Pharmacogenetics of codeine metabolism in an urban population of children and its implications for analgesic reliability

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Background. Codeine analgesia is wholly or mostly due to its metabolism to morphine by the cytochrome P450 enzyme CYP2D6, which shows significant genetic variation in activity. The aims of this study were to investigate genotype, phenotype and morphine production from codeine in children undergoing adenotonsillectomy, and to compare analgesia from codeine or morphine combined with diclofenac.

Methods. Ninety-six children received either codeine 1.5 mg kg⁻¹ or morphine 0.15 mg kg⁻¹ in a randomized, double-blind design. Genetic analysis was performed and plasma morphine concentrations at 1 h were determined. Postoperative analgesia and side-effects were recorded.

Results. Forty-seven per cent of children had genotypes associated with reduced enzyme activity. Mean (SD) morphine concentrations were significantly lower (P<0.001) after codeine [4.5 (0.3) ng ml⁻¹] than after morphine [24.7 (1.5) ng ml⁻¹], and morphine and its metabolites were not detected in 36% of children given codeine. There was a significant relationship between phenotype and plasma morphine (P=0.02). More children required rescue analgesia after codeine at both 2 (P<0.05) and 4 h after administration (P<0.01). Fifty-six per cent of children vomited after morphine and 29% after codeine (P<0.01). Neither phenotype nor morphine concentration was correlated with either pain score or the need for rescue analgesia (r=−0.21, 95% confidence interval −0.4, −0.01).

Conclusions. Reduced ability for codeine metabolism may be more common than previously reported. Plasma morphine concentration 1 h after codeine is very low, and related to phenotype. Codeine analgesia is less reliable than morphine, but was not well correlated with either phenotype or plasma morphine in this study.

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Codeine is still widely prescribed for postoperative pain relief and is frequently recommended for paediatric use.¹ ² The reputedly lower incidence of opioid-related side-effects has made codeine popular for younger age groups, including neonates, especially in situations where airway management and neurological assessment are critical.³–⁵ However, there is some doubt about the efficacy and reliability of codeine.⁶ Both animal and adult human laboratory experimental studies have shown that there is significant variability in both the pharmacokinetics and pharmacodynamics of codeine. These studies also suggest that the analgesic effect of codeine is either wholly or mostly dependent on its metabolism to morphine.⁶ ⁷ Metabolism of codeine to morphine is catalysed by the cytochrome P450 enzyme CYP2D6. Over 50 different genetic variants are known to exist for CYP2D6, which leads to a wide spectrum of
metabolic capabilities within populations. Individuals are normally classified as either poor metabolizers (PM) or extensive metabolizers (EM), depending on the activity of the enzyme, although this is known to be an oversimplification. PMs will produce little or no morphine from codeine whereas EMS will produce morphine, although the actual amount may show wide variation.

The aims of the current studies were to investigate: (i) genotype, phenotype and morphine production from codeine in a UK urban population of children undergoing adenotonsillectomy; and (ii) the efficacy, reliability and side-effects of codeine as part of a postoperative analgesic regimen.

Methods

All procedures were carried out at the Royal National Throat, Nose and Ear Hospital, London. After obtaining local research ethics committee approval, we recruited 98 children aged from 3 to 12 yr, ASA I and II, undergoing adenotonsillectomy. Informed written parental consent was sought for each child before enrolment in the study. The children were randomized into two groups: group C received codeine 1.5 mg kg⁻¹ and group M morphine 0.15 mg kg⁻¹ i.m. shortly after the induction of anaesthesia. Both groups also received diclofenac 1 mg kg⁻¹ per rectum at the same time. Blood for analysis was taken from an i.v. cannula inserted for the procedure. After induction, 2–4 ml blood was collected for genetic analysis, and a further 1–2 ml was taken 1 h after injection of the study drug for the measurement of plasma morphine and morphine metabolites.

