A STUDY OF CONJUGATION AND DRUG ELIMINATION IN THE HUMAN NEONATE

A.J. CUMMINGS & A.G.L. WHITELOW

Division of Perinatal Medicine, Clinical Research Centre and Northwick Park Hospital, Harrow, Middlesex

- 1 An investigation has been made of the excretion of α -methyldopa, α -methyldopa sulphate, lorazepam and lorazepam glucuronide in the urine of neonates.
- 2 The rate of elimination of both of the drugs in the newborn is slow compared with the adult rate, and apparent half-life being 3 to 4 times longer than the reported adult values.
- 3 The newborn appear able to readily metabolise α -methyldopa to α -methyldopa sulphate and to slowly conjugate lorazepam with glucuronic acid. α -Methyldopa tends to be conjugated to a greater extent and lorazepam to about the same or slightly lesser extent in the newborn than in adults.
- 4 It is postulated that elimination in the neonate is mainly controlled by the rate of renal excretion in the case of α -methyldopa and by the rate of conjugation in the case of lorazepam.

Introduction

The elimination of many drugs in the newborn is slow compared with elimination in adults and older children. This slow rate of elimination of drugs has been ascribed to the immaturity of physiological and anatomical factors including the relative inefficiency of renal function and the low capacity of drug metabolising enzyme systems in the newborn (Morselli, 1976). Evidence has been presented that renal functional development is closely related to the gestational age plus the postnatal age of the infant and that tubular function appears to be less mature than glomerular function at birth (Arant, 1978). Reports differ with regard to the maturity of drug conjugating systems, but glucuronidation enzyme systems are usually considered to be less mature at birth than the sulphating enzyme systems. Miller, Roberts & Fischer (1976) reported that paracetamol (acetaminophen), directly administered to neonates, is eliminated at rates similar to those in adults, but that the conjugation pattern is very different, with a larger proportion excreted as sulphate and only a small proportion as glucuronide. Rane (1974) reported that transplacentally acquired phenytoin was eliminated by neonates by hydroxylation followed by conjugation with glucuronic acid, to an extent only slightly less than in adults and at a similar rate. Conjugation in the newborn has been the subject of a review by Dutton (1978).

Studies with two drugs predominantly eliminated by urinary excretion and conjugation are now reported. The excretion of unchanged drug and of conjugated drug has been determined in the urine of newborn babies born to women who were receiving

treatment with α -methyldopa or lorazepam during the final stages of their pregnancy and during labour.

In adults, α -methyldopa is predominantly eliminated by excretion in urine and by conjugation with sulphate (Buhs *et al.*, 1964); after intravenous dosage the extent of conjugation is greatly reduced. The elimination half-life approximates 2–3 h (Kwan *et al.*, 1976). The babies born to women receiving the drug have plasma levels of α -methyldopa similar to the maternal levels, but which decline slowly over 2–4 days after birth; elimination is by excretion and conjugation in the newborn (Jones *et al.*, 1979).

Lorazepam is almost entirely eliminated by conjugation with glucuronic acid in adults, the metabolite being excreted in urine with a half-life of about 12–15 h (Greenblatt *et al.*, 1979). No studies in the newborn appear to have been reported. However, Tomson *et al.* (1979) have reported that after a single oral dose of the similar drug, oxazepam, to mothers before delivery, both oxazepam and oxazepam glucuronide were excreted in the urine of their newborn babies, but at a slower rate than in the mothers.

Methods

The babies from whom the urine samples used in these studies were obtained, received the drugs transplacentally; details of the babies are given in Table 1. They were born to women receiving treatment for hypertension with oral α -methyldopa, 0.75–2.0 g/day for several weeks, or with lorazepam oral or intravenous 3–8 mg/day for some days before delivery.

Table 1 Details of babies

Infant	Sex	Gestational age (weeks)	Birth weight (kg)	Apgar score	
				1 min	5 min
AS	M	32	1.42	4	7*
HN	M	38	3.54	8	10
WL	M	27	0.94	8	9†
GE	F	34	1.82	4	7
HI	M	38	2.02	2	7
HU	F	33	1.62	3	5
MC	M	39	3.48	9	9
PA	F	32	0.65	2	3

*At 10 min; †Neonatal death

Collections of urine from the babies was with urine bags (Abbot Laboratories, Queensborough, UK). Although some consecutive collections were made a single collection of urine over 2–5 h per day was usual. A sample of umbilical cord blood and of maternal blood at delivery (10 ml into lithium heparin) was also collected and the plasma separated. The plasma and urine samples were stored at -20°C until analysed.

