Br. J. clin. Pharmac. (1993), **35**, 314–317

Morphine glucuronidation in premature neonates

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The glucuronidation of morphine was investigated in 10 premature neonates (postnatal age < 24 h at initiation of treatment) following 24 h of therapy (2 h loading infusion, followed by a constant rate infusion). Morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) were measured in plasma obtained at 24 h in all babies. Plasma concentrations of M3G and M6G correlated significantly with morphine concentration ($P < 0.01$ in both cases), and with each other ($P < 0.001$), suggesting that the capacity for morphine glucuronidation in premature neonates is not saturated at the infusion rates used in this study. M3G/morphine and M6G/morphine plasma concentration ratios were independent of morphine infusion rates ($P > 0.05$) and morphine plasma concentrations ($P > 0.05$), providing further evidence of linear kinetics. However, M3G/morphine and M6G/morphine plasma concentration ratios increased significantly with increasing birth weight ($P < 0.05$ in both cases). This probably reflects increase in liver weight with increasing birth weight. Although morphine glucuronidation is deficient in premature neonates, significant concentrations of the respiratory stimulant M3G are achieved rapidly (20% of morphine plasma concentrations at 2 h). At this time, the respiratory depressant M6G could not be detected.

Keywords morphine glucuronidation neonates development

Introduction

Although the metabolism and pharmacokinetics of morphine have been studied extensively in adults (Boerner *et al.*, 1975; Osborne *et al.*, 1990) and, to a lesser degree, in children (Lynn & Slattery, 1987; Nahata *et al.*, 1985), data from premature neonates are limited (Barrett *et al.*, 1991; Bhat *et al.*, 1990; Choonara *et al.*, 1989; Mercurio *et al.*, 1989). However, the increasing use of opioid analgesics in neonatal intensive care has stimulated considerable interest in the disposition of morphine and its metabolites within this patient group. Studies in premature newborns have shown a trend towards an increase in plasma clearance (CL) with increasing gestational age (Barrett *et al.*, 1991; Bhat *et al.*, 1990) and a decrease in the half-life ($t_{1/2}$) of morphine with increasing gestational age (Barrett *et al.*, 1991; Bhat *et al.*, 1990) or post conceptional age (Mercurio *et al.*, 1989).

Morphine is metabolised predominantly by the liver to glucuronides which are subsequently excreted by the kidney (Boerner *et al.*, 1975). Whilst the major metabolite, morphine-3-glucuronide (M3G), appears to be an opiate antagonist (Smith *et al.*, 1990; Watt *et al.*, 1990), morphine-6-glucuronide (M6G) has potent analgesic properties in animals (Shimomura *et al.*, 1971) and man (Osborne *et al.*, 1988, 1990). Furthermore, in animal studies M3G has been shown to stimulate ventilation (Gong *et al.*, 1991; Pelligrino *et al.*, 1989) whereas in man M6G appears to be a respiratory depressant (Hasselstrom *et al.*, 1989; Osborne *et al.*, 1988). Glucuronidation of morphine has been demonstrated in neonates, but the metabolic capacity (based on M3G/morphine plasma concentration ratios) was reduced in comparison with that in children aged 1–16 years (Choonara *et al.*, 1989). Furthermore, although M6G plasma concentrations exceeded those of morphine in adults (Sawe *et al.*, 1983; 1985) and children (Choonara *et al.*, 1989), the latter study showed this was not the case in neonates since M6G plasma concentrations were below the detection limit of the assay (< 40 ng ml^{-1}).

Since M6G may contribute significantly to the efficacy of morphine (Osborne *et al.*, 1988, 1990), the lower capacity for conversion of morphine to M6G in premature neonates may compromise the efficacy of the drug within this patient group. In our experience, approximately 30% of babies with respiratory distress syndrome receiving morphine to reduce stress and help synchronise breathing during mechanical ventilation continued to 'fight the ventilator' despite relatively high

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doses (50–100 μg kg^{-1} h^{-1} for 4 h). This study investigates the patency of the glucuronidation pathway from measurements of plasma concentrations of the drug and its metabolites in premature neonates. We report plasma concentrations of M6G in preterm infants for the first time and describe the influence of birth weight on the relative concentrations of morphine and its glucuronide metabolites in neonatal plasma.