Surgery was performed using electrocautery for tonsillar dissection and haemostasis. The anaesthetist and surgeon present varied depending on which day and on which operating list the procedure was performed. All staff involved in the procedure and the perioperative care of the child were blinded as to which opioid was administered. No premedication was given. Topical local anaesthesia to facilitate i.v. cannulation was applied before anaesthesia, which was induced either by the i.v. route (propofol 2–5 mg kg⁻¹) or by inhalation (oxygen, nitrous oxide and sevoflurane). Once adequate anaesthesia had been established, a laryngeal mask of appropriate size was inserted into the airway. Anaesthesia was maintained with oxygen, nitrous oxide and sevoflurane with spontaneous ventilation. Analgesia (study drug plus diclofenac) was administered as soon as convenient after induction. Hartman’s solution 10 ml kg⁻¹ was given during the procedure and continued into the postoperative period at a maintenance rate appropriate to each individual child. At the end of the procedure the patient was allowed to breathe 100% oxygen, and the laryngeal mask was removed once airway reflexes had returned. Patients were kept in the recovery area until they were awake and comfortable and able to return to the ward. Further analgesia was not given during the procedure unless indicated on the basis of clinical judgement. Ondansetron 0.1–0.2 mg kg⁻¹ was administered if required.

The analysis was performed at the Department of Molecular Biology at University College London. Whole blood was stored at −70°C until analysis and all the samples were processed in one batch. DNA was extracted from the whole blood, which was then split using a two-stage polymerase chain reaction (PCR). The PCR products were labelled fluoroscopically to allow detection electronically using an ABI-377 analyser (Applied Biosystems, Foster City, CA, USA). Over 50 variants of the CYP2D6 gene have been identified and most are classified as rare, 87% of genotypes being accounted for by five variants. It is impractical to look for all these different variants in each sample of DNA; thus, the most common polymorphisms causing alteration in enzyme activity were targeted. These were CYP2D6*1, *2, *3, *4, *5, *9, *10 and *17. CYP2D6*1 is considered the normal or wild-type gene and has normal activity. Variants *3, *4, *5 and *9 have no activity and account for over 90% of PMs, whilst *2, *10 and *17 have reduced activity. Four possible phenotypes were identified. If a patient was homozygous for polymorphisms known to show no activity, he or she was classified as a PM, whilst a combination of a polymorphism of reduced activity with one of no activity classified the patient as an intermediate/poor metabolizer (IM/PM). An intermediate metabolizer (IM) was a patient with either a combination of two polymorphisms with reduced activity or a combination of one normal polymorphism with one of no activity, and an extensive metabolizer (EM) was a patient who was either homozygous for the normal gene or who had a combination of a normal polymorphism with one of reduced activity. Genotyping identified all the polymorphisms apart from *1 so, if none was found, the sample was considered to be from a patient with the normal gene. Thus, there was a chance that a PM patient with another polymorphism could have been considered an EM (false negative), though as these polymorphisms are rare the estimated risk of this occurrence was very small.

For measurement of plasma morphine and metabolites, the blood samples were centrifuged at 3000 r.p.m. for 10 min within 15 min of being taken. The plasma was stored before processing at −70°C. Plasma morphine and its glucuronide metabolites were measured by high-performance liquid chromatography at the Institute of Child Health, London. Analysis of the samples was in two batches, and limits of detection were 5 (sd 2) ng ml⁻¹ for morphine and 10 (2) ng ml⁻¹ for morphine-6-glucuronide (M6G) in both. The coefficient of variation for morphine was 4% at 20 ng ml⁻¹. The limit of detection for morphine-3-glucuronide (M3G) was 30 (8) ng ml⁻¹ in the first batch and 60 (8) ng ml⁻¹ in the second.

Time to first analgesia (time from the injection of the opioid to the first dose of supplementary analgesia) and pain scores (using self-report and behavioural pain assessment tools) both at rest and on swallowing were used to assess...
analgesia. The need for extra analgesia was assessed on clinical grounds by the anaesthetist and/or the nurse responsible for the patient. Further analgesia was given as an i.v. bolus of morphine 20 mg kg⁻¹, oral morphine solution 0.1 mg kg⁻¹ or oral acetaminophen 20 mg kg⁻¹ at their discretion and the patient was subsequently excluded from the analysis.

Two methods of pain scoring were used, both of which have been verified in this age group. A ‘faces’ type self-reporting pain tool was used in the recovery ward at the time of blood sampling for measurement of plasma morphine concentrations and 1, 2, 3 and 4 h after the end of the procedure, and the Children’s Hospital of Eastern Ontario Pain Score (CHEOPS) in the recovery ward. The scores were taken at each time point, both at rest and on swallowing, to provide static and dynamic measures of analgesia. The children were introduced to the self-reporting pain scale at the preoperative visit and shown how to use it. All nursing staff involved in the care of the children enrolled in the study had been taught previously how to use both pain scores.