The studies were conducted with the approval and under the rules of the Northwick Park Hospital and Clinical Research Centre Ethical Committee.

Determination of α -methyldopa

The unconjugated and total (unconjugated plus conjugated) α -methyldopa concentration in urine and plasma was determined by the gas chromatography mass spectrometric method of Setchell, Lawson & Cummings (1978).

Determination of lorazepam

The unconjugated and total lorazepam concentrations in urine and plasma were determined by an electron-capture, gas-liquid chromatographic method similar to that of Greenblatt, Franke & Schader (1978), using flunitrazepam as internal standard (Howard *et al.*, 1977).

Flunitrazepam was found preferable to oxazepam (Greenblatt *et al.*, 1978) as an internal standard, being less subject to interference, particularly from materials in baby urine and plasma.

Determination of creatinine in urine

The creatinine concentrations was determined by an alkaline picrate method.

Results

The concentrations of α -methyldopa, α -methyldopa-

sulphate, lorazepam and lorazepam glucuronide in the umbilical cord plasma and corresponding maternal plasma at delivery, are given in Table 2. Similar free and conjugated α -methyldopa concentrations were obtained in umbilical cord and maternal plasma, but the lorazepam and particularly the lorazepam glucuronide, tend to be lower in umbilical cord plasma than in maternal plasma.

For comparison, the average ($\pm$ s.d.) concentrations in twelve pairs of umbilical cord and maternal plasma for each drug were respectively 880 ($\pm$ 670) and 690 ($\pm$ 570) ng/ml α -methyldopa, 1030 ($\pm$ 611) and 1240 ($\pm$ 910) ng/ml α -methyldopa sulphate, 32 ($\pm$ 12) and 42 ($\pm$ 22) ng/ml lorazepam and 26 ($\pm$ 12) and 52 ($\pm$ 15) ng/ml lorazepam glucuronide.

The results indicate that in both cases the babies are born with an appreciable body load of the drug, of which a substantial proportion may be conjugated.

Examples of the pattern of excretion of α -methyldopa and α -methyldopa sulphate in the urine of three babies are given in Figure 1 and of lorazepam and lorazepam glucuronide in the urine of five babies in Figures 2 and 3 respectively. The amount of drug excreted per mmol creatinine excreted, is plotted against time after birth. The excretion of α -methyldopa and its conjugate was followed for about 50 h and the excretion of lorazepam glucuronide for up to about 12 days after birth.

The rate of excretion of α -methyldopa, lorazepam and their conjugates are considered to decline logarithmically with time, at least over the terminal part of the plots and the half-life has been calculated from the slope of the best straight line that can be obtained from these data; the values obtained are given in Table 3. Within the limits of the data from which they have been calculated, the values of the half-life of lorazepam and of lorazepam glucuronide appear to be similar for an individual infant. The half-life of α -methyldopa sulphate, however, tends to be slightly longer than that of α -methyldopa and this would be consistent with the previous report that the ratio of the concentration of conjugate to free α -methyldopa in the urine of neonates tend to increase with time after birth (Jones *et al.*, 1979). These results show α -methyldopa to be eliminated considerably more rapidly than lorazepam in the newborn.

Discussion

The excretion in neonatal urine of α -methyldopa, lorazepam and their conjugates has been related to creatinine excretion. This method of expressing the urinary excretion results is open to criticism in that the creatinine excretion can be variable in the post-natal period (Sertel & Scopes, 1973; Talafant, Hoskova & Pojerova, 1977), however, it is considered better than the only alternative, expressing the results

Table 2 The concentrations of unconjugated and conjugated α -methyldopa and lorazepam in umbilical cord plasma and in maternal plasma at delivery.

Infant	Umbilical cord		Maternal	
	Unconjugated	Conjugated	Unconjugated	Conjugated
		(ng/ml)		
		α -Methyldopa		
AS	870	500	660	750
HN	300	260	250	330
WL	270	380	370	530
		Lorazepam		
GE	17	3	15	43
HI	48	11	41	53
HU	31	9	75	25
MC	25	30	17	44
PA	46	26	55	82

as concentrations. The collection of urine from newborn babies over timed intervals is only feasible for very limited periods and there are problems associated with incomplete emptying of the bladder (Osborne *et al.*, 1977) and with the collection of small volumes of urine without significant loss.

The results of these studies indicate that babies exposed to α -methyldopa or lorazepam *in utero*, eliminate these drugs by renal excretion and metabolism to conjugates, apparently in a similar manner to adults but at rates which are 3 to 4 times slower.