Methods

Protocol

Informed consent was obtained from the parents and the study was approved by the Leeds West Hospital ethics committee. Blood was obtained from 10 neonates through an umbilical arterial catheter after 24 h of morphine therapy (2 h loading infusion followed by constant rate infusion). Three dose regimes were used, the low and intermediate regimens comprised of a 100 μg kg^{-1} h^{-1} loading infusion for 2 h followed by constant rate infusions of 12.5 and 25 μg kg^{-1} h^{-1}, respectively; the high dose regimen was a 200 μg kg^{-1} h^{-1} loading infusion for 2 h and a constant rate infusion of 50 μg kg^{-1}. Using the low dose regime, steady state was achieved after 2 h. Simulated plasma concentration-time profiles derived from population mean pharmacokinetic data (CL 2.4 ml min^{-1} kg^{-1} and half-life 8.8 h, unpublished) indicated that the intermediate and high dose regimes achieve approximately 90% of target steady state plasma drug concentrations within 24 h (about 90% of 174 and 347 ng ml^{-1}, respectively). All babies (postnatal age < 24 h at initiation of treatment) were receiving morphine for sedation during mechanical ventilation. Clinical details of individual patients and dosages are shown in Table 1.

Analytical methods

Morphine and its glucuronide metabolites were extracted from plasma using solid phase extraction columns (1 ml Bond Elut C8, 50 mg). These were conditioned with 2 × 1 ml of methanol, then 1 ml of water followed by 1 ml 0.01M NH_4HCO_3, pH 9.3 prior to loading the sample. Plasma (200 μl) and internal standard solution (200 μl, 1 μg ml^{-1} 10,11-dihydrocarbamazepine (DHCBZ) in 0.01M NH_4HCO_3, pH 9.3) were added to 200 μl of 0.01M NH_4HCO_3, pH 9.3 at the head of the column. The sample was then drawn slowly through the column under vacuum. The column was subsequently washed with 1 ml of 0.01M NH_4HCO_3, pH 9.3 and eluted with 250 μl of methanol. This was evaporated to dryness at 55° C and reconstituted in 400 μl of mobile phase for injection into the h.p.l.c. chromatograph (100–250 μl). Separation of morphine and its metabolites was achieved using a Waters Nova-Pak C18 column (300 × 3.9 mm i.d.) and a mobile phase consisting of 2 mM sodium dodecyl sulphate in 0.05% v/v H_3PO_4–acetonitrile (71.5:28.5 by volume) flowing at 1.2 ml min^{-1}. Retention times for morphine, M3G, M6G and DHCBZ were 14.7, 6.5, 8.3 and 10.5 min, respectively. Fluorescence detection (Merck-Hitachi Model F-1050) with excitation and emission wavelengths of 245 nm and 335 nm, respectively provided determination limits of < 10 ng ml^{-1} for all analytes. The within-batch precision of the method is indicated by coefficients of variation of < 9.5% at a plasma concentration of 10 ng ml^{-1} for morphine and M6G, and < 4.3% at a plasma concentration of 20 ng ml^{-1} for M3G. Plasma samples containing 50 ng ml^{-1} of morphine and M6G, and 100 ng ml^{-1} of M3G were stable when stored up to 6 weeks at −20° C (between-batch coefficients of variation < 7.9%).

Calculations

Statistical analysis was by the Mann-Whitney U test (*P* value) or least-squares linear regression analysis (Pearson's correlation coefficient, *r* and *P* value).

