Physiologically based modelling of inhibition of metabolism and assessment of the relative potency of drug and metabolite: dextromethorphan vs. dextrorphan using quinidine inhibition

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Aims To define the relative antitussive effect of dextromethorphan (DEX) and its primary metabolite dextrorphan (DOR) after administration of DEX.

Methods Data were analysed from a double-blind, randomized cross-over study in which 22 subjects received the following oral treatments: (i) placebo; (ii) 30 mg DEX hydro-bromide; (iii) 60 mg DEX hydro-bromide; and (iv) 30 mg DEX hydro-bromide preceded at 1 h by quinidine HCl (50 mg). Cough was elicited using citric acid challenge. Pharmacokinetic data from all non-placebo arms of the study were fitted simultaneously. The parameters were then used as covariates in a link PK–PD model of cough suppression using data from all treatment arms.

Results The best-fit PK model assumed two- and one-compartment PK models for DEX and DOR, respectively, and competitive inhibition of DEX metabolism by quinidine. The intrinsic clearance of DEX estimated from the model ranged from 59 to 1536 l h⁻¹, which overlapped with that extrapolated from *in vitro* data (12–261 l h⁻¹) and showed similar variation (26- vs. 21-fold, respectively). The inhibitory effect of quinidine ([I]/K_i) was 19 (95% confidence interval of mean: 18–20) with an estimated average K_i of 0.017 µM. Although DEX and DOR were both active, the potency of the antitussive effect of DOR was 38% that of DEX. A sustained antitussive effect was related to slow removal of DEX/DOR from the effect site (k_{eo} = 0.07 h⁻¹).

Conclusions Physiologically based PK modelling with perturbation of metabolism using an inhibitor allowed evaluation of the antitussive potency of DOR without the need for separate administration of DOR.

Keywords: active metabolite, CYP2D6, population pharmacokinetics–pharmacodynamics

Introduction

Assessing the contribution of drug and its metabolite(s) to the overall pharmacological effect in circumstances where the parent drug and its metabolite(s) are both active can be difficult. However, a number of experimental approaches are possible involving deliberate or natural perturbation of the drug/metabolite ratio. These include administration of the metabolite alone [1], inhibition of

the metabolic pathway that is responsible for the formation of the metabolite [2, 3], and investigation of pharmacological effect in subpopulations who are polymorphic with respect to the biotransformation pathway that produces the active metabolite [4, 5]. PK–PD modelling can be used as a complementary step in all of the above methods [6–8], where inter- or intra-individual differences in plasma concentration–time profiles between the parent drug and its metabolite are used to quantify the effect of each chemical moiety. We have recently evaluated CYP2D6 activity in relation to the antitussive effect of dextromethorphan (DEX) [3]. Inhibition of DEX metabolism by quinidine resulted in contrasting ratios of dextromethorphan/dextrorphan (DEX/DOR) in different arms of this study. Administration of 30 mg DEX with quinidine pretreatment produced a similar antitussive effect to that of 60 mg DEX, implying

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a greater effect of DEX. However, without the application of a modelling approach, the results were not conclusive regarding the concentration–effect relationship and the relative activity of DEX and DOR [3].

DEX, a codeine analogue devoid of opiate side-effects, is widely available over the counter as a cough suppressant. Its effectiveness has been confirmed in both clinical [3, 9–13] and experimental cough challenge studies [14, 15]. The metabolism of DEX is largely by CYP2D6-mediated O-demethylation to DOR. DOR itself is mainly metabolized by glucuronidation [16] and partly by oxidation to 3-hydroxy-morphinan mainly by CYP3A4 with some contributions from other cytochrome P450 enzymes [17, 18].

Suggestions that DOR has antitussive activity date from studies in 1953 using unanaesthetized dogs [19]. More recent animal studies with guinea pigs have also indicated that the antitussive [20] and antiepileptic [21] activities of DEX might be related to its metabolite DOR. Although studies in humans have attributed the abuse liability of DEX to its metabolite DOR [22] and consider this compound to be responsible for neuromodulatory [23], antinociceptive [23] and anticonvulsive [24] effects, there has been no systematic assessment of the relative potency of DEX and DOR in humans. It has been implied that a lack of CYP2D6 activity or a bypass of hepatic first-pass metabolism using appropriate dosage forms may, therefore, have implications for therapeutic effects [25]. Some investigators have advocated the administration of DOR on its own as an antitussive instead of DEX [26].

Genetic polymorphism of the CYP2D6 enzyme can be an important component of variability in response to drug therapy [27–29], although controlled prospective studies to evaluate its clinical significance and pharmacoeconomic impact are few [30]. Since the overall disposition of DEX is highly dependent upon CYP2D6 activity [4], selective inhibition by a small dose of quinidine [31–33] can be used to perturb the DEX/DOR ratio [3, 34]. Different DEX/DOR ratios are also expected when different routes and formulations associated with varying degrees of first-pass metabolism are used. The objective of the present study was to use PK–PD modelling to assess the relative antitussive potency of DEX and DOR without separate administration of DOR.