The presence of α -methyldopa sulphate and lorazepam glucuronide in the umbilical cord plasma (Table 2) may indicate the formation of these metabolites in the foetus, but the possibility of some contribution by transfer from the mother cannot be ex-

Figure 1 The excretion of α -methyldopa and α -methyldopa sulphate in the urine of three newborn infants.

(α -methyldopa—open symbols; α -methyldopa sulphate—solid symbols).

Figure 2 The excretion of lorazepam in the urine of five newborn infants.

Figure 3 The excretion of lorazepam glucuronide in the urine of five newborn infants.

(The results from an infant have the same symbol in both Figures 2 and 3).

cluded. More definite evidence for the metabolism of these drugs in the neonate is provided by the much higher ratios of conjugated to unconjugated drug concentration in the urine than initially in the umbilical cord plasma. The overall ratios in urine were approximately, α -methyldopa sulphate to α -methyldopa, 5:1 and lorazepam glucuronide to lorazepam 8:1, these are respectively higher and rather lower than are usually found in adults after treatment with these drugs.

In adults, the formation of α -methyldopa sulphate appears to occur mainly during absorption of α -methyldopa from the gastro-intestinal tract, after absorption and following intravenous dosage, elimination is reported to be mainly by renal excretion (Kwan *et al.*, 1976). However, a greater proportion of the α -methyldopa has been reported to be conjugated in adults and in children with some renal insufficiency and accumulation of the metabolite was observed (Myhre *et al.*, 1972; O'Dea & Mirkin, 1980). The elimination of lorazepam is predominantly by

metabolism to a glucuronide (Greenblatt *et al.*, 1979), with the rate of metabolism apparently rate limiting.

Thus the results of these studies might be interpreted as indicating that α -methyldopa elimination in the newborn is governed mainly by the rate of renal excretion, whereas the elimination of lorazepam in the newborn is governed mainly by the rate of its metabolism to a glucuronide, these being respectively the slowest processes involved.

It is of some interest that the very premature baby, WL, seemed able to metabolise α -methyldopa as well as the term baby, NH, and the very small and premature infant, PA, appeared able to conjugate lorazepam.

The elimination of lorazepam in the neonate appears to follow a similar pattern, to the elimination of oxazepam, as reported by Tomson *et al.* (1979) and these results provide additional evidence of the ability of the newborn to effect the conjugation of drugs with glucuronic acid.

The data which are now available allow some comment to be made on the conjugation of drugs in the newborn. In the newborn as in the adult, the rate of elimination of a drug is a composite of the rate of renal excretion and of the metabolic processes operating upon it, the relative rates of these processes control the extent of metabolism. In the neonate, sulphate formation is well developed compared with renal function and glucuronide formation and competes more effectively with these latter processes than is usual in the adult. Thus α -methyldopa and paracetamol are conjugated with sulphate to a greater extent than in adults. Similarly, a greater proportion of the α -methyldopa metabolite, α -methyldopamine, is excreted in urine as the sulphate by neonates than by adults (Lawson *et al.*, 1980). The capacity for glucuronidation is limited in the newborn, nevertheless those drugs which are slowly metabolised to products excreted as glucuronides, may be conjugated to much the same extent as in adults, for example lorazepam, oxazepam and phenytoin. In the case of lorazepam where the rate of elimination appears to be controlled by the rate of glucuronidation, evidence for saturation kinetics might be expected to be discernable in the excretion pattern in the immediate postnatal period, but no definite indication can be seen in the present results (Figure 3).

Table 3 The half-life of the decline with time after birth of the drug: creatinine ratios in baby urine.

Drug	AS	HN	WL	Infant				
				GE	HI	HU	MC	PA
				Half-life (h)				
α -Methyldopa	9	8	10	—	—	—	—	—
α -Methyldopa sulphate	19	9	12	—	—	—	—	—
Lorazepam	—	—	—	50	65	28	46	43
Lorazepam glucuronide	—	—	—	53	73	53	43	34

However, the excretion pattern could be affected by changes in other physiological parameters occurring around this time.

We gratefully acknowledge the help of Sister Cathy Lawrence and the co-operation of the nursing staff on the Delivery Suite and Special Care Baby Unit of Northwick Park Hospital.

