Morphine, morphine-6-glucuronide and morphine-3-glucuronide pharmacokinetics in newborn infants receiving diamorphine infusions

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- 1 The pharmacokinetics of morphine, morphine-6-glucuronide (M6G) and morphine-3-glucuronide (M3G) were studied in 19 ventilated newborn infants (24–41 weeks gestation) who were given a loading dose of $50 \mu\text{g kg}^{-1}$ or $200 \mu\text{g kg}^{-1}$ of diamorphine followed by an intravenous infusion of $15 \mu\text{g kg}^{-1} \text{ h}^{-1}$ of diamorphine. Plasma concentrations of morphine, M3G and M6G were measured during the accrual to steady-state and at steady state of the diamorphine infusion.
- 2 Following both the $50 \mu\text{g kg}^{-1}$ or $200 \mu\text{g kg}^{-1}$ loading doses the mean steady-state plasma concentration ($\pm$ s.d.) of morphine, M3G and M6G were $86 \pm 52 \text{ ng ml}^{-1}$, $703 \pm 400 \text{ ng ml}^{-1}$ and $48 \pm 28 \text{ ng ml}^{-1}$ respectively and morphine clearance was found to be $4.6 \pm 3.2 \text{ ml min}^{-1} \text{ kg}^{-1}$.
- 3 M3G formation clearance was estimated to be $2.5 \pm 1.8 \text{ ml min}^{-1} \text{ kg}^{-1}$, and the formation clearance of M6G was estimated to be $0.46 \pm 0.32 \text{ ml min}^{-1} \text{ kg}^{-1}$.
- 4 M3G metabolite clearance was $0.46 \pm 0.60 \text{ ml min}^{-1} \text{ kg}^{-1}$, the elimination half-life was $11.1 \pm 11.3 \text{ h}$ and the volume of distribution was $0.55 \pm 1.13 \text{ l kg}^{-1}$. M6G metabolite clearance was $0.71 \pm 0.36 \text{ ml min}^{-1} \text{ kg}^{-1}$, the elimination half-life was $18.2 \pm 13.6 \text{ h}$ and the volume of distribution was $1.03 \pm 0.88 \text{ l kg}^{-1}$.
- 5 No significant effect of the loading dose ($50 \mu\text{g kg}^{-1}$ or $200 \mu\text{g kg}^{-1}$) on the plasma morphine or metabolite concentrations or their derived pharmacokinetic parameters was found.
- 6 We were unable to identify correlations between gestational age of the infants and any of the determined pharmacokinetic parameters.
- 7 M3G:morphine and M6G:morphine steady-state plasma concentration ratios were 11.0 ± 10.8 and 0.8 ± 0.8 , respectively.
- 8 The metabolism of morphine in neonates, in terms of the respective contributions of each glucuronide pathway, was similar to that in adults.

Keywords diamorphine morphine morphine-6-glucuronide morphine-3-glucuronide pharmacokinetics metabolism premature newborn intravenous infusion dose regimen

Introduction

Increased use of the opiates morphine and diamorphine in neonatal intensive care units has resulted from their recommendation for sedation and analgesia [1–3]. There is, however, some concern regarding what constitutes a safe and appropriate dosage regimen, compounded by studies demonstrating that diamorphine

and morphine are biotransformed to produce metabolites which possess analgesic pharmacological activity [4]. A number of recent studies have concentrated on defining the relative pharmacological importance of morphine and its active conjugated metabolite—morphine-6-glucuronide (M6G) [5–7]. It is now believed that a significant proportion of the analgesic activity of morphine resides with M6G [8, 9]. Another conjugated

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metabolite of morphine is morphine-3-glucuronide (M3G) which possesses significant opioid antagonistic activity in animal models [10, 11] whilst M6G has respiratory suppressant activity along with its established analgesic potency [12].

Several studies have examined the pharmacokinetics of the opioid analgesics in both infants and neonates [13–19]. However, as can be seen above, the role of active metabolites in the pharmacology of morphine necessitates a more comprehensive study of the disposition of opiates and their metabolites [20]. This is particularly true for premature neonates, a group which are likely to be sensitive to opiate side-effects but who, as reported above receive such drugs as part of their treatment during intensive care.