Methods

Subjects

The data that form the basis of this investigation were collected as part of an investigation of the antitussive effect of DEX. Details of the study design, subjects, drugs and cough challenge test were described previously [3].

In summary, this was a double-blind, randomized, cross-over study in which 22 subjects (12 male, 10 female, mean age 24 years) received the following oral treatments: (i) placebo; (ii) 30 mg DEX hydro-bromide; (iii) 60 mg DEX hydro-bromide; and (iv) 30 mg DEX hydro-bromide preceded at 1 h by quinidine HCl (50 mg). Blood samples were taken for assay of plasma DEX and DOR (conjugated and unconjugated) by the method of Chen *et al.* [35]. Intra-assay coefficients of variation for the analyses at 2.5 ng ml^{-1} were between 6% and 13%. Cough was produced by five inhalations of 3 ml 10% w/v citric acid (placed in a nebulizing chamber) over 5 min. The frequency of cough response was measured at baseline (prior to quinidine or placebo, $t = -1 \text{ h}$) and at regular intervals up to 12 h. All volunteers gave written informed consent to the investigation, which was approved by the South Sheffield Research and Ethics Committee.

PK–PD modelling

Model selection was based on the lowest value of the Akaike Information Criteria (AIC) [36] after visual inspection of residuals for systematic error. Different weighting schemes were explored ($1/y^2$ for PK and uniform weighting for PD were chosen in the final models). The P-Pharm software package (version 1.5; InnaPhase, Champs sur, France) was used both for PK and PD analyses.

Selection of PK models

Various models were examined and fit to the PK data of DEX (see Table 1). These models were based on one- or two-compartment kinetics where the elimination process was defined by metabolic and nonmetabolic routes. The metabolic route also contributed to the formation of metabolite during hepatic first pass [37], and a well-stirred liver was assumed (see Figure 1 and Appendix). The inhibitory effect of quinidine was considered to be competitive in nature and inversely related to the inhibition constant (K_i) and directly proportional to the time-averaged unbound concentration of quinidine in plasma ($[I]$). This approach reduced the complexity of possible time variant change in the clearance of DEX and it was justified on the basis that the impact of the most variable region of the quinidine concentration–time profile, i.e. the absorption phase, was avoided by taking quinidine 1 h before the DEX dose. Thus, the intrinsic clearance of DEX was decreased by $(1 + [I]/K_i)$ -fold in the presence of quinidine and the $[I]/K_i$ ratio, as a single model parameter, represented the potency of quinidine inhibition in each individual. The calculated $[I]/K_i$ ratio was used to estimate the *in vivo* K_i knowing the average

Table 1 The results of pharmacokinetic model building and selection*.

Chemical moiety	Model	Number of model parameters	Model description	-LL	AIC†
DEX	PK (1)	4	1st order absorption with monophasic disposition§	1011	2.654
	PK (2)	7	As above with addition of a peripheral distribution compartment	957	2.514
	PK (3)	8	As above with variable absorption between quinidine arm and DEX	937	2.468
	PK (4)	9	As above with variable absorption between DEX 30 mg and DEX 60 mg	922	2.436
	PK (5)	10	As above with variable clearance between DEX 30 mg and DEX 60 mg	913	2.416
DOR	PK (6)	11	As above with variable non-metabolic clearance between study arms	890	2.363
	PK (7)	13	As above with variable t_{lag} between DEX30 and DEX60 study arms	865	2.309
	PK (8)	2	Link to PK (7) with monoexponential disposition of DOR	805	2.133
	PK (9)	3	As above with variable t_{lag} for DOR	738	1.964

*For more details see Appendix. †The best fit model (lowest AIC) is shown in *italics*. §Elimination was via both metabolic and non-metabolic routes; metabolic clearance was defined by a well-stirred liver model. CL_{int} (intrinsic clearance) was allowed to vary for different dose levels; effects of quinidine on DEX clearance were modelled assuming a competitive inhibition of CL_{int} .

Figure 1 The best-fit PK-PD link model for DEX and DOR.

1–13-h post-dose plasma concentration of quinidine from *in vivo* studies [38] and its unbound fraction in plasma [39].

Thus, PK parameters of DEX were determined from the simultaneous fitting of concentration–time profiles in all three non-placebo arms of the study (see Appendix for equations). These parameters were then used as individual constants for the analysis of DOR data (a two-stage link model for DOR and DEX).

In addition to the AIC, the χ^2 test for significance of improvement in log-likelihood (-LL) was applied to the outcomes. The critical values for declaring significant improvement of likelihood were based on $\alpha=0.05$. Thus, for example, decreases in -LL of 3.8, 6.0 and 7.8 were required to accept significant improvements following addition of 1, 2 and 3 model parameters, respectively.