Respiratory rate and a five-point sedation score (1=awake, 5=hard to rouse) were also recorded at the same time points as above. Postoperative vomiting was assessed by counting the number of vomiting episodes for each patient in a number of set time periods (<1, 1–2, 2–4 and 4–8 h). Again, patients were removed from analysis once further analgesia had been administered. Other unexpected complications and adverse effects were also recorded. Patients were monitored closely until discharge from the hospital.

All data were handled electronically and calculations performed using either Microsoft Excel 2000 or Graphpad Prism 3.0. Comparisons between continuous variables were made using either analysis of variance with Bonferroni post-test corrections or t-tests as appropriate. Survival analysis was performed using Kaplan–Meier survival curves. Categorical data were analysed using the χ² test and correlation between independent variables was tested with Pearson’s correlation coefficient. For all comparisons, P<0.05 was considered significant.

Results

Ninety-eight children were recruited into the study but two were excluded: one child was given an incorrect dose of the study opioid, and consent for the other was withdrawn after the procedure had been postponed on the first attendance. Data were therefore included from a total of 96 patients; of these, 48 received morphine and 48 received codeine. Patient characteristics are shown in Table 1. There was no significant difference between the two groups for age, sex or duration of surgery, or in the make-up of the ethnic groups of the patients randomized to the two arms of the study.

The total number of patients with phenotypes indicating reduced enzyme activity (PM, IM/PM and IM) in the study population was 46 (47%). The total number of PMs, who would not be expected to produce morphine from codeine at all, was 4 (4.1%). The distribution of these phenotypes in each ethnic group and in the two respective treatment groups is shown in Tables 2 and 3.

One sample from group C was lost by the laboratory. Results of the measurement of plasma concentrations of morphine and its glucuronide metabolites 1 h after morphine or codeine are shown in Figure 1. In group M, morphine and its metabolites were detected in all the patients. In group C, morphine and/or its metabolites were not detected in 17 patients (36%). The mean (sd) plasma concentrations of morphine, M3G and M6G were 24.7 (10.1), 96.58 (40.5) and 29.3 (13) ng ml⁻¹ respectively after morphine, and 4.5

| Table 1 Patient characteristics for the codeine and morphine groups. Data are mean (sd or range) or number. *P=0.044 |
|-----------------|-----------------|
| **Codeine group (n=48)** | **Morphine group (n=48)** |
| Sex (male/female) | 24/24 | 23/25 |
| Age (yr) | 7.31 (3–12) | 6.23 (3–12) |
| Weight (kg) | 32.39 (19.2) | 25.79 (11.49) |
| Surgical time (min) | 33.52 (10.7) | 33.06 (11.48) |
| Caucasian | 21 | 15 |
| Asian | 12 | 10 |
| Afro-Caribbean | 5 | 5 |
| Turkish | 5 | 3 |
| Arab | 0 | 2 |
| Caucasian/Asian | 3 | 1 |
| Caucasian/Afro-Caribbean | 4 | 7 |
| Caucasian/Arab | 1 | 1 |
| Asian/Afro-Caribbean | 0 | 0 |

| Table 2 Ethnicity in relation to phenotype. Data are numbers of patients. EM=extensive metabolizer; IM=intermediate metabolizer; IM/PM =intermediate/poor metabolizer; PM=poor metabolizer |
|-----------------|-----------------|-----------------|
| **EM** | **IM** | **IM/PM** | **PM** |
| Caucasian | 13 | 15 | 7 | 1 |
| Asian | 12 | 5 | 2 | 2 |
| Afro-Caribbean | 8 | 3 | 5 | 1 |
| Turkish | 3 | 2 | 3 | 1 |
| Arabian | 1 | 1 | 1 | 1 |
| Caucasian/Asian | 4 | 2 | 3 | 1 |
| Caucasian/Afro-Caribbean | 3 | 2 | 3 | 1 |
| Caucasian/Arabian | 1 | 1 | 1 | 1 |
| Asian/Afro-Caribbean | 1 | 1 | 1 | 1 |

| Table 3 Phenotype distribution (type of metabolizer) |
|-----------------|-----------------|-----------------|
| **Phenotype** | **Morphine group (n=48)** | **Codeine group (n=48)** |
| Poor | 2 | 2 |
| Intermediate/poor | 8 | 9 |
| Intermediate | 12 | 17 |
| Extensive | 26 | 19 |
There was a significant difference between the two treatment groups for the plasma concentrations of morphine and both glucuronide metabolites ($P<0.01$). There was no difference in M3G:M6G ratio, which was 4.2 (3.17) in group M and 3.8 (3.5) in group C ($P=0.6$).