Diamorphine (3,6-diacetylmorphine) is a semi-synthetic derivative of morphine which is rapidly metabolized to morphine via hydrolytic ester cleavage; it has therefore been considered as a pro-drug for morphine [21, 22]. Diamorphine is used by almost a third of newborn intensive care units in the United Kingdom [23]. When compared with an equivalent dose of morphine, an intravenous dose of diamorphine is believed to have a more rapid onset of action and a greater potency. A previous study conducted by our group concluded that a $50 \mu\text{g kg}^{-1}$ loading dose followed by a $15 \mu\text{g h}^{-1} \text{kg}^{-1}$ infusion was safe [18]. However, it was suggested that an increase in the loading dose to $200 \mu\text{g kg}^{-1}$ would provide a more rapid onset of action. A comparison of the $50 \mu\text{g kg}^{-1}$ and $200 \mu\text{g kg}^{-1}$ loading doses has recently been made and concluded that the higher dose confers no benefits and may produce undesirable side-effects [3].

This present study was designed to measure the plasma concentrations of morphine and its two primary conjugated metabolites, M3G and M6G, achieved when using two dosage regimens of diamorphine, and to evaluate the pharmacokinetics of morphine and its metabolites during such infusions in ventilated infants.

Methods

Patients

Twenty-seven infants were entered into the study. Fourteen patients were allocated to receive the higher loading dose and thirteen to receive the lower loading dose. Sufficient blood samples for pharmacokinetic analysis were obtained from nineteen out of the twenty-seven infants participating in the study, data from which are reported here. The nineteen neonates had a gestational age 24 to 41 weeks (mean 29.7 ± 3.7), postnatal age of 4 to 43 h (mean 18.5 ± 13.5) and birth weight of 0.68 to 3.04 kg (mean 1.4 ± 0.6 kg). The clinical details of these patients are shown in Table 1. Diamorphine was used to treat distress and as sedation to synchronize the infants' breathing with that of the ventilator. Approval for the study was obtained from the Hospital Ethics Committee and written parental consent was obtained for each infant prior to randomization.

Table 1 Details of infants participating in the study

Patient	Postnatal age (h)	Gestational age (weeks)	Weight (kg)
<i>50 $\mu\text{g kg}^{-1}$ loading dose</i>			
1	4	29	1.12
2	34	31	1.68
3	7	41	2.30
4	15	28	1.10
5	19	28	1.33
6	9	29	1.30
7	18	27	0.84
8	6	28	1.10
<i>200 $\mu\text{g kg}^{-1}$ loading dose</i>			
9	37	30	1.54
10	21	25	0.78
11	26	28	1.24
12	7	35	3.04
13	38	24	0.68
14	13	32	1.81
15	38	28	1.14
16	6	29	0.84
17	6	32	2.02
18	43	29	1.20
19	5	32	2.14
Mean ($n = 19$)	18.5	29.7	1.4
$\pm$ s.d.	13.5	3.7	0.6

Dosing regimen

Patients received a diamorphine loading dose of $50 \mu\text{g kg}^{-1}$ or $200 \mu\text{g kg}^{-1}$ as an infusion over 30 min followed by a continuous infusion of $15 \mu\text{g kg}^{-1} \text{h}^{-1}$. For clinical reasons, the infusion rate was changed during the study for certain patients. These changes were taken into account when calculating the pharmacokinetic parameters. The duration of the infusion ranged from 10 to 216 h (mean 94.9 ± 59.4 h).

Blood sampling and analysis

Arterial blood samples (0.5 ml) were collected *via* an indwelling catheter before the study, after the loading dose, at 2, 6, 12, 24, and every further 24 h during the continuous infusion. Blood samples were centrifuged and the separated plasma was stored at -25°C until analysed. Samples were analysed by an h.p.l.c. method for morphine, morphine-6-glucuronide (M6G) and morphine-3-glucuronide (M3G) [24] which was modified as follows. The plasma sample (0.2 ml) was mixed with the internal standard (codeine-6-glucuronide) and was added to a mixture of carbonate buffer (0.4 ml, pH 9.4, 0.2 M) and tetrabutylammonium hydrogen sulphate (0.1 ml, 20 mM). The mixture was then extracted using Bond-Elut C18 cartridge which had previously been pre-wetted with 1 ml methanol followed by 1 ml water followed by 1 ml of carbonate buffer (pH 9.2, 5 mM). The cartridge was washed with 2×1 ml of carbonate buffer (pH 9.2, 5 mM) and the analytes eluted with 0.5 ml

