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Short communication

Determination of dexamethasone in plasma of premature neonates using high-performance liquid chromatography

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Abstract

A high-performance liquid chromatographic assay is described for dexamethasone in neonatal plasma. Samples (100 μl) were extracted with ethyl acetate (1.0 ml) containing 50 ng of beclomethasone. The mobile phase of acetonitrile–0.01 *M* phosphate buffer (pH 7.0) (45:55, v/v) was pumped at a flow-rate of 1.0 ml/min through an Ultrasphere ODS column (5.0 μm particles). Detection was by UV absorbance at 240 nm. Calibration plots were linear ($r^2 > 0.999$) from 10–1000 ng/ml. Within-day and between-day precision (C.V.%, coefficient of variation) between 15–900 ng/ml was 1.21–20.6%. Inaccuracy was 7.7%, or less. The minimum quantifiable concentration (C.V. $\approx$ 20%) was 15 ng/ml. Recovery (20–1000 ng/ml) exceeded 75%. Concomitant neonatal medication did not interfere.

1. Introduction

Dexamethasone has been shown to benefit premature infants with bronchopulmonary dysplasia (BPD), a serious form of chronic lung disease in premature infants [1]. However, dexamethasone has a number of potentially serious adverse effects such as hypertension, adrenal suppression, pneumothorax, hyperglycaemia and increased risk of sepsis. Accordingly, knowledge of the pharmacokinetics of dexamethasone in preterm babies is desirable when attempting to develop adequate dosing strategies for the treatment of BPD.

Previously published high-performance liquid chromatographic (HPLC) assays for dexamethasone are unsuitable for application in pharmacokinetic studies in premature infants due to the large amount (2–6 ml) of blood required at each sampling time [2–6]. On the other hand, radioimmunoassay (RIA) techniques have been developed which offered good sensitivity from small plasma sample volumes [7,8]. However, a drawback is that the reagents for these methods are not available commercially which necessitates specialised expertise and equipment for the production of the dexamethasone immunogen and antisera together with the potential quality control problems inherent in this methodology. A selective and sensitive combined HPLC–RIA method is also available but it is complex and laborious requiring precipitation of serum proteins, chromatog-

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raphy, collection and solvent extraction of the chromatographic fractions followed by RIA [9]. A very recent alternative approach involved pre-column derivatisation of dexamethasone in 100-μl plasma to form fluorescent adducts with 2-(4-carboxyphenyl)-5,6-dimethyl benzimidazole, CBD [10]. While sensitive, the chemical derivatisation step, lack of commercial availability of the CBD reagent, relatively long chromatographic elution time (>30 min), and possible interference from other steroids (*e.g.* corticosterone) limit the usefulness of this technique.

We now describe a simple, robust HPLC method capable of quantitating dexamethasone to 15 ng/ml with UV absorbance detection following ethyl acetate extraction from 100 μl of plasma from extremely low birth weight (ELBW) neonates.

2. Experimental

2.1. Reagents and materials

Dexamethasone, beclomethasone, hydrocortisone and bovine serum albumin (BSA, Fraction V) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Ethyl acetate and acetonitrile (ChromAR HPLC) were purchased from Mallinckrodt Specialty Chemicals Co. (Paris, KY, USA). All other reagents were AR grade and were purchased from BDH Chemicals Australia (Kilsyth, Vic, Australia). Freshly glass-distilled water was used to make up all aqueous reagents.

2.2. Instrumentation

Analyses were performed on a modular system consisting of a Model 501 pump, a Model 712 WISP injector, a Model 484 variable wavelength detector (Millipore, Waters Chromatography Division, Milford, MA, USA), and a chart recorder (Omniscribe, Houston Instruments, Austin, TX, USA).

2.3. Sample preparation

Plasma (100 μl) was pipetted into 1.5-ml polypropylene tubes (Bio Rad Laboratories, Sydney, Australia) and extracted by vortex-mixing with 1.0 ml of ethyl acetate containing 50 ng of beclomethasone. The tubes were centrifuged at 11 000–12 300 *g* for 5 min, and the clear supernatants transfered into glass tubes and evaporated under nitrogen at 50–60°C. The residue was dissolved in 100 μl of mobile phase of which 20–80 μl was injected onto the chromatograph.

2.4. Chromatography and quantitation

A mobile phase of acetonitrile–0.01 *M* phosphate buffer (pH 7.0) (45:55, v/v) was pumped at a flow-rate of 1.0 ml/min (20–24°C) through an Ultrasphere (Beckman Instruments, San Ramon, CA, USA) ODS column (250 × 4.6 mm I.D.) containing 5-μm particles. The mobile phase was filtered (0.45-μm membrane pore size) and degassed under reduced pressure before use. Detection was at 240 nm, the experimentally determined spectral maximum for dexamethasone in the mobile phase, and the sensitivity was set at 0.005 AUFS.

Calibrations were obtained by weighted regression [11] of the peak-height ratio of dexamethasone to beclomethasone, on dexamethasone concentration in the standards. Dexamethasone content was determined by inverse prediction from the calibration equation. Standards (10, 20, 50, 100, 250, 500, 1000 ng/ml in 3% w/v aqueous BSA), controls (15, 300, 900 ng/ml in 3% w/v aqueous BSA), and unknowns were processed identically.