References

- ARANT, B.S. (1978). Developmental patterns of renal function maturation compared in the human neonate. *J. Pediatr.*, **92**, 705–712.
- BUHS, R.P., BECK, J.L., SPETH, O.C., SMITH, J.L., TRENNER, N.R., CANNON, P.J. & LARAGH, J.H. (1964). The metabolism of methyldopa in hypertensive human subjects. *J. Pharmac. exp. Ther.*, **143**, 205–214.
- DUTTON, G.J. (1978). Developmental aspects of drug conjugation with special reference to glucuronidation. *Ann. Rev. Pharmac. Tox.*, **18**, 17–35.
- GREENBLATT, D.J., FRANKE, K. & SHADER, R.I. (1978). Analysis of lorazepam and its glucuronide metabolite by electron-capture gas-liquid chromatography. *J. Chromatog.*, **146**, 311–320.
- GREENBLATT, D.J., SHADER, R.I., FRANKE, K., MACLAUGHLIN, D.S., HARMATZ, J.S., ALLEN, M.D., WERNER, A. & WOO, E. (1979). Pharmacokinetics and bioavailability of intravenous, intramuscular and oral lorazepam in humans. *J. pharm. Sci.*, **68**, 57–63.
- HOWARD, P.J., LILBURN, J.K., DUNDEE, J.W., TONER, W. & McILROY, P.D.A. (1977). Estimation of plasma lorazepam by gas-liquid chromatography and a benzene extraction. *Anaesthesia*, **32**, 767–770.
- JONES, H.M.R., CUMMINGS, A.J., SETCHELL, K.D.R. & LAWSON, A.M. (1979). A study of the disposition of α -methyldopa in newborn infants following its administration to the mother for the treatment of hypertension during pregnancy. *Br. J. clin. Pharmac.*, **8**, 433–440.
- KWAN, K.C., FOLTZ, E.L., BREAUULT, G.O., BAER, J.E. & TOTARO, J.A. (1976). Pharmacokinetics of α -methyldopa in man. *J. Pharmac. exp. Ther.*, **198**, 264–277.
- LAWSON, A.M., MESWANI, P., SETCHELL, K.D.R. & CUMMINGS, A.J. (1980). The measurements of the anti-hypertensive drug α -methyldopa and some of its metabolites using GCMS and selected ion monitoring. In *Advances in Mass Spectrometry*. Vol. 8, ed. Quayle, A., pp. 1211–1218. London: Hayden & Sons.
- MILLER, R.P., ROBERTS, P.J. & FISCHER, L.J. (1976). Acetaminophen elimination kinetics in neonates, children and adults. *Clin. Pharmac. Ther.*, **19**, 284–294.
- MORSELLI, P.L. (1976). Clinical pharmacokinetics in neonates. *Clin. Pharmacokin.*, **1**, 81–98.
- MYHRE, E., STENBAEK, O., BRODWALL, E.K. & HANSEN, T. (1972). Conjugation of methyldopa in renal failure. *Scand. J. clin. Lab. Invest.*, **29**, 195–199.
- O'DEA, R.G. & MIRKIN, B.L. (1980). Metabolic disposition of methyldopa in hypertensive and renal insufficient children. *Clin. Pharmac. Ther.*, **27**, 37–43.
- OSBORNE, J., DuMONT, G., BEECROFT, M. & AYRES, A.B. (1977). Bladder emptying in neonates. *Arch. Dis. Child.*, **52**, 896–898.
- RANE, A. (1974). Urinary excretion in diphenylhydantoin metabolites in newborns. *J. Pediatr.*, **85**, 543–545.
- SERTEL, H. & SCOPES, J. (1973). Rates of creatinine clearance in babies less than one week of age. *Arch. Dis. Child.*, **48**, 717–720.
- SETCHELL, K.D.R., LAWSON, A.M. & CUMMINGS, A.J. (1978). The measurement of α -methyldopa in urine and plasma as its n-butylester-pentafluoropropionate derivative using stable isotope dilution gas chromatography-mass spectrometry. In *Quantitative Mass Spectrometry in Life Science II*, ed. DeLeenkeer, A., pp. 263–273. Amsterdam: Elsevier Scientific Publishing Company.
- TALAFANT, E., HOSKOVA, A. & POJEROVA, A. (1977). Negative feedback control of neonatal serum creatine and increased urinary creatinine excretion after enzyme-inducing drugs. *Pediat. Res.*, **11**, 880–883.
- TOMSON, G., LUNELL, N.-O., SUNDWALL, A. & RANE, A. (1979). Placental passage of oxazepam and its metabolite in mother and newborn. *Clin. Pharmac. Ther.*, **25**, 74–81.

(Received December 9, 1980)