of the mobile phase. Aliquots (0.1 ml) of the extracted sample were injected onto the h.p.l.c. The analysis was performed using a Hypersil ODS2 Excel column (250 mm $\times$ 4.6 mm) and a mobile phase comprising 22% acetonitrile and 78% phosphate buffer (pH 2.3, 0.01 M) containing 1.75 mM sodium dodecyl sulphate. Detection of analytes was achieved by either ultraviolet absorption (M3G) or coulometric detection (M6G and morphine) as appropriate. The intra-day assay coefficients of variation were 4.2%, 3.8% and 7.0% for M3G, M6G and morphine respectively at a plasma concentration of 20 ng ml⁻¹. There was no evidence of any interference with the h.p.l.c. assay from co-administered drugs present in the plasma samples.

Pharmacokinetic analysis

Morphine clearance was calculated from the infusion rate divided by the steady-state plasma drug concentration. The steady-state concentration was taken as the plasma drug concentration at the end of the infusion, or as a mean of values during infusion where these values were within $\pm 10\%$ of each other. A mono-exponential model was fitted to the individual plasma metabolite accrual concentration-time data by non-linear least squares regression analysis using the MINIM 2.0.2 software package (R.D. Purves, Department of Pharmacology, University of Otago, New Zealand). This procedure provided estimates for the metabolite elimination rate constant ($k(m)$). Metabolite elimination half-life ($t_{1/2(m)}$) was calculated by dividing 0.693 by $k(m)$. Metabolite formation clearance (CL_f) was calculated from the product of the fraction of the administered morphine dose (f_m) converted to each metabolite and the individual morphine clearance (CL). It was not possible during this study to directly estimate f_m . Hence, literature values for f_m of 0.55 for M3G and 0.10 for M6G were used [25]. The individual metabolite elimination clearance (CL_(m)) values were then calculated from the product of the formation clearance (CL_f) and the morphine steady state concentration (C_{ss}) divided by the steady state concentration of the respective metabolites ($C(m)_{ss}$) [26]. The steady-state concentrations were taken as the plasma or metabolite drug concentrations at the end of the infusion, or as means of values during infusion where these values were within $\pm 10\%$ of each other. Individual metabolite volumes of distribution ($V(m)$) were estimated by dividing CL_(m) by $k(m)$ [27].

Data analysis

Statistical analysis was performed using an unpaired two-tailed Student's *t*-test to determine any significant differences in the accrual kinetics of the two loading doses. Correlations were performed using least squares linear regression analysis (Pearson's correlation coefficient, *r* and *P* value).

Results

Morphine, M3G and M6G were detected in the plasma of all study subjects and the mean steady-state concentrations of each compound (86 ± 52 ng ml⁻¹, 703 ± 400 ng ml⁻¹ and 48 ± 28 ng ml⁻¹ respectively) during the diamorphine infusion are shown in Table 2. The plasma concentration data for all subjects for morphine, M3G and M6G are shown in Figure 1. The solid lines on these figures join together the mean concentrations of these compounds at 0.5, 2, 6, 12, 24, 48, 72 h. Figure 2 shows the typical morphine, M3G and M6G plasma concentrations in an individual infant receiving diamorphine infusion together with the lines of best fit (monoexponential accrual for M3G and M6G; linear regression for morphine). The pharmacokinetic parameters for morphine and its metabolites M3G and M6G, in each individual subject, are shown in Table 3. We were unable, due to the large inter-patient variability, to discern any significant difference (Student's *t*-test) in pharmacokinetics for morphine and its metabolites between the two loading dose regimens. We have therefore combined the two sets of data in all the reported pharmacokinetic parameters.

Figure 1 Morphine (a), M3G (b) and M6G (c) plasma concentrations *vs* time for 19 premature infants receiving diamorphine infusion. The solid lines join mean concentrations at 0.5, 2, 6, 12, 24, 48, and 72 h after the start of the infusion.