Results

Morphine, M3G and M6G achieved measurable concentrations in all plasma samples obtained at 24 h. Plasma concentrations in individual patients, and

Table 1 Clinical details of patients

Patient	*Sex*	*Birth weight (kg)*	*Gestation (weeks)*	*Dose regimen (μg kg^{-1} h^{-1})*	*Bilirubin (μmol l^{-1})*	*Creatinine (μmol l^{-1})*	*Urea (mmol l^{-1})*	*Additional drugs*
1	M	1.70	31	200[a]/50[b]	188	65	1.6	1, 2, 3
2	M	1.72	31	200/50	88	100	3.5	1, 2, 3
3	F	1.35	31	200/50	252	50	3.0	1, 2, 3
4	M	1.46	29	200/50	51	53	2.9	1, 2, 3
5	F	1.01	29	100/12.5	172	68	4.5	3, 4, 5
6	M	0.91*	31	100/12.5	124	107	4.6	1, 2, 3
7	F	1.26	28	100/12.5	76	98	7.8	1, 2, 3
8	F	1.34	31	100/12.5	10	50	—	1, 2, 3, 6
9	M	1.24	28	100/12.5	51	116	4.3	1, 2, 6, 7, 8
10	M	0.98*	31	100/25	119	161	7.7	1, 2, 3, 9, 10, 11

Postnatal age < 24 h on initiation of morphine treatment.
[a] 2 h loading infusion.
[b] Constant rate infusion.
* Birth weight low for dates.
Additional drugs: 1 Curosurf, 2 Ampicillin, 3 Gentamicin, 4 Pancuronium, 5 Vancomycin, 6 Vitamin K, 7 Cefotaxime, 8 Cloxacillin, 9 Metronidazole, 10 Caffeine, 11 Dopamine.

Table 2 Plasma concentrations and plasma concentration ratios after 24 h of morphine infusion

Patient	*Plasma concentration (24 h) ng ml^{-1}*			*Plasma concentration ratio*		
	Morphine	*M3G*	*M6G*	*M3G/Morphine*	*M6G/Morphine*	*M6G/M3G*
1	298	260	68	0.87	0.23	0.26
2	298	345	97	1.16	0.33	0.28
3	258	180	49	0.70	0.19	0.27
4	422	309	98	0.73	0.23	0.32
5	96	75	18	0.78	0.19	0.24
6	93	28	14	0.30	0.15	0.50
7	94	101	24	1.07	0.26	0.24
8	98	80	28	0.82	0.29	0.35
9	101	25	12	0.25	0.12	0.48
10	269	74	19	0.28	0.07	0.26

plasma concentration ratios are listed in Table 2. Mean plasma concentrations (s.d.) of morphine, M3G and M6G were 96 (3), 62 (34) and 19 (7) ng ml^{-1} and 319 (71), 274 (71) and 78 (24) ng ml^{-1} for the low and high dose regimes, respectively. Significant positive correlations between plasma concentrations of morphine and its main metabolites, M3G and M6G were observed (Pearson's correlation coefficient, $r = 0.839$ and 0.850, respectively; 8 df, $P < 0.01$ in both cases). Similarly, plasma concentrations of the two glucuronides correlated significantly (Pearson's correlation coefficient, $r = 0.987$; 8 df, $P < 0.001$).

Mean M3G/morphine, M6G/morphine and M6G/M3G plasma concentration ratios (s.d.) were 0.64 (0.36), 0.20 (0.07) and 0.36 (0.13), and 0.87 (0.21), 0.25 (0.06) and 0.28 (0.02), for the low and high dose regimes, respectively. Comparisons between mean M3G/morphine, M6G/morphine and M6G/M3G values in samples obtained from babies on the high and low dose regimes showed no significant difference (Mann-Whitney U test, $P > 0.05$). Since these ratios appeared to be independent of morphine infusion rate, data were pooled to examine the influence of morphine plasma concentration or birth weight on plasma concentration ratios using least-squares linear regression analysis. All plasma concentration ratios appeared to be independent of morphine plasma concentration (Pearson's correlation coefficient, $r = 0.191$, 0.151 and -0.369 for M3G/morphine, M6G/morphine and M6G/M3G, respectively; 8 df, $P > 0.05$). Significant positive correlations were found between birth weight and the M3G/morphine, and the M6G/morphine plasma concentration ratios (Pearson's correlation coefficient, $r = 0.673$ and 0.712, respectively; 8 df, $P < 0.05$ in both cases). The relationship between birth weight and the M6G/M3G plasma concentration ratio, however, gave a negative correlation but this was not significant (Pearson's correlation coefficient, $r = -0.306$; 8 df, $P > 0.05$).