Two-way ANOVA (subjects, treatments) with Tukey's *post hoc* test was used to compare Bayesian estimates of individual PK parameters in the different arms of the study.

Selection of PD models

Initially, we assumed no effects of DEX or DOR on cough response and applied a placebo effect model, as described by Rostami-Hodjegan *et al.* [40], to data from all four arms of the study. In brief, the model consisted of a first-order rate constant for nonlinear suppression of cough response, which also determined the first-order rate of return to baseline cough level together with a scale factor to determine the size of the placebo effect (Rostami-Hodjegan *et al.* [40] and Appendix). Once model parameters for placebo effect were estimated in each arm of study, any parameter values that were significantly different between the arms were noted. Since this preliminary analysis indicated a significant difference between the study arms and rejected the hypothesis of a random placebo antitussive effect, a dose-related effect of DEX could be implied. Therefore, formal PK-PD models were applied assuming a relationship between the concentrations of DEX and/or DOR and antitussive effect.

Individual Bayesian PK parameter values obtained during the PK analysis served as covariates for the PD analysis (i.e. a two-stage PK-PD link model was used). The link between plasma concentration or concentration at a remote effect compartment and antitussive effect was defined by E_{max} and sigmoidal E_{max} models with competitive interactions between DEX and DOR. The PK-PD model, assuming an effect compartment, is shown in Figure 1. The effect of placebo [40] was nested in all PD models. Interstudy variation of placebo model parameter values was allowed in some submodels (see Appendix). The relative success of different PK-PD models in explaining the antitussive effect, based on DEX alone, DEX plus DOR and DOR alone, was examined using the AIC as well as the *F*-test for significance of improvement in log-likelihood.

Table 2 Mean pharmacokinetic parameter values of dextromethorphan (DEX) and dextrorphan (DOR) according to the best fit models 9 and 7 (Table 1)*.

Treatment groups	Total clearance ($l\ h^{-1}$)	Intrinsic metabolic clearance ($l\ h^{-1}$)	Non-metabolic clearance ($l\ h^{-1}$)	V (l)	k_s (h^{-1})	t_{ag} (h)	F_H (%)	$[I]/[K_i]$	$t_{1/2}[I]$ (h)	$t_{1/2}[2]$ (h)	$k(DOR)$ (h^{-1})	$[V(DOR)]/F(DOR)]$ (l)
DEX (30 mg)	75 (13) [39–85]	523 (257) [59–910]	3 (0)	961 (167) [585–1292] As above	2.6 (0.9) [0.8–3.9]	0.8 (0.1) [0.5–1.2]	20 (15) [9–60]	NA	3.1 (0.3) [2.3–3.7]	17 (4) [10–24]	0.51 (0.21) [0.21–1.07]	3776 (1382) [1222–6441] As above
DEX (60 mg)	78 ^a (12) [43–88]	788 ^a (528) [73–1536]	3 (0)	As above	2.0 ^a (0.9) [0.5–3.4]	0.6 ^{ab} (0.1) [0.5–1.0]	16 ^c (14) [6–55]	NA	3.1 (0.3) [2.2–3.8]	16 (4) [9–26]	As above	As above
DEX (30 mg) + Quinidine (50 mg)	20 ^c (9) [3–35]	27 ^c (14) [3–56]	3 (0)	As above	1.1 ^c (0.7) [0.3–2.4]	0.7 (0.3) [0.3–1.7]	78 ^c (10) [62–97]	19 (2) [13–23]	3.6 ^c (0.5) [2.7–5.2]	58 ^c (38) [24–145]	0.34 ^c (0.14) [0.11–0.63]	As above

*The numbers in parentheses indicate SD and those in square brackets are the range for each parameter. For a full description of parameters see Appendix. NA, Not applicable. ^aSignificantly different from 30-mg arm ($P < 0.02$); ^bsignificantly different from quinidine arm ($P < 0.05$); ^csignificantly different from corresponding parameter value in absence of quinidine ($P < 0.001$).

Results

PK modelling

The results of fitting different models are shown in Table 1. Parameter values for the final PK models (models 7 and 9, Table 1) are listed in Table 2. Individual Bayesian and population fits to plasma concentration–time profiles of DEX and DOR are shown in Figure 2a and b, respectively. A significant ($P < 0.001$) decrease in the clearance of DEX was observed in the quinidine study arm compared with the DEX arms (Table 2). Other PK parameters of DEX which were influenced by quinidine included the absorption rate constant (slower absorption; $P < 0.01$), the fraction escaping first-pass metabolism (higher F_H ; $P < 0.001$) and the elimination half-life of DEX (longer half-life; $P < 0.001$). Furthermore, quinidine had a significant effect on the elimination rate constant of DOR [$k(DOR)$; $P < 0.001$] and decreased its apparent volume of distribution [$V(DOR)/F(DOR)$].