Table 2 Steady-state plasma concentration ratios for morphine, M3G and M6G after diamorphine infusion

Patient	Morphine C _{ss} (ng ml ⁻¹)	M3G C _{ss} (ng ml ⁻¹)	M6G C _{ss} (ng ml ⁻¹)	M3G:Morphine C _{ss} ratio	M6G:Morphine C _{ss} ratio	M3G:M6G C _{ss} ratio
50 µg kg ⁻¹ loading dose						
1	20	928	35	46.4	1.8	26.5
2	215	1237	91	5.8	0.4	13.6
3	76	234	85	3.1	1.1	2.8
4	153	599	38	3.9	0.2	15.8
5	41	676	30	16.5	0.7	22.5
6	109	667	59	6.1	0.5	11.3
7	151	1067	64	7.1	0.4	16.7
8	96	135	37	1.4	0.4	3.6
200 µg kg ⁻¹ loading dose						
9	61	810	71	13.3	1.2	11.4
10	41	681	120	16.6	2.9	5.7
11	104	533	41	5.1	0.4	13.0
12	86	311	49	3.6	0.6	6.3
13	88	952	11	10.8	0.1	86.5
14	14	45	39	3.2	2.8	1.2
15	43	124	21	2.9	0.5	5.9
16	80	794	27	9.9	0.3	29.4
17	146	1240	39	8.5	0.3	31.8
18	55	1401	19	25.5	0.3	73.7
19	46	926	43	20.1	0.9	21.5
Mean	86	703	48	11.0	0.8	21.0
± s.d.	52	400	28	10.8	0.8	22.8

Figure 2 Typical morphine (▲), M3G (■) and M6G (●) plasma concentrations in an individual infant receiving diamorphine infusion. Solid lines represent lines of best fit.

There was no significant difference (one-way ANOVA) between the mean plasma morphine concentrations for both loading dose patient cohorts at the following times after the commencement of the infusion; 2 h (53.2 ± 37.9 ng ml⁻¹), 6 h (57.1 ± 36.7 ng ml⁻¹), 12 h (64.1 ± 37.4 ng ml⁻¹), and 24 h (65.1 ± 43.2 ng ml⁻¹). This indicates that the plasma morphine concentration reaches a plateau value very soon after the commencement of the infusion.

The mean plasma clearance of morphine for all patients was 4.6 ± 3.2 ml min⁻¹ kg⁻¹ ($n = 19$), and, using

the fm literature data of Hasselström & Säwe [25], this provides mean formation clearance (CL_f) values of 2.5 ± 1.8 ml min⁻¹ kg⁻¹ for M3G and 0.46 ± 0.32 ml min⁻¹ kg⁻¹ for M6G. The mean elimination half-lives of M3G and M6G were found to be 11.1 ± 11.3 h and 18.2 ± 13.6 h respectively. The mean metabolite elimination clearances (CL(m)) for M3G and M6G were calculated to be 0.46 ± 0.60 ml min⁻¹ kg⁻¹ and 0.71 ± 0.36 ml min⁻¹ kg⁻¹ respectively and the metabolite volumes of distribution (V(m)) were 0.55 and 1.03 l kg⁻¹ for M3G and M6G.

Discussion

The range of plasma morphine concentrations reported here compares well with those reported previously [18] for a similar diamorphine infusion regimen administered to neonates. Diamorphine is known to be very rapidly hydrolysed in blood to produce 6-acetylmorphine and subsequently morphine [21, 22, 28] and we have previously demonstrated that diamorphine can effectively be viewed as a pro-drug for morphine [18].

We were not able to find any significant difference between the steady-state morphine plasma concentrations with either the 50 µg kg⁻¹ or the 200 µg kg⁻¹ loading doses. We also noted that both regimens rapidly produced effective steady state concentrations of morphine—there was no significant difference (using ANOVA) between the mean plasma morphine concentrations at 2 h, 6 h, 12 h and 24 h. This is in contrast to

Table 3 Morphine and metabolite pharmacokinetics in premature infants following diamorphine infusion