Figure 2 shows the relationships of plasma morphine, M3G and M6G with phenotype for group C. Morphine concentrations were generally very low and could not be detected in an increasing proportion of patients with phenotypes associated with declining metabolic capacity. No morphine was found in the plasma of the two PMs (Fig. 2A). Plasma morphine concentrations were also related to phenotype, with a significant trend ($P<0.02$) across the groups (Fig. 2B). There was no difference in plasma morphine between the IM and IM/PM groups, but mean concentrations for both these groups were very low and close to the lower limit of measurement. Plasma concentrations of metabolites are shown in Figure 2C and D. The ratio of M3G:M6G was 4.5 (5.02) in the EM group and 3.4 (2.07) and 2.95 (2.39) in the IM and IM/PM groups respectively ($P>0.05$).

Comparisons of time to first analgesia were made using Kaplan–Meier survival curves 2 and 4 h after administration of either opioid (Fig. 3). In the first 2 h, the number of patients who required extra analgesia was eight (16.7%) in group C and two (4.2%) in group M ($P<0.05$). By 4 h, 20 of the 48 (41.7%) patients in group C required extra analgesia compared with nine of the 48 (18.8%) patients in group M ($P<0.01$). No relationship could be established between the group C patients of each phenotype who needed rescue analgesia. PMs did not differ significantly from other

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**Fig 1** Plasma morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) concentrations in the morphine and codeine groups ($n=48$ for each group). Data are mean and SD. *$P<0.01$; **$P<0.001$.**

**Fig 2** Relationship between plasma morphine, metabolites and phenotype for patients treated with codeine. P=poor metabolizers; IM/P=intermediate/poor metabolizers; E=extensive metabolizers. Data are mean and sd. (a) Number of patients of each phenotype with undetectably low plasma morphine concentration. (b) Plasma morphine concentration in relation to phenotype. *$P<0.02$. (c) Morphine-3-glucuronide (M3G) concentration in relation to phenotype. (d) Morphine-6-glucuronide (M6G) concentration in relation to phenotype.
phenotypes despite a demonstrated lack of plasma morphine. Overall, there was no relationship between plasma morphine, metabolite concentrations or M3G:M6G ratio and the requirement for extra analgesia.

Comparison of both behavioural and self-reporting pain scores, at rest and on swallowing, did not show a difference between groups C and M at any time point. Pain scores were generally higher on swallowing than at rest ($P<0.05$), with larger differences seen between pain on swallowing and at rest in group C compared with group M. Pain scores were also generally higher in recovery than at subsequent time points in children from both groups ($P<0.05$). Children in group C who received supplementary analgesia in the recovery room had higher pain scores at rest compared with the other children in group C ($P<0.05$). There was no difference in pain scores between the phenotypes in group C.

The percentage of patients who vomited in the first 4 h after operation was 56.3 for group M and 29.2 for group C ($P<0.01$). Figure 4 shows a comparison between groups M and C for the number of patients who vomited in each time period. There was no difference between the two groups in the proportion of patients who vomited at <1, 1–2 and 4–8 h but the difference was significant at 2–4 h ($P<0.01$), with more vomiting in group M. In group C, neither of the PM patients vomited but otherwise postoperative nausea and vomiting was evenly distributed between the phenotypes ($P>0.05$). There was no difference between the two groups in the time to oral intake, which was measured from the end of surgery. Intravenous fluids were continued because of vomiting in two patients in group M and one in group C, but discharge from hospital was not delayed.

Figure 5 shows sedation scores at each of the time points for each group. Patients who received morphine were more sleepy at 2 h ($P<0.01$). By 4 h the degree of sedation was similar. There was no difference in respiratory rate between the two groups at any of the time points during the study.

Five patients in the group C and none in group M required replacement of the laryngeal mask airway with a tracheal tube. On three occasions this was because of inability to maintain the airway on insertion of the surgical retractor, on one occasion it was because of an unsatisfactory airway in the anaesthetic room, and on the other occasion the tube was placed after an episode of laryngospasm. Pain scores on swallowing were not increased in this subgroup. One patient in each group had a prolonged stay in recovery (>1 h) because of slow awakening after anaesthesia. One patient in each group had postoperative bleeding that required a return to theatre some hours later. One morphine patient had significant swelling and pain the next day which delayed discharge, which in all other cases was the morning after the procedure.