Correlations between the observed and individual Bayesian predicted plasma concentrations of DEX are shown in Figure 3a ($P < 0.001$); corresponding correlations for DOR are shown in Figure 3b ($P < 0.01$).

The intrinsic clearance of DEX estimated from the model ranged from 59 to 1536 $l\ h^{-1}$. Assuming an average plasma quinidine concentration of 2.2 μM between 1 and 13 h after quinidine dose [38] (corresponding to 0–12 h post DEX dose) and $86 \pm 6\%$ protein binding [39], the *in vivo* K_i of quinidine was estimated to be 0.017 (± 0.002 SD) μM based on unbound drug.

PD modelling

The results of PD model building are shown in Table 3. A placebo model with varying parameter values in each arm of the study indicated that the intensity (as defined by 'scale', see Appendix) and the pattern of response (as defined by lag time, $t_{lag}(\text{Placebo})$ and the rate constant for appearance/disappearance of effect (k_{cough} , see Appendix), but not the baseline cough values, were different between treatment and placebo arms (data not shown). However, these differences were not random. The change in intensity, as defined by a 'scale' value of 1.37–2.00-fold observed with actual placebo, was related to the change in dose of DEX, although there was no difference in intensity of effect between the 60-mg study arm and that for 30 mg preceded by quinidine. In general, the kinetics of effect (as indicated by the rate constant of appearance and disappearance of effect, k_{cough} , see Appendix) were slower (0.69–0.81-fold) than after actual placebo. The values of k_{cough} were similar for the 30-mg and 60-mg DEX arms but differed for 30 mg DEX preceded by quinidine. Following rejection of a random placebo

Figure 2 Bayesian (thin lines) and population (thick line) predicted and observed (symbols) plasma DEX (a) and DOR (b) concentrations. Note the different scales of concentrations for DEX and DOR; also note that many individuals had only one to two measurable DOR plasma concentrations when they were pretreated with quinidine (i.e. QDEX30 study arm).

effect, a mechanistic concentration-related effect model for DEX was explored (Table 3).

The best-fit mechanistic PD model assumed a sigmoidal $E_{\max}$ function, with DEX and DOR both being active but with DOR having only 38% of the antitussive potency of DEX, and a 10-h equilibration half-life for the effect (Table 4). The individual Hill coefficient for antitussive effect varied from 0.2 to 36.

Administration of DEX 60 mg and DEX 30 mg preceded by quinidine produced maximum responses of 50% cough suppression compared with 25% after placebo. Predictions of response from the best-fit model correlated significantly with observed cough data ($P < 0.001$; Figure 4), and the final model performed better than a nonmechanistic variable placebo effect model (Table 3).

Discussion

The plasma drug concentration–time data obtained from the three different arms of our previous study, one with quinidine pretreatment, were fitted successfully using a physiologically based PK model. Incorporating these data into a link PK–PD model, we were then able to assess the contributions of DEX and DOR to the overall antitussive effect following DEX administration. This was

possible since the metabolic pathway responsible for formation of the metabolite of interest was selectively inhibited, producing variable relative levels of DEX and DOR in different arms of the study. A physiologically based representation of hepatic clearance for model fitting purposes has been proposed by Piotrovskij *et al.* [41]. Using this approach, DEX intrinsic clearance was defined as a primary model parameter and the effect of quinidine inhibition on first-pass metabolism and on subsequent passes was considered simultaneously. To test the reliability of this approach we compared our estimated values of *in vivo* intrinsic DEX clearance and the K_i of quinidine with those reported from *in vitro* studies. These comparisons showed good consistency in both cases. The estimated metabolic intrinsic clearance of DEX ($59\text{--}1536\text{ l h}^{-1}$) overlapped with that extrapolated from *in vitro* data in CYP2D6 extensive metaboliser subjects ($12\text{--}261\text{ l h}^{-1}$; unpublished data) and showed similar variation (26- vs. 21-fold, respectively). A possible under-prediction of DOR concentrations at higher values (see Figure 3b) could be related to subjects with high CYP2D6 activity presenting with DEX concentrations below the level of quantification in many samples, leading to misspecification of individual PK parameters. However, these concentrations represented a negligible fraction of the data,

Figure 3 Population-derived individual Bayesian predictions *vs.* observed plasma concentrations of DEX (a) and DOR (b) according to the best-fit PK models applied to combined data from all study arms. The diagonal line represents a perfect match of the values.

and considering the ‘simultaneous’ nature of the fit to three study arms, the results were acceptable.

