Indices and graphical approaches for the detection of interindividual and interethnic variations in codeine metabolism

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Aims To evaluate the use of different graphical methods and statistical tests in the detection of interindividual and interethnic variations in codeine metabolism. Various urinary metabolic ratios (MR) for codeine O-demethylation were also compared for their ability to determine phenotype.

Methods Frequency histograms, normal test variable (NTV) plots and admixture analysis were used to examine the distributions of the urinary MRs for codeine O-demethylation, N-demethylation and glucuronidation in 132 Caucasian and 222 Chinese subjects.

Results In the Caucasian population, apparent bimodality was shown in both a frequency histogram and NTV plot of the log MR of codeine O-demethylation (codeine/(morphine (M) + M-3 and M-6-glucuronide (M3G and M6G) + normorphine (NM)). Admixture analysis confirmed the co-segregation of codeine O-demethylation and debrisoquine hydroxylation. The antimode for the codeine O-demethylation MR between extensive and poor metabolisers was located between 5.5 and 8.3. A simplified MR for codeine O-demethylation (codeine/M3G) demonstrated a similar correlation with the debrisoquine MR to the more complex MR, allowing a simplification of the analytical method for phenotyping. The Chinese population had significantly higher median MRs for codeine N-demethylation, O-demethylation and glucuronidation, which was shown clearly in the frequency histograms, but not in the NTV plots.

Conclusion A histogram seems preferable over a NTV plot for assigning phenotype using the codeine O-demethylation MR, because it is clear and simple. Interethnic difference in the metabolism of codeine are also better visualised from the histograms.

Keywords: codeine metabolism, polymorphism, debrisoquine, metabolic ratio, ethnic groups, Caucasian, Chinese, graphical methods, admixture analysis

Introduction

The O-demethylation of codeine has been shown to cosegregate with the polymorphic hydroxylation of debrisoquine in both Caucasian [1, 2] and Chinese populations [3]. Apart from indicating a low prevalence of poor metabolizers (PM) in Chinese, the distribution of the urinary metabolic ratio (MR) for the O-demethylation of codeine showed a marked shift to the right in the Chinese population compared with Caucasians [4]. A molecular explanation of this phenomenon has been provided [3, 5, 6].

Various methods have been used to detect multiple modes in population distributions of data. Graphical methods include histograms and probit plots [7, 8] as well as a new normal test variable (NTV) plot [9]. The utility and limitations of the first two methods and the choice of different experimental indices have been discussed in detail [7, 8, 10, 11].

The aims of the present study were to evaluate the use of different graphical methods and statistical tests in the detection of interindividual and interethnic variations in

codeine metabolism. Various urinary MRs for codeine O-demethylation were also compared for their ability to determine phenotype.

Methods

Subjects

One hundred and thirty-two Caucasians and 222 Chinese were studied. The Caucasian subjects were students and staff at the Karolinska Institute and Huddinge University Hospital. They had all been phenotyped with debrisoquine on an earlier occasion. Some of the Caucasian data on phenotyping with debrisoquine and codeine have been published earlier together with information on the characteristics of the volunteers [1]. The Chinese subjects were recruited from students living temporarily (less than 8 years) in Sweden. Data on genotype and phenotype for debrisoquine hydroxylase for some of the Chinese were also published previously [3–6].

Information on ethnic origin and health was obtained by interview. No alcohol or medication except oral contraceptives was allowed during the study. All subjects were

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unrelated volunteers. The studies were approved by the Ethics Committee at the Huddinge Hospital.

Protocol

All subjects received a single oral dose of 25 mg codeine phosphate (Kodein[®], ACO) at bedtime after emptying the bladder. Urine was then collected for the following 8 h. The urine volume and pH were measured and an aliquot was stored frozen at -20°C until analysed.

The Caucasian subjects were given a single oral dose of 10 mg debrisoquine hemisulphate (Declinax; Hoofman-La Roche Inc., Basel, Switzerland) on an earlier occasion and urine samples were collected for the following 6 h.

Drug analysis

Codeine and its seven metabolites namely morphine (M), M-3- and M-6-glucuronide (M3G, M6G), normorphine (NM), norcodeine (NC), NC-glucuronide (NCG) and codeine-6-glucuronide (C6G) in urine were measured using a published h.p.l.c. method [12]. The minimum detectable concentrations in urine were 30 nmol l^{-1} for M3G, 50 nmol l^{-1} for C6G, 200 nmol l^{-1} for NC and codeine, 4 nmol l^{-1} for M6G, 10 nmol l^{-1} for NM and 5 nmol l^{-1} for M. The day-to-day coefficients of variation were 5.0% for M3G, 2.0% for C6G, 2.1% for NC, 4.7% for codeine, 10.5% for M6G, 9.3% for NM and 5.6% for M. The metabolic pathways of codeine are shown in Figure 1.

