Abstract

A micro method for determination of indomethacin in plasma was developed. Following deproteinization of plasma with acetonitrile containing internal standard (mefenamic acid), the separation of indomethacin and internal standard was achieved by high-performance liquid chromatography using a 7 μm LiChrosorb-RP18 column (250×4 mm I.D.) at 50°C. The mobile phase was 6 m*M* phosphoric acid–acetonitrile (50:50). The flow-rate was kept at 2.0 ml/min and the column effluent was monitored at 205 nm. The coefficients of variation of the method estimated at 0.2 and 1.0 μg/ml were 4.2 and 2.3%, and the detection limit of the drug was about 0.05 μg/ml ($S/N=5$). The method requires minimum pretreatment of the plasma with a small sample volume (25 μl), and is very suitable for therapeutic drug monitoring of indomethacin in premature infants with symptomatic patent ductus arteriosus.

Keywords: Indomethacin

1. Introduction

Indomethacin (IDM) has been widely used as a pharmacologic agent to induce closure of the patent ductus arteriosus (PDA) in premature infants [1,2]. The plasma concentrations of IDM have been closely related to its therapeutic effects [1,2]. The wide intersubject variability in plasma concentration and half-life of IDM was also observed [1]. Therefore, measurement of plasma concentrations of IDM may provide valuable information for IDM treatment in premature infants with symptomatic PDA. Normally, doses ranging from 0.1 to 0.3 mg/kg are intravenously administered for closure of the ductus. Because of the low doses and small sample sizes, a sensitive method is required to monitor IDM in the infants.

Previous HPLC methods [3–6] require relatively large plasma samples (more than 0.1 ml) and are less suitable for routine clinical analysis. Although a HPLC method recently developed by Kubo et al. [7] is sensitive and selective with a small sample size (20 μl), this method requires an additional reaction system for in-line alkaline hydrolysis. The method described in this paper is simple and sensitive, and is adapted for routine monitoring plasma IDM in infants using a small amount (25 μl) of plasma.

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2. Experimental

2.1. Materials and reagents

IDM was obtained from Sigma (St. Louis, MO, USA). Mefenamic acid (MA) was supplied by Sankyo (Tokyo, Japan). Acetonitrile (HPLC grade, Cica-Merck) was obtained from Kanto Chemical (Tokyo, Japan). Purified water was obtained using a Milli-Q reagent water system (Millipore). The other reagents were of analytical-reagent grade.

Stock solutions of both IDM and MA were prepared at concentrations of 2.0 and 0.2 μg/ml in acetonitrile, respectively. These solutions were stable at 4°C for at least six months.

2.2. Apparatus and chromatographic conditions

A Model LC-3A liquid chromatograph equipped with a loop-type injector (Model SIL-1A) and a variable-wavelength ultraviolet absorbance detector (Model SPD-2A) (all from Shimadzu, Kyoto, Japan) was used. A data-processor (Model C-R1A Chromatopac; Shimadzu) was used for peak-area integration and calculations. Analysis was performed on a 7 μm LiChrosorb RP-18 column (250 mm×4 mm I.D.; Cica-Merck) from Kanto Chemical, operated at 50°C. The mobile phase was 6 m*M* phosphoric acid–acetonitrile (50:50, v/v). The flow-rate was kept at 2.0 ml/min and the column effluent was monitored at 205 nm.

2.3. Sample preparation

A 25-μl volume of plasma was pipetted into a 1.5-ml tapered polypropylene tube, and then 125 μl of internal standard solution was added. After vortex mixing for 30 s, the sample was centrifuged at about 8000 *g* for 2 min. A 125-μl aliquot of the supernatant was transferred into a 10-ml glass-stoppered centrifuge tube and was evaporated to dryness under reduced pressure. The residue was dissolved in 60 μl of the mobile phase and a 30-μl aliquot was injected onto the HPLC system.

2.4. Quantitation

Quantitation was performed by the peak-area method with MA as internal standard. Calibration graphs were obtained by analysing control plasma samples spiked with various amounts of IDM solutions (0.1, 0.2, 0.5, 1.0 and 2.0 μg/ml). The within-run variation was determined by analysing by two plasma samples containing 0.2 and 1.0 μg/ml IDM.

3. Results and discussion

Most of the previous HPLC methods for determination of plasma IDM require relatively large amounts of plasma [3–6]. Although the method described by Kubo et al. [7] is sensitive and selective with a 20-μl volume of plasma, this method requires a reaction system equipped with a stainless-steel reaction coil in a heating bath (140°C) and a stainless-steel coil in a cooled box (15°C) for in-line alkaline hydrolysis and is less convenient for routine clinical analysis. Therefore, we developed a simple HPLC procedure for the determination of IDM in a small volume (25 μl) of plasma from which proteins were removed by acetonitrile precipitation. To improve the separation of IDM, MA and plasma constituents, the supernatant fraction after the precipitation of plasma proteins was evaporated to dryness, reconstituted with the mobile phase and then injected onto the HPLC system.