The estimated value of the *in vivo* K_i of quinidine (0.017 μM) was also consistent with *in vitro* values (0.015–0.04 μM) reported using yeast and human liver microsomes [42–45]. Another outcome of the physiologic PK modelling was that the fraction of first-pass metabolism of DEX could be estimated without having intravenous data. Thus, following 30 mg DEX 82% of the circulating DOR was estimated to originate from first-pass metabolism. The corresponding value following 60 mg DEX administration was 67%. A faster initial decline of the metabolite compared with parent drug, as seen in this study, also confirms the major contribution

of first-pass metabolism to the formation of circulating DOR.

There was no suggestion of a dose-dependent change in the clearance of DEX as the values were similar in the DEX30 and DEX60 study arms. However, quinidine decreased the clearance of DEX by 75% and prolonged its half-life to 58 h (from 17 and 16 h in the DEX30 and DEX60 arms, respectively). Dose–response, but not concentration–response, relationships for the inhibitory effect of quinidine on CYP2D6 are well documented [31]. Our data could not add to this information as only one dose level of inhibitor and substrate had been used and plasma quinidine concentrations were not measured. The inhibitory effect of quinidine observed in the DEX30 study arm is expected to be similar for other doses of DEX. Theoretically, only K_m is affected by competitive inhibition. Therefore, as long as the DEX concentration is below its K_m (as evident from dose linearity), the proportional change in intrinsic clearance will be constant regardless of DEX concentration.

The increased absorption rate constant of the DEX when subjects were pretreated with quinidine ($P < 0.001$) might be due to an effect on stomach emptying. This possibility is also evident in the data reported by Capon *et al.* [34], in that the absorption rate constant of DEX (as estimated from the change in t_{max}) was 25% faster in the quinidine arm of their study. A greater effect in our study (63%) could reflect the differences in the dosage forms of DEX used (syrup *vs.* tablets). Thus, acceleration of stomach emptying is expected to affect liquid formulations more than solid dosage forms.

The elimination of DOR is determined by renal function and non-CYP2D6-mediated metabolism. The latter is largely by glucuronidation [46] with some conversion to 3-hydroxymorphinan, mainly by CYP3A4 [17, 18]. As quinidine preferentially inhibits CYP2D6, no effect on the elimination of DOR would be expected. However, a prolonged elimination of DOR was observed which could be related to a haemodynamic effect of quinidine in decreasing blood flow to the kidney and liver [47, 48].

By applying PK–PD modelling to a set of data where the metabolism of DEX was inhibited, a prolonged concentration-dependent antitussive effect could be explained by a greater potency for DEX than DOR. The equilibration half-life of 10 h for DEX (and even longer for DOR) seems much longer than can be attributed to simple elimination of the active drug from the effect site. Such a prolonged effect might be related to a mechanism of action involving endogenous mediators. There are two implications of the lower antitussive potency of DOR. Firstly, individuals with high CYP2D6 activity (upper bound of extensive metabolizers or ultra-rapid metabolizers) may show less antitussive effect following DEX

Table 3 The results of pharmacodynamic model building and selection*.

Active moiety	Model	Number of model parameters	Model description	-LL	AIC†
Placebo	PD (1)	6	Only placebo effect, with variable scale and shape	1294	2.198
	PD (2)	9	As above with variable baseline	1245	2.126
	PD (3)	12	As above with variable t_{lag}	1240	2.128
DEX	PD (4)	3	E_{max} with effect compartment	1235	2.351
	PD (5)	4	Sigmoidal E_{max}	1233	2.350
	PD (6)	7	As above with variable baseline placebo effect§	1247	2.344
	PD (7)	10	As above with variable extent of placebo effect§	1218	2.344
	PD (8)	13	As above with variable t_{lag} of placebo effect§	1208	2.337
DOR	PD (9)	4	As (5)	1397	2.365
	PD (10)	7	As (6)	1268	2.158
	PD (11)	10	As (7)	1315	2.247
	PD (12)	13	As (8)	1250	2.151
DEX/DOR	PD (13)	4	As (4) with competitive effect between DEX and DOR	1332	2.263
	PD (14)	5	As (5) with competitive effect between DEX and DOR	1315	2.230
	PD (15)	6	As above with variable k_{e0} for DEX and DOR	1318	2.238
	PD (16)	12	As above with variable baseline and effect of placebo§	1214	2.066

*For more details see Appendix. †The best-fit model with the lowest AIC value is indicated in *italics*. ‡P-value for the statistical significance of improvement in -LL compared with the previous model on the list. §These variable effects refer to inter-occasion (IOV) (intra-individual) differences in placebo parameter values between different study arms.

Table 4 Population pharmacodynamic parameter estimates from the best fit model*.