3. Results and discussion

Beclomethasone acted as a suitable internal standard in the analysis as it is not used in neonatal therapeutics and has the same structure as dexamethasone except for a 9α-chloro substituent in place of the 9α-fluoro group on dexamethasone. Thus, both compounds have similar solubilities, stabilities, UV spectra and chromatographic properties. Dexamethasone and beclomethasone were eluted at 18 and 22 min, respectively, and both peaks were free from interference by endogenous peaks as shown in

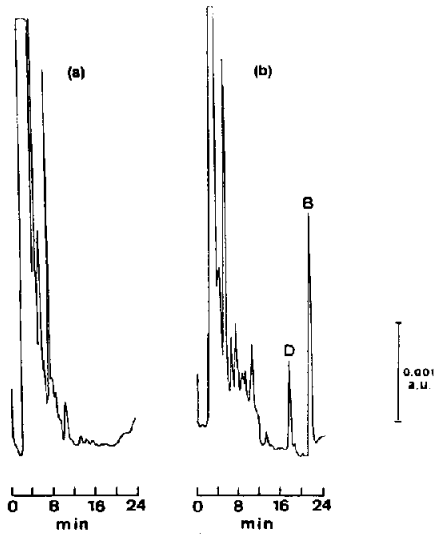

Fig. 1. Chromatograms of (a) drug-free neonatal plasma, and (b) plasma containing 168.5 ng/ml from blood drawn 2 h after an intravenous bolus of 0.385 mg/kg dexamethasone to an ELBW neonate (weight, 790 g); D = dexamethasone, B = beclomethasone (internal standard).

the chromatograms of blank and dexamethasone-containing neonatal plasma (Fig. 1). The following drugs and their metabolites did not interfere as assessed by the lack of interference in plasma samples drawn before commencement of dexamethasone treatment from infants who were prescribed these medications: amoxycillin, ceftriaxone, erythromycin, furosemide, gentamicin, indomethacin, midazolam, morphine, nystatin, salbutamol, theophylline, vancomycin.

Hydrocortisone was eluted after 9 min but was not quantitated since premature infants have extremely low concentrations of this steroid. Presently, concentrations of hydrocortisone were barely detectable in the plasma of most preterm babies even before administration of dexamethasone.

The mean regression equation for 5 replicate calibrations on different days: $y = 2.067x - 0.00052$, showed excellent linearity ($r^2 > 0.999$) and analysis of variance revealed insignificant ($p > 0.4$) nonlinearity in the error sums of squares. Precision and inaccuracy data (20–900 ng/ml) are shown in Table 1. Inaccuracy was less than 8% while the minimum quantifiable concentration was set to 15 ng/ml (between-day C.V. $\approx 20\%$).

Plasma from term and preterm neonates contains 2–5% (w/v) albumin [12] and, in view of meagre supplies of drug-free neonatal plasma, 3% BSA solution was used as the matrix for dexamethasone standards and seeded controls. Peak-height ratios of dexamethasone/beclomethasone were similar in 3% BSA and neonatal plasma containing varying albumin concentrations. Recoveries from the extraction of dexamethasone (20–1000 ng/ml) in 3% BSA were greater than 75%.

The clinical application of this method was established by measurement of dexamethasone concentrations in plasma samples drawn periodically over 24 h from 7 extremely low birth weight infants (birth weight: 459–949 grams; postconceptional age: 26–29 weeks) on day 1 of a Cummings *et al.* regime [1] for the treatment of BPD. These data are presented in Fig. 2 and show that the assay can be readily used to describe dexamethasone kinetics following in-

Table 1
Precision and inaccuracy of HPLC dexamethasone analysis

Dexamethasone concentration (ng/ml)	Precision (C.V.) (%)		Inaccuracy[a] (%)	
	Within-day ($n = 8$)	Between-day ($n = 3$)	Day 1	Day 2
15	11.3	20.6	2.2	4.3
300	1.21	7.7	7.7	7.7
900	1.48	10.6	2.3	7.6

[a]Defined as: [(measured conc. − target conc.)/target conc.] · 100%.

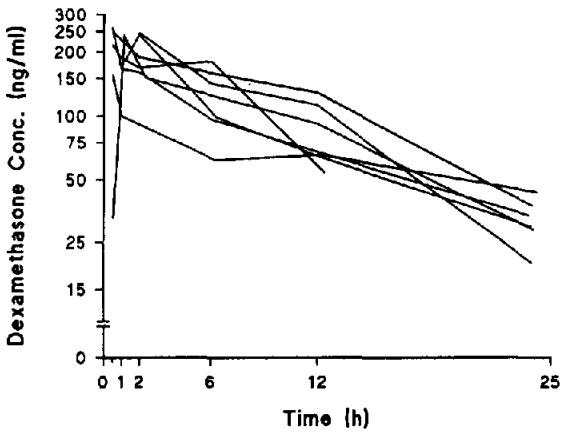

Fig. 2. Plasma dexamethasone concentrations over time measured after administration of dexamethasone (0.276–0.4 mg/kg) to 7 ELBW neonates.

travenous bolus doses in the range 0.276–0.40 mg/kg.

In conclusion, a simple, sensitive, precise, and robust HPLC method has been described which is suitable for the analysis of dexamethasone in neonatal samples and which has potential for other clinical applications, including bioavailability studies.

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