Influence of CYP2D6 activity on pre-emptive analgesia by the N-methyl-D-aspartate antagonist dextromethorphan in a randomized controlled trial of acute pain

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Abstract
There is some evidence that dextromethorphan (DM) is effective as a preemptive analgesic agent. DM is mainly metabolized to dextrorphan (DOR) by CYP2D6 whose activity can be inhibited by pharmacologic intervention.

Reference

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Influence of CYP2D6 Activity on Pre-emptive Analgesia by the N-Methyl-D-Aspartate Antagonist Dextromethorphan in a Randomized Controlled Trial of Acute Pain

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Background: There is some evidence that dextromethorphan (DM) is effective as a pre-emptive analgesic agent. DM is mainly metabolized to dextrorphan (DOR) by CYP2D6 whose activity can be inhibited by pharmacologic intervention.

Objectives: To investigate the efficacy of DM as a pre-emptive analgesic agent and describe the population pharmacokinetics in the presence of normal and poor CYP2D6 metabolism in acute post-operative pain.

Study Design: Double blind, randomized, placebo-controlled trial

Setting: Post-surgical analgesic consumption after knee ligament surgery, a setting of acute pain.

Methods: Forty patients were randomized to a single oral dose of 50 mg quinidine or placebo, administered 12 hours before 50 mg DM. Patients were genotyped for the major CYP2D6 and ABCB1 variants and phenotyped for CYP2D6 using urine DM/DOR metabolic ratios and blood samples for population pharmacokinetic modeling.

Results: Quinidine was effective in inhibiting CYP2D6 activity, with 2-fold reduction of DM to DOR biotransformation clearance, prolonged DM half-life, and increased DM systemic availability. Patients in the quinidine group required significantly less often NSAIDs than patients in the placebo group (35.3% vs. 75.0%, P = 0.022). The odds ratio for NSAID consumption in the placebo vs. quinidine group was 5.5 (95% confidence interval (CI) 1.3 - 22.7) at 48 hours after surgery.

Limitations: While this study shows an impact of DM on pre-emptive analgesia and is mechanistically interesting, the findings need to be confirmed in larger trials.

Conclusion: CYP2D6 inhibition by quinidine influenced the pre-emptive analgesic effectiveness of DM confirming that CYP2D6 phenotypic switch increases the neuromodulatory effect of oral dextromethorphan.

Key words: Pre-emptive analgesia, dextromethorphan, population kinetics, quinidine, cytochrome 2D6

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Pain is an adaptive process with the objective to conserve the organism's integrity (1). Several mechanisms are involved in increasing the excitability of the central nervous system after an acute and intense nociceptive stimulation. Central sensitization, a process in which the memory of pain is retained, leads to an increased pain response in case of repeated stimulation, and has been shown to be an important contributing factor for developing chronic pain (2). The pathophysiology of central sensitization involves N-methyl-D-aspartate (NMDA) neuro-receptors, activated by several excitatory neuromessengers (3) and pre-emptive analgesia using an NMDA antagonist is aimed at reducing this phenomenon.

Dextromethorphan (DM) is a synthetic opioid derivative, devoid of opioid analgesic activity. It has been widely used as an anti-cough medication for several decades and has a favorable safety profile. DM's anticonvulsant, anti-nociceptive, and neuroprotective properties are related to antagonism at the NMDA receptor (4,5). CYP2D6 is the main enzyme responsible for DM elimination by its principal metabolite dextrophan (DOR). Inhibition of CYP2D6 by quinidine comes close to the genetic absence of CYP2D6 activity, observed in about 10% of individuals of European origin (6,7).

The analgesic effect and analgesic sparing potential of DM have been investigated in several clinical settings and results have been equivocal (8). Our previous work has suggested that a CYP2D6 poor metabolizer (PM) phenotype increases the neuromodulatory efficacy of DM in healthy volunteers (4). A multicenter randomized controlled study analyzed the analgesic effectiveness of a DM/quinidine combination therapy (9) for diabetic neuropathic pain. The same drug combination developed to increase DM bioavailability in the central nervous system has also been demonstrated to be effective to treat the pseudobulbar effect in multiple-sclerosis (10-12) and is FDA approved for this indication. The variability of the CYP2D6 phenotype might be one reason for the conflicting results observed in different controlled randomized trials on pre-emptive analgesia using DM only. The use of a combination of DM and a CYP2D6 inhibitor might lead to an improved outcome in acute pain and other neurological conditions (4,8,13). We therefore performed a double blind, randomized, controlled trial to investigate the pre-emptive neuromodulatory effect of a DM/quinidine vs. DM/placebo combination on post-surgical analgesic consumption after knee ligament surgery, a setting of acute pain.

METHODS

The study was designed and conducted by the investigators. Approval of the protocol was obtained from the institutional review board of our institution (ID: 01-192 - APSIC 01-020). The trial was registered with the Swiss Agency for Therapeutic Products in accordance with national guidelines and conducted in accordance with the International Conference on Harmonization, the principles of the Declaration of Helsinki, Good Clinical Practice, and Swiss regulatory requirements. Written informed consent was obtained from each subject prior to inclusion.

Patient Recruitment

Patients were recruited pre-operatively before ligament reconstruction of the knee at Geneva University Hospital. Inclusion criteria were

1) age 16 to 65 years,
2) surgery under epidural anesthesia,
3) otherwise healthy individuals, and
4) written informed consent obtained.

Exclusion criteria were

1) planned general anesthesia or post-operative peripheral block,
2) contraindication to epidural anesthesia,
3) chronic use of opioids, quinidine, or other CYP2D6 inhibitors, and
4) moderate to severe pre-operative pain or chronic pain syndrome.