Debrisoquine and 4-OH-debrisoquine concentrations in urine were determined by the method of Lennard *et al.* [13]. The minimum detectable concentrations of debrisoquine and 4-OH-debrisoquine were about 100 nmol l^{-1} . The day-to-day coefficients of variation were $<10\%$ for debrisoquine and $<5\%$ for 4-OH-debrisoquine at concentrations $1\text{--}80\text{ }\mu\text{mol l}^{-1}$.

Admixture analysis [14], was used to evaluate the O-demethylation of codeine in both ethnic groups and also for describing the distribution of debrisoquine MR values in the Caucasians. The parameters estimated are the common variance, the overall mean, the square root of the proportion for the distribution with the higher mean (q), the distance between the two extreme distributions (t), and the degree of displacement of the middle distribution (d). In genetic terms, q is the gene frequency, t is the displacement between the homozygous means, and d is the degree of dominance. P is the skewness parameter to be estimated. Student's t -test

was used to compare log MR values for *N*-demethylation and glucuronidation between Caucasians and Chinese subjects.

Spearman rank correlation was used to assess relationships between the various MRs for codeine O-demethylation.

Frequency histograms were constructed for the log MRs of codeine. NTV plots [9] were also constructed. Both of the graphical methods were used to compare Caucasian and Chinese data.

Results

Table 1 shows the urinary recovery of codeine and its seven metabolites in the Caucasian and Chinese subjects.

O-demethylation of codeine

The MR for codeine O-demethylation (codeine/the sum of the O-demethylated metabolites M, M3G, M6G and NM) in the 222 Chinese ranged between 0.35–51 with a median of 1.8, and in the 132 Caucasians ranged between 0.38–60 with a median of 1.3. As shown in Figure 2a the frequency distribution of the MR in Chinese was shifted to the right compared with that in the Caucasians.

The frequency distribution of the MR for O-demethylation in Caucasians appeared to be bimodal on visual inspection of the plots (Figure 2a). Two separate groups were apparent, one with a MR between 0.4–5.5 ($n=114$; 86.4%) and the other between 8.3–55 ($n=18$; 13.6%), suggesting an antimode between 5.5 and 8.3. The NTV plot showed a steep descent into the negative regions at a value of 0.86 on the abscissa which is consistent with the presence of two separate groups representing 14% and 86% of the population (Figure 3a).

The histogram of the distribution of the log MR for O-demethylation in Chinese indicated only one subject separated from a main group (Figure 2a). The NTV plot showed no steep negative descent (Figure 3a).

The results of the admixture analysis are presented in Tables 2–4. For codeine O-demethylation in Caucasians, P (the skewness factor) was not significantly different from 1 ($\chi^2=328.16\text{--}327.66=0.5$; $P>0.05$) and the data were best described by a mixture of two normal distribution. The parameter estimates for these distributions (Table 2) were similar to those obtained for debrisoquine hydroxylation in

Figure 1 Metabolic pathways of codeine. M-3-G = morphine-3-glucuronide, M-6-G = morphine-6-glucuronide, NM = normorphine, NCG = norcodeine glucuronide.

Table 1 0–8 h urinary recoveries of codeine and its seven metabolites in 132 Caucasians and 222 Chinese healthy volunteers (% of dose, mean $\pm$ s.d.).

	Caucasians	Chinese
Codeine	4.7 ± 2.1	6.7 ± 2.8
C-6-G	62 ± 12	43 ± 11
NC	2.3 ± 1.1	2.4 ± 1.3
NCG	2.5 ± 0.8	1.4 ± 0.7
M3G	2.3 ± 1.6	2.4 ± 1.5
M6G	0.40 ± 0.26	0.68 ± 0.68
NM	0.70 ± 0.43	0.91 ± 0.57
Total	75 ± 13	58 ± 14

Figure 2 Frequency distribution of the log 0-8 h urine MRs for codeine O-demethylation (codeine/M + M3G + M6G + NM) (a), N-demethylation (codeine/NC + NCG + NM) (b) and glucuronidation (codeine/C6G) (c) in 132 Caucasians and 222 Chinese subjects.

Figure 3 NTV plots of the log 0-8 h urine MRs for codeine O-demethylation (a), N-demethylation (b) and glucuronidation (c) in 132 Caucasians and 222 Chinese subjects.