Discussion

The mechanism of codeine analgesia is not completely understood but there is convincing evidence from both animal and human laboratory studies that it is wholly or substantially dependent on metabolism to morphine.6 7 17 18 The activity of the enzyme complex responsible for this reaction is known to be genetically regulated, with a spectrum of phenotypes between normal or ‘extensive’ and

![Fig 3 Time to first analgesia (Kaplan–Meier survival curves).](https://academic.oup.com/bja/article-abstract/89/6/839/261766/25-January-2019)

![Fig 4 Postoperative vomiting. *$P<0.01$.](https://academic.oup.com/bja/article-abstract/89/6/839/261766/25-January-2019)
absent or ‘poor’. In this study we investigated the genotypes of a population of children who were having adenotonsillectomy and assigned them to phenotypes that should reflect this range of metabolic capability. A high proportion (46%) of the children studied had phenotypes associated with reduced activity of the enzyme. Plasma morphine and metabolite concentrations in children given the parent drug, codeine, were very low or absent in greater proportions of those in the low-activity groups, the actual values reflecting the range predicted by the phenotype.

Plasma morphine concentration has not been directly related to analgesia, but pharmacokinetic studies have suggested that a range of 5–65 ng ml\(^{-1}\) may be therapeutic in children.\(^{19-21}\) One hour after administration of codeine in this study, mean plasma morphine was below this range in all phenotypes after codeine, including normal and extensive metabolizers. The half-life of both codeine and morphine after i.m. injection is 2–3 h, and the usual recommended dosing regimen for both is 4- to 6-hourly.\(^{1, 22, 23}\) However, such low concentrations at 1 h imply that analgesia from codeine-derived morphine after that time would be unlikely.

In the second part of the present study, the analgesia and side-effects of codeine and morphine combined with diclofenac were compared. Diclofenac was included for ethical reasons because the concurrent use of opioid and NSAID (non-steroidal anti-inflammatory drug) analgesia is standard practice after adenotonsillectomy. Significantly more children needed rescue analgesia at 2 and 4 h in the group given codeine (group C) than in the group given morphine (group M). However, behavioural and self-report pain assessments did not differentiate between the groups at any stage of the study. All the mean scores were within the satisfactory range. However, the codeine subgroup who needed supplementary analgesia had significantly higher scores in recovery than the rest of that group. Although within group C plasma morphine was related to phenotype, no relationship could be found between phenotype and analgesia; as morphine concentrations were very low and differences in morphine production between phenotypes were small, this may not be surprising. The concurrent use of diclofenac may also have masked any differences between the groups, as such analgesic combinations are known to increase efficacy substantially in comparison with monotherapy (see below).

In the first 4 h after operation, 29.2% of the patients vomited in group C compared with 56.3% of patients in group M. Vomiting is common after adenotonsillectomy in children, with a reported incidence of between 30 and 75%.\(^{24, 25}\) The causes are multiple and may include stimulation of laryngeal and pharyngeal reflexes, gastric irritation as a result of swallowed blood, anaesthetic agents and analgesic administration.\(^{26, 27}\) Broadly, there was little difference between the two drug groups in terms of sedation and respiratory rate. Previous studies in both adults and children have suggested that, although the incidence of side-effects may be low, the overall efficacy of codeine is also low and analgesia may be inadequate for postoperative pain in some circumstances.\(^{3, 28, 29}\) Indeed, in the adult some single-dose studies have shown no difference between codeine and placebo, and a quantitative systemic review suggests that codeine 60 mg has a number needed to treat (NNT) of 18, which is very high when compared with 5.0 for acetaminophen 600 mg and 3.1 for the combination.\(^{30-32}\)

In conclusion, we have found that reduced ability to metabolize codeine to morphine was common in this population of children, and that plasma concentrations of morphine are generally very low 1 h after i.m. codeine for patients of all phenotypes, even those who have normal metabolic capability. Codeine analgesia with diclofenac was less reliable than morphine, but a clear relationship between plasma morphine or phenotype and analgesia could not be established. Uncertainties regarding the efficacy and any possible advantages of codeine over morphine or other opioids mean that its use cannot be recommended without reservation. Situations in which pain is known to be significant and pain assessment problematic present particular difficulties, which will only be heightened by the use of codeine.

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