Parameter	Value	CV(%)
E_{max} (%)	37.7	49
EC_{50} (ng ml ⁻¹)	3.2	20
k_{e0} (DOR) (h ⁻¹)	0.07	56
$k_{e0}(\text{DOR})/k_{e0}(\text{DEX})$	0.17	28
Pot_{DOR} (potency DOR relative to DEX) (%)	38	69
n	3.9	195

*The best-fit PD model (PD (16)) was described by equation 12 in the Appendix and inter-occasional variability (IOV) associated with the placebo effects were 6–10% and 54–63% for baseline cough and magnitude of placebo effect, respectively. E_{max} , maximum cough reduction (%) from placebo adjusted baseline; EC_{50} , concentration at the effect site associated with half E_{max} ; k_{e0} , apparent inter-compartmental transfer rate for removal of active compound from the effect compartment; Pot_{DOR} , relative potency (%) of DOR compared with DEX; n , Hill coefficient.

administration. Secondly, higher DEX/DOR ratios following administration of DEX in dosage forms associated with a lower hepatic first-pass metabolism may lead to a greater antitussive effect from these formulations.

A possible bias in over-predicting lower and under-predicting higher cough values (Figure 4) might be decreased by the application of more complex mechanistic models of effect. However, apparent mismatch between observed and predicted PD outcome is commonly observed in many PK–PD models involving a baseline effect. The literature on PK–PD modelling rarely reports correlations between observed and predicted values, and

where these have been reported systematic bias is often apparent (e.g. [49–51]). Such bias may reflect differences in the nature of PD and PK measurements, in that negative values are not possible for the latter while measured but not model-predicted responses can go below baseline (or placebo) values. Accounting for error does not resolve this major difference between PK and PD data.

Our conclusion that DOR is significantly less potent than DEX as an antitussive requires validation by a prospective study in which DOR is administered *per se*. If this is confirmed, the PD information generated by the current analysis may be used to refine the delivery and dosage of DEX for optimal therapeutic effect.

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Appendix

PK modelling Equation 1 describes plasma DEX concentrations based on a one-compartment model beyond the absorption lag (PK Model 1):

$$C(t) = [(ka \times F \times D)/V_c] \times \{ [1/(k_a - k)] \times e^{-k(t-t_{lag})} + [1/(k - k_a)] \times e^{-ka(t-t_{lag})} \} \quad (1)$$

$C(t)$, D , F , V_c , k_a , and k are plasma dextromethorphan concentration at time t , dose, bioavailability, central volume of distribution, first-order absorption rate constant and first-order elimination rate constant ($k = CL/V_c$), respectively. Bioavailability was a composite factor determined by the product of the fraction available to the

Figure 4 (a) Population (line) predicted and observed (symbols) number of coughs applied to combined data from all study arms. DEX 30 (▽); DEX 60 (△); QDEX 30 (○). (b) Population-derived individual Bayesian prediction *vs.* observed number of coughs according to the best-fit PK-PD model. The diagonal line represents a perfect match of the values.

portal vein (f_a ; assumed to be 1) and the fraction escaping hepatic first pass metabolism, F_H :

$$F = f_a + F_H = f_a \times (1 - F_H)$$

and defined with respect to intrinsic clearance (CL_{int}) and liver blood flow (Q_H) according to a well-stirred liver model:

$$E_H = CL_{int} / (CL_{int} + Q_H)$$

Overall DEX clearance, CL , is the sum of hepatic ($E_H \times Q_H$) and non-metabolic clearance, CL_{NH} :

$$CL = (E_H \times Q_H) + CL_{NH}$$

Equation 2 describes plasma DEX concentrations beyond the absorption lag time (t_{lag}) assuming a two-compartment PK model (PK Models 2–7):

$$C(t) = [(k_a \times F \times D) / V_c] \times (A1 + A2 + A3) \quad (2)$$

where $A1$, $A2$ and $A3$ are:

$$A1 = \{[k_{21} - \alpha] / [(k_a - \alpha)(\beta - \alpha)]\} \times e^{-\alpha(t-t_{lag})}$$

$$A2 = \{[k_{21} - \beta] / [(k_a - \beta)(\alpha - \beta)]\} \times e^{-\beta(t-t_{lag})}$$

$$A3 = \{[k_{21} - k_a] / [(\beta - k_a)(\alpha - k_a)]\} \times e^{-k_a(t-t_{lag})}$$

and k_{21} is the transfer rate constant from peripheral to central compartment and α and β are the hybrid rate constants associated with distribution and elimination phases, respectively.

Metabolism of DEX to DOR represents 96% of its total metabolism [52]. Therefore, assuming that DOR is the only primary metabolic product of DEX and quinidine competitively inhibits DEX metabolism, the E_H of DEX following quinidine is given by:

$$E_H = \{Clu_{int} / [1 + [I] / K_i]\} / \{Clu_{int} / (1 + [I] / K_i) + Q_H\} \quad (3)$$

where $[I] / K_i$ is the ratio of time-averaged unbound plasma quinidine concentration ($[I]$) (1–13 h after quinidine consumption) to the inhibition constant (K_i).