Discussion

In contrast with earlier studies in preterm infants (Barrett *et al.*, 1991; Bhat *et al.*, 1990; Choonara *et al.*, 1989; Mercurio *et al.*, 1989), we were able to measure plasma concentrations of morphine as well as those of its two main metabolites (M3G and M6G). The mean plasma morphine concentration (96 ng ml^{-1}) for the low dose regime was in good agreement with the predicted steady state plasma drug concentration (87 ng ml^{-1}) calculated using the population mean CL (2.4 ml min^{-1} kg^{-1}). The mean plasma morphine concentration (319 ng ml^{-1}) for the high dose indicated that this regime achieved about 92% of the predicted steady state plasma drug concentration (347 ng ml^{-1}) after 24 h. Only one infant received the intermediate dose regime and the plasma concentration of morphine (269 ng ml^{-1}) was much higher than the predicted steady state concentration (174 ng ml^{-1}). This infant was 'small for dates' and a low hepatic glucuronidation (M3G/morphine plasma concentration ratio 0.28, Table 2) may have contributed to the discrepancy between predicted and measured values. Plasma concentrations of of M3G and M6G correlated significantly with plasma concentrations of morphine and were significantly related to each other, suggesting that the capacity for glucuronidation of morphine in premature neonates is not saturated at the infusion rates used in this study. This is also supported by the observations that the plasma concentration ratios (M3G/morphine and M6G/morphine) were independent of plasma morphine concentration and differences between mean values for the high and low dose regimes were not significant. Choonara *et al.*, (1989) reported a mean M3G/morphine plasma concentration ratio of 5.0 (4.6) in premature neonates a value 6–7 times greater than those noted in this study (0.64 (0.36) and 0.87 (0.21), for the low and high dose regimes, respectively). This could be due to differences in gestational age (24–37 weeks), post-natal age (2–12 days) or birth weight (not reported), but the most significant factor is likely to be the longer duration of morphine infusion (at least 48 h, with 1 exception). Thus, it appears that although plasma morphine concentrations approach steady state after 24 h, plasma M3G concentrations may continue to increase up to (and possibly beyond) 48 h.

Since the M3G/morphine and M6G/morphine plasma concentration ratios increased significantly with birth weight and since the gestational age range of the infants studied (28–31 weeks) was insufficient for developmental changes, the changes may reflect increases in liver weight with increasing birth weight. Previous studies in adults have shown that renal function influences excretion of the glucuronides (Hasselstrom *et al.*, 1989; Osborne *et al.*, 1988). However, assessment of renal function in preterm infants is difficult during the first 48 h of life because plasma creatinine concentrations change rapidly from values reflecting maternal renal clearance to those

which represent independent neonatal renal function. It is not possible, with the present data, to interpret how renal excretion influences M3G/morphine and M6G/morphine plasma concentration ratios in premature neonates, but any 'developmental' changes in renal function would tend to lower these ratios. In the present study, birth weight was chosen as the measure of neonatal 'development' because the gestational age range of the infants studied was not sufficient for significant developmental changes, two infants were 'small for dates' (birth weight below the 10th percentile for gestational age), liver weight is proportional to body weight, and birth weight is a continuous variable and an accurate measure is routinely available.

The relationships between the plasma concentration ratios and birth weight and the significant correlation between plasma concentrations of M3G and M6G point to a common regulatory mechanism for the formation of the two glucuronides, as suggested previously (Choonara *et al.*, 1989). However, the M6G/M3G plasma concentration ratio showed a trend towards a decrease with increasing birth weight.

Although the glucuronidation of morphine is deficient in premature neonates, significant plasma concentrations of the respiratory stimulant M3G (Gong *et al.*, 1991; Pelligrino *et al.*, 1989) are achieved rapidly (20% of morphine plasma concentrations at 2 h). At this time, the respiratory depressant M6G (Hasselstrom *et al.*, 1989; Osborne *et al.*, 1988) cannot be detected (Hartley *et al.*, 1992). This imbalance may be a contributary factor in infants who continue to 'fight the ventilator' despite high doses of morphine (Quinn *et al.*, 1992).

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(Received 22 June 1992, accepted 29 September 1992)