Table 2 Admixture analysis of the Caucasian data for the codeine/the sum of O-demethylated metabolites urine ratio used as an index of codeine O-demethylation ($n=132$).

	p	q	t	d	$-2 \ln L + C$
One normal distribution	(1)	(0)	—	—	373.6
One skewed distribution	-2.30	(0)	—	—	335.66
Two normal distributions	(1)	0.378	2.31	(0)	328.16
Two skewed distributions	0.43	0.391	2.10	(0)	327.66

p =skewness parameter; q =gene frequency; t =the distance between the two extreme distributions; d =degree of dominance. In the expression $-2 \ln L + C$, L is likelihood and C is a constant.

the same individuals (Table 3), particularly the gene frequency (q) which were almost identical (0.378 for codeine O-demethylation in Table 2 and 0.377 for

debrisoquine hydroxylation in Table 3) and which correspond to an incidence of PM phenotype of about 14% (0.378×0.378).

Table 3 Admixture analysis of the Caucasian data for the debrisoquine MR ($n=132$).

	p	q	t	d	$-2 \ln L + C$
One normal distribution	(1)	(0)	—	—	373.60
One skewed distribution	-2.83	(0)	—	—	321.22
Two normal distributions	(1)	0.377	2.45	(0)	299.80
Two skewed distributions	0.49	0.386	2.25	(0)	299.24
Three normal distributions	(1)	0.364	2.72	0.17	299.38
Three skewed distributions	0.38	0.362	2.59	0.23	298.33

Table 4 Admixture analysis of the Chinese data for the urine codeine/the sum of *O*-demethylated metabolites ratio used as an index for codeine *O*-demethylation ($n=222$).

	p	q	t	d	$-2 \ln L + C$
One normal distribution	(1)	(0)	—	—	629.01
One skewed distribution	0.00005	(0)	—	—	620.14
Two normal distributions	(1)	0.070	4.04	(0)	622.97
Two skewed distributions	0.23	0.065	3.04	(0)	619.84
Three normal distributions	(1)	0.112	4.44	0.33	619.18
Three skewed distributions	1.82	0.108	5.69	0.31	616.93

The Chinese data for codeine *O*-demethylation (Table 4) were better fitted by one skewed than by one normal distribution. A mixture of two normal distributions did not show a better fit than one skewed distribution.

In the 132 Caucasian subjects, the Spearman-rank correlation coefficient between the MR of debrisoquine and that for codeine *O*-demethylation (codeine/(M3G + M6G + M + NM)) was 0.77 ($P < 0.001$; Table 3). An equally strong correlation was also obtained when the simplified MR for *O*-demethylation (codeine/M3G) was used ($r_s = 0.77$, $P < 0.001$; Table 5).

The ratio of the sum of the *O*-demethylated metabolites divided by the sum of the metabolites of all three pathways showed a slightly better correlation with the debrisoquine MR ($r_s = 0.82$; $P < 0.001$; Table 5).

N-demethylation

The distribution of the MR for codeine *N*-demethylation (codeine/NC + NCG + NM) was normal as shown in Figure 2b, with a skewness of 0.085 in Chinese and of -0.057 in the Caucasians. The frequency distribution was shifted to the right in Chinese compared with that in Caucasians, with the former group having a significantly high ($P < 0.001$) median MR.

The NTV plots showed flat curves for data from both

ethnic groups without substantial negative values, suggesting a normal distribution in both groups (Figure 3b).

Glucuronidation

A unimodal distribution was apparent for the MR for codeine glucuronidation (codeine/C6G) (Figure 2c). The skewness was about 0.2 in both ethnic groups. The mean MR was significantly higher in the Chinese ($P < 0.001$) than in the Caucasian population.

Discussion

Admixture analysis has been used previously to analyse debrisoquine and desipramine metabolic ratios in Caucasian and Chinese populations [15, 16]. We now show that an apparent bimodality exists in the distribution of the MRs for codeine *O*-demethylation in Caucasians. Analysis of debrisoquine data in the same individuals indicated an almost identical gene frequency (q -value) giving an incidence of PM of 14%. Thus, there is no dissociation between debrisoquine hydroxylation and codeine *O*-demethylation in Caucasians. Since codeine fulfills the criteria for a probe drug for polymorphic debrisoquine-type oxidation, namely wide availability and lack of toxicity at a single low oral dose (25 mg), it may prove to be a suitable index of the

Table 5 Spearman-rank correlation coefficients between log MR for debrisoquine hydroxylation and various log MRs for codeine *O*-demethylation in 132 Caucasian subjects.