Equation 4 describes the plasma concentrations of DOR (PK Models 8, 9):

$$C(t)_{DOR} = C(t)_{DOR(1st\ pass)} + C(t)_{DOR(systemic)} \quad (4)$$

where $C(t)_{DOR(1st\ pass)}$ and $C(t)_{DOR(systemic)}$ are first-pass and systemic components. These components were described by equations 5 and 6, respectively:

$$C(t)_{DOR(systemic)} = \{[D \times F \times k_a \times (E_H \times Q_H / V_c)] / [V(DOR) / F(DOR)]\} \times (A1_m + A2_m + A3_m + A4_m) \quad (5)$$

Since the proportion of DOR that is lost during first-pass metabolism to secondary metabolites is unknown, $V(DOR) / F(DOR)$ represents a hybrid function of both the volume of distribution of DOR and its hepatic metabolism. $A1_m$, $A2_m$, $A3_m$ and $A4_m$ are given by:

$$A1_m = \{[k_{21} - \alpha] / [(k_a - \alpha)(\beta - \alpha)] \times (k(DOR) - \alpha)\} \times e^{-\alpha(t-t_{lag})}$$

$$A2_m = \{[k_{21} - \beta] / [(k_a - \beta)(\alpha - \beta)] \times (k(DOR) - \beta)\} \times e^{-\beta(t-t_{lag})}$$

$$A3_m = \{[k_{21} - k_a] / [(\beta - k_a) \times (\alpha - k_a)(k(DOR) - k_a)]\} \times e^{-k_a(t-t_{lag})}$$

$$A4_m = (k_{21} - k(DOR)) / \{[k_a - k(DOR)] \times [\beta - k(DOR)][\alpha - k(DOR)]\} \times e^{-k(DOR)(t-t_{lag})}$$

where $k(\text{DOR})$ is the elimination rate constant of DOR. The plasma concentration of DOR contributed by its first-pass formation is given by:

$$C(t)_{\text{DOER (1st pass)}} = ([k_a \times \text{Dose} \times E_H] / \{V(\text{DOR})/F_H(\text{DOR})\} \times [k_a - k(\text{DOR})]) \times [e^{-k(\text{DOR})(t-\text{tlag})} - e^{-k(\text{DOR})(t-\text{tlag})}] \quad (6)$$

In the absence of i.v. data for DOR, and hence lack of information on $V(\text{DOR})$ and $F(\text{DOR})$, the fraction of DEX that is converted to DOR is unknown. By perturbation of first-pass metabolism and assuming that DOR is the only metabolic product of DEX, values of E_H can be estimated. However, only a composite value of $V(\text{DOR})/F(\text{DOR})$ could be calculated not knowing the first-pass effect on DOR itself. The assessment of the identifiability of metabolite models similar to ours has been discussed by Evans *et al.* [53]. They concluded that, if the volume of distribution of a metabolite is known *a priori* then all of the model parameters are globally identifiable. Otherwise, only the relative rate of drug conversion to metabolite from first pass and systemic metabolism can be determined.

To incorporate the possibility of inter-occasional variability (IOV) (or intra-individual variability) in model parameters [54, 55], each relevant parameter from the non-DEX30 study arm was described relative to their values in DEX30 study arm by the following equation:

$$\text{Parameter value} = \text{Parameter value in DEX30 study arm} \times (1 + \% \text{IOV})$$

If the population distribution of IOV was significantly different from zero the effect was declared not to be random.

PD modelling The placebo effect was differentiated from drug effect using the dynamic effect model described by Rostami-Hodjegan *et al.* [40]. Variables included scale, baseline, appearance/disappearance rate constant (k_{cough}) and t_{lag} (placebo). Each of these parameters could have random IOV (PD Model 1–3):

$$\text{Placebo effect} = \text{Baseline} - (\text{scale} \times k_{\text{cough}}) \times [t - t_{\text{lag}}(\text{Placebo})] \times e^{-k_{\text{cough}} \times (t - t_{\text{lag}}(\text{Placebo}))} \quad (7)$$

where the first-order rate constant for nonlinear suppression of cough response and return to baseline, k_{cough} , was sex dependent and was described by the following equation [40]:

$$k_{\text{cough}}(h^{-1}) = 0.049 \times \text{Sex} (\text{Male} = 1; \text{Female} = 2) + 0.238$$

The following equation was used to calculate the concentration of DEX in a hypothetical effect compartment:

$$C_e(\text{DEX}) = (k_a F_{\text{DOR}} \cdot k_{e0} / V_c) \times (A1 + A2 + A3 + A4) \quad (8)$$

where:

$$A1 = \{(k_{21} - \alpha) / [(k_a - \alpha)(\beta - \alpha)(k_{e0} - \alpha)]\} \times e^{-\alpha(t - \text{tlag})}$$

$$A2 = \{(k_{21} - \beta) / [(k_a - \beta)(\alpha - \beta)(k_{e0} - \beta)]\} \times e^{-\beta(t - \text{tlag})}$$

$$A3 = \{(k_{21} - k_a) / [(\beta - k_a)(\alpha - k_a)(k_{e0} - k_a)]\} \times e^{-k_a(t - \text{tlag})}$$

$$A4 = \{(k_{21} - k_{e0}) / [(k_a - k_{e0})(\beta - k_{e0})(\alpha - k_{e0})]\} \times e^{-k_{e0}(t - \text{tlag})}$$

and k_{e0} is a rate constant defining removal of DEX from the effect compartment.