Patient	CL Morphine	CL _f (ml min ⁻¹ kg ⁻¹)		t _{1/2(m)} (h)		CL(m) (ml min ⁻¹ kg ⁻¹)		V (m) (l kg ⁻¹)	
	(ml min ⁻¹ kg ⁻¹)	M3G	M6G	M3G	M6G	M3G	M6G	M3G	M6G
50 µg kg ⁻¹ loading dose									
1	9.7	5.3	0.97	7.0	1.7	0.11	0.55	0.07	0.08
2	3.2	1.8	0.32	12.2	33.5	0.31	0.76	0.33	2.21
3	5.8	3.2	0.58	7.2	19.3	1.03	0.51	0.64	0.86
4	1.3	0.7	0.13	6.5	25.8	0.18	0.51	0.10	1.13
5	4.7	2.6	0.47	3.5	18.0	0.16	0.64	0.05	1.01
6	1.8	1.0	0.18	4.4	25.0	0.16	0.33	0.06	0.71
7	1.3	0.7	0.13	4.2	14.7	0.10	0.30	0.04	0.38
8	4.0	2.2	0.40	30.7	39.8	1.57	1.04	4.18	3.60
200 µg kg ⁻¹ loading dose									
9	6.3	3.5	0.63	10.3	17.5	0.26	0.54	0.23	0.83
10	9.4	5.2	0.94	13.6	53.7	0.31	0.32	0.37	1.50
11	3.7	2.0	0.37	6.7	17.0	0.40	0.94	0.23	1.38
12	2.2	1.2	0.22	3.8	5.9	0.34	0.39	0.11	0.20
13	2.2	1.2	0.22	4.7	0.9	0.11	1.75	0.05	0.13
14	13.8	7.6	1.38	0.4	1.7	2.36	0.49	0.08	0.07
15	4.5	2.5	0.45	43.6	8.6	0.86	0.92	3.23	0.69
16	3.4	1.9	0.34	28.6	19.4	0.19	1.00	0.46	1.68
17	2.6	1.5	0.26	14.3	23.1	0.17	0.99	0.21	1.98
18	3.5	1.9	0.35	2.1	7.8	0.08	1.02	0.01	0.69
19	4.2	2.3	0.42	7.4	13.3	0.11	0.45	0.07	0.52
Mean	4.6	2.5	0.46	11.1	18.2	0.46	0.71	0.55	1.03
±s.d.	3.2	1.8	0.32	11.3	13.6	0.60	0.36	1.13	0.88

our earlier predictions that a 200 µg kg⁻¹ loading dose would provide a more rapid achievement of steady-state morphine plasma concentrations [18]. We have as yet, no simple explanation for this observation. We have attempted to identify any pharmacokinetic parameter which would successfully discriminate between the 50 µg kg⁻¹ and the 200 µg kg⁻¹ loading dose patient cohorts but we were unable to find any significant differences between these groups for any parameter. Physiological data from this study reported elsewhere [3] have demonstrated that there is no advantage in using the higher loading dose regimen and that the 50 µg kg⁻¹ loading dose appears to be safe and effective.

The dosage regimens employed in these studies therefore result in relatively constant concentrations of morphine within an individual. If such a situation exists then the drug metabolites will be produced at a constant rate dependent upon the fraction of a dose of the drug converted to each metabolite (*f_m*) and the drug infusion rate [26]. Therefore, the approach of a metabolite to its steady state concentration is controlled solely by the half-life of that metabolite. It is therefore possible to estimate directly from this accrual profile the half-life of the drug metabolites. This process can be seen in Figure 1 for M3G and M6G. Such data on the elimination half-life of individual metabolites of morphine have not previously been reported in this patient group. However, since the metabolites of morphine are known to play a significant role in the pharmacological activity of the drug it is important to be able to characterize the pharmacokinetics of M3G and M6G in order to provide a better understanding of the

pharmacodynamics of morphine based analgesia in this patient group.