Typical chromatograms of plasma samples are shown in Fig. 1. The retention times for IDM and MA were 5.9 min and 9.4 min, respectively. Ten drug-free plasma samples were analysed for possible interferences from endogenous constituents. As shown in Fig. 1a, no background interference was observed. Since unknown substances contained in blank plasma were eluted at 12 and 15 min in the present conditions, the actual chromatographic time was about 20 min in multiple sample analyses. Several drugs, such as theophylline, caffeine, phenytoin, phenobarbital and furosemide, which are commonly used during IDM therapy in premature infants, did not interfere with the assay results.

The linearity of the method was studied for five different concentrations of IDM in spiked plasma samples. The regression lines were linear ($y=0.643x+0.00238$, $r=0.9999$) over the concentration range examined (0.1–2.0 μg/ml) and nearly passed through the origin.

The within-run precision was established for two concentrations of IDM in spiked plasma samples. As

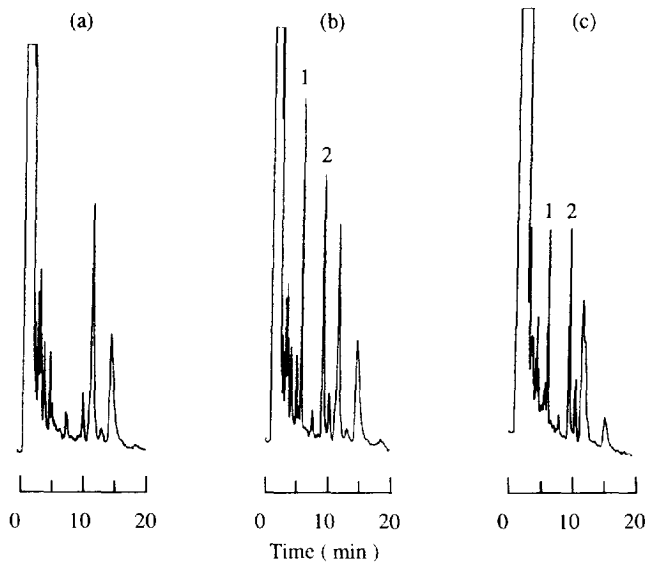

Fig. 1. Chromatograms of (a) blank plasma, (b) blank plasma spiked with indomethacin (1.0 μg/ml) and (c) plasma obtained from a premature infant undergoing indomethacin therapy. Peaks: 1=indomethacin; 2=mefenamic acid (internal standard).

shown in Table 1, the recoveries of the drug were nearly 100% and the coefficients of variation were less than 4.2%. The detection limits of the drug, estimated for a signal-to-noise ratio of 5, was about 0.05 μg/ml in plasma.

Fig. 2 shows a plasma concentration–time profile of IDM after intravenous infusion of the drug over 30 min to a premature infant with symptomatic PDA. In this patient, the second dose was administered at 24 h after the first dose and the ductus closure was achieved at 24 h after the second dose. The plasma concentration of IDM at 1, 5 and 120 h after the second dose was 0.87, 0.83 and 0.27 μg/ml, respectively. The plasma half-life of IDM estimated from log–linear elimination phase after the second dose was about 80 h, which is extremely longer than the mean values reported previously for premature infants [2].

Table 1
Within-run variability of the method

Added (μg/ml)	Found (mean±S.D.; $n=5$) (μg/ml)	Coefficient of variation (%)
0.20	0.20±0.0084	4.2
1.00	1.00±0.023	2.3

In conclusion, the proposed method, requiring only a small volume of plasma and with rapid sample preparation, is very suitable for therapeutic drug monitoring of IDM in premature infants with symptomatic PDA and also in patients undergoing conventional IDM therapy.

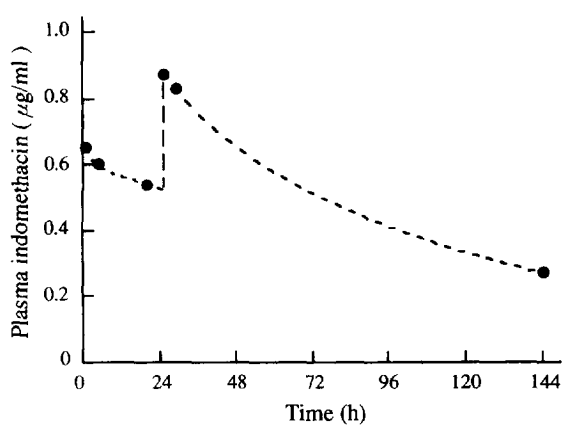

Fig. 2. Plasma concentration–time profile of indomethacin after intravenous administration of the drug to a premature infant with a symptomatic patent ductus arteriosus. The drug solutions (Indacin® IV, Banyu Pharmaceutical) were infused over 30 min at time 0 (dose; 0.2 mg/kg) and at 24 h (dose; 0.1 mg/kg) after the first dose.

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