The following equations were used to calculate the concentration of DOR in the same hypothetical effect compartment as DEX:

$$C_e(\text{DOR})_{\text{systemic}} = \{[D \times F \times k_a \times k_{e0}(\text{DOR}) \times (E_H \times G_H / V_c)] / [V(\text{DOR})/F(\text{DOR})]\} \times (A1_m + A2_m + A3_m + A4_m + A5_m) \quad (9)$$

where $A1_m$, $A2_m$, $A3_m$ and $A4_m$ are:

$$A1_m = \{(k_{21} - \alpha) / [(k_a - \alpha)(\beta - \alpha)] \times (k(\text{DOR}) - \alpha)(k_{e0}(\text{DOR}) - \alpha)\} \times e^{-\alpha(t - \text{tlag})}$$

$$A2_m = \{(k_{21} - \beta) / [(k_a - \beta)(\alpha - \beta)(k(\text{DOR}) - \beta)(k_{e0}(\text{DOR}) - \beta)]\} \times e^{-\beta(t - \text{tlag})}$$

$$A3_m = \{(k_{21} - k_a) / [(\beta - k_a)(\alpha - k_a)(k(\text{DOR}) - k_a)(k_{e0}(\text{DOR}) - k_a)]\} \times e^{-k_a(t - \text{tlag})}$$

$$A4_m = (k_{21} - k(\text{DOR})) / \{[k_a - k(\text{DOR})] \times (\beta - k(\text{DOR}))(\alpha - k(\text{DOR})) \times (k_{e0}(\text{DOR}) - k(\text{DOR}))\} \times e^{-k(\text{DOR})(t - \text{tlag})}$$

$$A5_m = (k_{21} - k_{e0}(\text{DOR})) / \{[k_a - k_{e0}(\text{DOR})] \times (\beta - k_{e0}(\text{DOR}))(\alpha - k_{e0}(\text{DOR})) \times (k(\text{DOR}) - k_{e0}(\text{DOR}))\} \times e^{-k_{e0}(\text{DOR})(t - \text{tlag})}$$

and $k_{e0}(\text{DOR})$ is a rate constant defining removal of DOR from the effect compartment.

The contribution of the first-pass formation of DOR to its effect compartment concentrations was described by:

$$C_e(\text{DOR})_{1st \text{ pass}} = \{[k_a \times D \times k_{e0}(\text{DOR}) \times E_H] / [V(\text{DOR}) / F_H(\text{DOR})] \times (k_a - k(\text{DOR}))\} \times A \quad (10)$$

where:

$$A = \{e^{-k(\text{DOR})(t - \text{tlag})} / (k_{e0}(\text{DOR}) - k(\text{DOR})) - e^{-k(\text{DOR})(t - \text{tlag})} / (k_{e0}(\text{DOR}) - k_a)\}$$

The PK and PD data were linked by assuming E_{max} ($n = 1$) or sigmoidal E_{max} ($n \neq 1$) models such that:

$$E = \text{Placebo Effect} \times \{1 - E_{\max} \times (C_e/EC_{50})^n / [1 + (C_e/EC_{50})^n]\} \quad (11)$$

where C_e , $E_{\max}$, and EC_{50} , and n are the concentration of active moiety (DEX, PD Models 4–8 and DOR, PD Models 9–12), maximal antitussive effect, concentration of active moiety in the effect compartment that is associated with half $E_{\max}$, and the Hill-coefficient, respectively.

The combined effects of DEX and DOR were modelled assuming a competitive interaction between the two chemical moieties at the same receptor site (PD Models 13–16):

$$E = \text{Placebo Effect} \times \{1 - E_{\max} \times (C_{\text{DEX}}/EC_{50}) + (Pot_{\text{DOR}}C_{\text{DOR}}/EC_{50})^n / [1 + ((C_{\text{DEX}}/EC_{50}) + (Pot_{\text{DOR}}C_{\text{DOR}}/EC_{50})^n)]\} \quad (12)$$

Pot_{DOR} is the relative potency of DOR to DEX [$EC_{50}(\text{DEX})/EC_{50}(\text{DOR})$].

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