The studies reported here did not permit us to make a satisfactory estimate for *f_m* or for *V(m)* and indeed such data have yet to be reported for neonates and are rarely obtained with confidence in adult studies [6, 25]. The most common approach to the estimation of *f_m* is to obtain the percentage of the administered dose excreted in the urine as a particular metabolite. Such data have, in the past, been of doubtful reliability due to analytical problems in measuring the glucuronide metabolites and studies to obtain such data would be extremely difficult to conduct in premature infants. It is clear, however, that this information is essential if one is to be able to calculate the formation and elimination clearances of the two principal morphine metabolites. Estimates for the volume of distribution of M6G are available, but only in adults [8, 9] but no values are available for the *V(m)* for M3G. In the absence of such published information we have chosen to use literature data [25] to provide our estimate of *f_m*. It should be clear that these must represent approximations of the true description of morphine metabolism in the neonate patient cohort but will serve until more appropriate data become available.

The formation clearances of M3G and M6G reflect the *f_m* values introduced from the literature and these data are then further used to calculate the metabolite elimination clearances. The half-life of M6G was not notably different from that of M3G, and this is also reflected in the metabolite elimination clearances for M6G and M3G. The absolute value for the half-lives of

these metabolites have not been previously reported in premature infants although a value of approximately 2–3 h has been obtained after the administration of M6G to adults [8, 9]. It has been shown that renal elimination is the primary route of excretion of the glucuronide metabolites of morphine and that renal function is well correlated with the renal clearance of M6G [9, 29]. Most studies examining glomerular filtration rate in infants with respiratory distress syndrome have shown that this is reduced [30]. Two studies have reported values for the elimination clearance of M6G, giving values of approximately 1.50–2.75 ml min⁻¹ kg⁻¹ [8, 9]. These values are in reasonable agreement with our estimates, taking into account the immaturity of our patient group. No literature data are available for the metabolite elimination clearance of M3G and our values are the first estimation in any subject group. Our data have also shown that the volume of distribution for M6G appears to be greater than that for M3G and both of these values are somewhat larger than the reported volume of distribution of 0.28–0.43 l kg⁻¹ in adults [8, 9]. One potential explanation for the differences in distribution between these metabolites may lie in the higher lipophilicity at physiological pH measured for M6G when compared with M3G [31].

We were unable to identify correlations between gestational age and any of the determined pharmacokinetic parameters. Unlike previous reports we did not observe a correlation between gestational age and morphine clearance [18] nor with M3G:morphine and M6G:morphine plasma concentration ratios [20]. Such correlations are difficult to achieve since the range of gestational age of patients participating in studies such as ours is restricted by the intake into neonatal intensive care units.

Glucuronidation of morphine has been demonstrated in premature neonates after administration of morphine [15, 20]. Studies by these workers have indicated that the morphine glucuronidation in neonates (in terms of M3G or M6G:morphine metabolite ratios) was reduced when compared with older children and adults. The use of single-point concentration determinations of morphine and its metabolites and relatively short infusion times suggest that these previous data are likely to be subject to considerable variability. Our data indicate that the mean half-lives of M6G and M3G are 18.2 h and 11.2 h and consequently that the metabolite concentrations will continue to increase up to and beyond the morphine infusions of 24 h or 48 h duration [15, 20]. Previous observations by these same workers have reported M6G:morphine concentration ratios which are reduced in the premature neonate; thereby producing steady-state plasma concentrations of M6G which are lower than those of morphine in the majority of patients. Our data concur with these findings and we observed a M6G:morphine ratio of less than unity in 14 out of 19 patients. However, the intravenous administration of morphine to adults has been reported to produce M6G:morphine plasma AUC concentration ratios of similar magnitude—0.7 [25] and 1.4 [6]. Our studies have found that the M3G:morphine plasma concen-

tration ratio is much higher than that for M6G—giving a mean value of 11.0. These data are not in agreement with those of Hartley *et al.* [20] who reported a value of approximately 0.7 but are closer to the figure obtained by Choonara *et al.* [15] of 5.0. Data obtained from adults who received intravenous morphine produced plasma AUC M3G:morphine ratios of 7.7 [25] and 7.9 [6].

Our data therefore suggest that the metabolism of morphine in neonates, in terms of the respective contributions of each pathway, is not markedly dissimilar from that in adults who have received intravenous morphine. This finding therefore adds weight to our use of literature values for *f_m*. In summary, we would conclude that the overall elimination of morphine is reduced, as would be predicted, in this patient population following intravenous diamorphine infusion, but that the relative contributions of the various routes of elimination remains similar between the neonates and adults.

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(Received 31 October 1995,
accepted 30 January 1996)