	Codeine/ (M3G + M6G + M + NM)	Codeine/ M3G	(M3G + M6G + M + NM)/ all metabolites	M3G/ all metabolites
Debrisoquine MR	0.77	0.77	0.82	0.83
Codeine/(M3G + M6G + M + NM)		1.00	0.83	0.84
Codeine/M3G			0.85	0.87
(M3G + M6G + M + NM)/all metabolites				0.99

activity of CYP2D6. The PM prevalence of 14% in this study was higher than that (7.4%) reported in European Caucasians calculated from pooled data [17]. This may be explained by a non-random selection of the Caucasian subjects in the present study and that some of the individuals included were known PMs of debrisoquine [1].

No clear bimodality was found in the distribution of the codeine MR for O-demethylation in Chinese in the present study. Only one subject (MR = 51) was separated from the rest of the Chinese population. A low prevalence of PM (1% or less) in Chinese or Japanese has been reported previously using other test drugs such as debrisoquine [15] and metoprolol [18]. Although the antimodes used in the present study were established originally in Caucasian populations, their value is not necessarily the same in other ethnic groups or for other probe drugs. In the present study, the apparent antimode for the O-demethylation of codeine MR in the Caucasian population was between 5.5 and 8.3. However, this is clearly not the antimode in the Chinese because of the shift to the right in the frequency distribution of the MR. The Chinese subjects with MR up to 14 still belong to the extensive metaboliser group. The reason for the increased MR for O-demethylation is a decreased formation of metabolites through this pathway. The basis of this metabolic deficiency in Chinese is a large RFLP XbaI mutation of *CYP2D6* [3, 6] or a *CYP2D6Ch* mutation [5]. While the *CYP2D6Ch* mutation is rare in Caucasians, its frequency is about 50% in Chinese [5]. It has also been shown that the low prevalence of PM in Chinese is due to the absence of the two most commonly occurring mutations causing the PM phenotype in Caucasians [3]. In addition, renal clearance, plasma drug binding and hepatic blood flow may all confound the interpretation of metabolic ratio data. Thus, ethnic differences in any of these variables may obscure ethnic differences in oxidative polymorphism [11, 19].

We have used two graphical methods to detect deviations from normality in frequency distributions of indices of the metabolism of codeine. Both histogram and NTV plots suggested a bimodal distribution of the MR for O-demethylation in the Caucasian population. Plotting the former on a log scale and allocating the data to 40 separate cells indicated an antimode between 5.5 and 8.3, giving a PM group representing 14% of the population. This percentage is the same as that indicated by the NTV plot. These results suggest that the histogram is a simple and clear graphical method for displaying the polymorphism in codeine O-demethylation.

The frequency distribution of this MR in Chinese showed both a lower incidence of PM and a higher median ratio. No ethnic differences were revealed clearly by the NTV plot.

The MR used for codeine O-demethylation was the urine recovery of codeine divided by the sum of the O-demethylated metabolites (M3G + M6G + M + NM). M3G, which accounts for 3% of the dose recovered in an 8 h urine, and codeine were measured by h.p.l.c. with u.v.-detection. However, detection of the other three metabolites required an electro-chemical detector [4, 12]. Accordingly, the analytically easier to determine codeine/M3G ratio was assessed as an alternative index of codeine O-demethylation. The codeine/M3G and codeine (M3G + M6G + M + NM)

ratios showed similar correlations with the debrisoquine MR. Using the codeine/M3G ratio simplifies phenotyping with codeine, since only one metabolite needs to be measured and only a u.v. detector is required.

A new ratio between the sum of the O-demethylated metabolites and the sum of all metabolites produced by O- and N-demethylation and glucuronidation is proposed, as it removes the influence of codeine excretion which is urine pH dependent [4, 20]. Another reason for using this ratio is to take the non-polymorphic pathways into account, since a previous study has demonstrated an increase in the metabolite formation through these pathways in PMs [1]. Although the pharmacokinetic basis for using this combined metabolite ratio has not been examined, its validity is suggested by the significant correlation with the debrisoquine MR.

In conclusion, a histogram seems preferable over a NTV plot for assigning phenotype using the codeine O-demethylation MR because of its clarity and simplicity of use. Interethnic differences in the metabolism of codeine are also better visualised from histograms. The urinary codeine/M3G ratio is as good an index of codeine O-demethylation as the codeine/M + M3G + M6G + NM ratio, allowing a simplification of the analytical method for phenotyping using codeine as the test drug.

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