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Note

Simultaneous determination of doxapram and 2-ketodoxapram in plasma of neonates by gas chromatography

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Idiopathic apnea of prematurity occurs in 25% of newborns less than 2.5 kg [1], the resultant hypoxia posing a threat to central nervous system integrity. Neonates resistant to tactile stimuli and conventional pharmacological treatment (theophylline, caffeine) require assisted ventilation — an aggressive and potentially problematic procedure. Doxapram (Fig. 1), a central nervous system stimulant, has demonstrated therapeutic value in treating neonatal apnea resistant to theophylline [2, 3] and may obviate the need for assisted ventilation.

The monitoring of plasma doxapram levels may be useful in the clinical management of neonates receiving this drug. The pharmacological significance of the metabolite 2-ketodoxapram (Fig. 1) has not been determined.

Analytical methods described for doxapram either require volumes of plasma unrealistic for routine neonatal collection [4, 5] or utilize sophisticated in-

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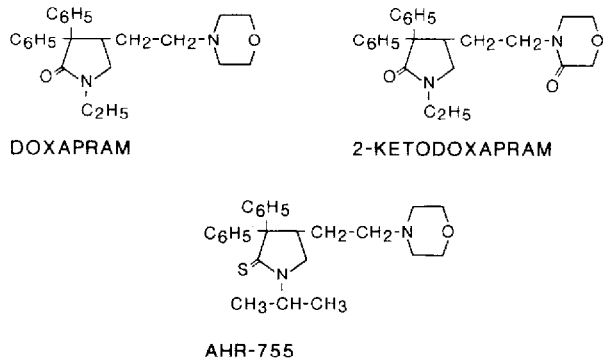

Fig. 1. Structural formulas of doxapram, 2-ketodoxapram and AHR-755 (internal standard).

strumentation (gas chromatography—mass spectrometry) [6]. The purpose of our work was to develop a simple, rapid method for simultaneous quantitation of doxapram and 2-ketodoxapram in neonatal plasma.

EXPERIMENTAL

To neonate plasma (0.2 ml) in a 4-ml Reacti-Vial® (Pierce, Rockford, IL, U.S.A.) was added internal standard (the doxapram analogue, AHR-755, Fig. 1) at a final concentration of 1.0 mg/l. Standards prepared in drug-free calf serum (Gibco Labs., Chagrin Falls, OH, U.S.A.) (0.1—10.0 mg/l doxapram and 2-ketodoxapram) were run simultaneously with each set of unknown samples. Saturated sodium tetraborate buffer, pH 9.0 (0.1 ml) was added and each sample was extracted with glass-distilled 1-chlorobutane (Caledon Labs., Georgetown, Canada) (0.5 ml) by vortexing for 30 s. After centrifugation, the upper organic layer was transferred to a Reacti-Vial (0.5 ml) and evaporated to dryness in a nitrogen stream. The residue was dissolved in ethyl acetate (40 μl), vortexed, and 1—3 μl aliquots were injected into the gas chromatograph (Hewlett-Packard Model 5710A with a nitrogen- phosphorus detector). The glass column (1.2 m × 2 mm I.D.) was packed with GP 3% SP-2250 on 80—100 mesh Supelcoport (Supelco Canada, Oakville, Canada). The carrier gas (helium), hydrogen and air flow-rates were 65, 3 and 50 ml/min, respectively. Injection port, column oven and detector temperatures were 300, 285 and 300° C, respectively.

RESULTS AND DISCUSSION

Under the chromatographic conditions described, doxapram, the internal standard (AHR-755) and 2-ketodoxapram chromatographed as shown in Fig. 2. Theophylline and caffeine, two drugs commonly co-prescribed with doxapram, did not interfere. Furthermore, no endogenous interferents were found (Fig. 2). Calibration graphs obtained by plotting peak-height ratios (doxapram/AHR-755; 2-ketodoxapram/AHR-755) versus concentration were not only linear for doxapram (r = 0.9929—0.9999) and 2-ketodoxapram (r = 0.9954—1.0000) over a concentration range of 0.1—10.0 mg/l, but also passed through the origin.

Recoveries of doxapram and 2-ketodoxapram (peak-height ratio comparisons

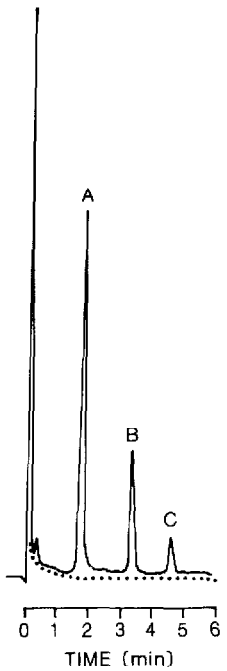

Fig. 2. Gas chromatograms of extracts of neonatal plasma before (· · ·) and following (——) an intravenous infusion of doxapram, 2.5 mg/kg/h. Peaks: A = doxapram (2.1 mg/l); B = AHR-755 (internal standard); C = 2-ketodoxapram (0.5 mg/l).

of extracted to directly chromatographed solutions) from human plasma averaged 96.8 and 94.5%, respectively, at the investigated concentrations (1.0 and 10.0 mg/l). Recoveries of doxapram and 2-ketodoxapram from drug-free calf serum were not statistically different (Student t test, $p > 0.5$) from human plasma recoveries. This permitted the construction of calibration curves using an inexpensive, easily accessible, drug-free biological pool.

The limit of determination for both compounds was 0.05 mg/l upon extraction of 0.2 ml of plasma (0.75 ng on-column). Repeatability (within-run precision) was determined by replicate analyses (n = 10) of a prepared doxapram (5.0 mg/l) and 2-ketodoxapram (1.0 mg/l) calf serum pool. The mean coefficients of variation for doxapram and 2-ketodoxapram were 3.8 and 8.3%, respectively. For assessment of reproducibility (between-day precision), multiple samples (n = 6) of the same serum pool were analyzed on different days. Coefficients of variation for doxapram and 2-ketodoxapram were 3.4 and 7.4%, respectively. The sample means for sera analyzed for reproducibility were 5.1 mg/l for doxapram and 1.1 mg/l for 2-ketodoxapram.

This assay is simple, accurate, precise, specific, sensitive and rapid (eighteen plasma samples analyzed in 4 h). Chromatograms of extracts of blank neonate plasma and plasma obtained from a neonate receiving an infusion of doxapram (2.5 mg/kg/h) are shown in Fig. 2. This method is currently being utilized in an investigation of the clinical efficacy of doxapram in neonatal apnea.

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REFERENCES

1 W.J.R. Daily, M. Klaus and H.B.P. Meyer, Pediatrics, 43 (1969) 510.
2 E. Sagi, F. Eyal, G. Alpan, D. Patz and I. Arad, Arch. Dis. Child., 59 (1984) 281.
3 G. Alpan, F. Eyal, E. Sagi, C. Springer, D. Patz and K. Goder, J. Pediatr., 104 (1984) 634.
4 R.B. Bruce, J.E. Pitts, F. Pinchbeck and J. Newman, J. Med. Chem., 8 (1965) 157.
5 R.H. Robson and L.F. Prescott, J. Chromatogr., 143 (1977) 527.
6 H. Nichol, J. Vine, J. Thomas and R.G. Moore, J. Chromatogr., 182 (1980) 191.