As indicated in Fig. 1, 192 patients were screened. The main reasons for exclusion were general anesthesia (47.2%), refusal to participate (20.1%), and planned peripheral block after surgery (11.8%). The overall inclusion rate was 25% (48 patients).

Study Design

We conducted a randomized, double-blind, placebo-controlled trial. Each patient was randomly assigned to receive a single dose of either 50 mg quinidine orally or placebo 8 to 12 hours before surgery. The Central Pharmacy of the Geneva University Hospital prepared randomized quinidine sulfate and placebo. Each fasting patient then received 50 mg DM (dextromethorphan hydrobromide) orally at one hour prior to surgery.

Of the 22 patients randomized to quinidine and the 26 patients randomized to placebo, 4 patients were excluded or dropped out in each group (failure to receive DM or general anesthesia; Fig. 1). Hence, the...
study includes 18 patients in the quinidine group and 22 patients in the placebo group. The trial was stopped after inclusion of 40 patients, a point of predefined intermediary analysis, because significance was reached for the primary endpoint.

Epidural anesthesia using bupivacaine (10 - 12mg) and fentanyl (25µg) was initiated 30 - 60 minutes before incision. In the recovery room, a patient-controlled analgesia device delivered morphine intravenously (maximum 1 mg per 5 minutes and 30 mg per 4 h) if pain was rated > 5 on a 0 - 10 visual analog scale (VAS). Intravenous ketorolac and/or oral acetaminophen were available at the patient discretion. If pain remained > 7 on the VAS despite intravenous ketorolac and morphine,
a peripheral block was performed and the patient dropped out of the study (one patient in the placebo group; see Fig. 1).

**Outcome Assessment**

Primary outcome variables were NSAID, acetaminophen, and morphine consumption over the 0 - 48 hour time interval after surgery. For 18 patients treated with analgesics other than intravenous ketorolac or oral acetaminophen, doses were converted to equivalent doses of intravenous ketorolac or oral acetaminophen on the basis of previous data (14) -400 mg ibuprofen equivalent to 15 mg intravenous ketorolac; intravenous propacetamol converted to oral acetaminophen in a 2:1 ratio.

We also recorded pain level and possible adverse effects (drowsiness, nausea, and dizziness) on a 0 - 10 VAS at 1, 3, 5, 8, 12, 24, and 48 hours after surgery.

**Blood Sampling and Urine Collection**

Blood samples were obtained from all patients in a 0 - 75 hour time interval after DM administration (2 to 7 samples per patient). Thirty-five patients had at least one DM or DOR concentration above the limit of quantification and were included in the pharmacokinetic analysis. Genomic DNA was extracted from 200 µl of whole blood using the QIAamp DNA blood mini kit (Quiagen, Hombrechtikon, Switzerland). Urine was collected for phenotype assessment in 33 patients during the 8 hours after DM administration.

**Analytical Methods**

DM and DOR quantification in plasma and urine was performed according to previously described methods using liquid chromatography coupled to fluorescence detection after liquid-liquid extraction (15,16).

CYP2D6 enzyme activity was determined using the urinary metabolic ratio (MR) DM/DOR (15,17,18). Individuals were classified as UM (ultra rapid metabolizer) when MR was below 0.003, EM (extensive metabolizer) between 0.003 and < 0.03, IM (intermediate metabolizer) between 0.03 and 0.3, and PM above 0.3, with increasing MR reflecting decreasing CYP2D6 activity.

CYP2D6 genotypes (alleles *3, *4, and *6) were determined by real-time PCR using allele-specific TaqMan™ probes (Applied Biosystems, Foster City, USA) according to instructions of the manufacturer. CYP2D6 gene duplication (*xN) and allele *5 (del) were determined by long-range real-time PCR with SYBR Green and allele-specific primers, as previously described (19).

Treatment by quinidine could also inhibit p-glycoprotein, the product of the ABCB1 gene, and influence transport in the gut and at the brain-blood barrier. Two common polymorphisms previously described in the ABCB1 gene could potentially influence the outcomes in this study and we therefore genotyped the ABCB1 C3435T and G2677T/A variants in all participants of this study. ABCB1 C3435T and G2677T/A genotypes were typed in a single multiplex PCR with fluorescent probe melting temperature analysis on a Light Cycler (Roche, Rotkreuz, Switzerland). For the G2677T/A variant, a sensor probe, modified with a locked nucleic acid, was used to improve allelic discrimination. A heterozygous sample was included for quality control in each run. The resulting melting curves were highly reproducible (SD on Tm < 0.3°C) and allowed a reliable discrimination between the alleles. To further confirm the reliability of the method, 6 samples were genotyped for the 2677 SNP using Taqman probes and 10 samples for the 3435 SNP by RFLP. All methods were in 100% concordance for the samples tested. The minor allele frequencies of the 2 ABCB1 variants tested were 0.47 in this study.

**Population Pharmacokinetic Model**

In order to strengthen parameter estimation, model development proceeded by considering the present sample (sample 1: 35 patients, 170 measurements, 1 to 12 per patient, 0 - 75 hour time interval) together with a sample of healthy volunteers who took part in an earlier study (4). The participants of the published study received 50 mg DM preceded by placebo or 50 mg quinidine sulfate 12 hours earlier according to a randomized crossover design (sample 2: 9 subjects, 281 measurements, 13 to 18 per subject, 0 - 24 hour time interval).

Concentration-time profiles of DM and DOR were fitted simultaneously using a 2-molecule linear pharmacokinetic model (20). Rate constants $k_a$, $k_{12}$, $k_{10}$, and $k_{20}$ described absorption, biotransformation of DM to DOR, elimination of DM by other routes (i.e., renal elimination and biotransformation to 3-methoxymorphinan), and total elimination of DOR (i.e., renal elimination, glucuronidation, and biotransformation to 3-hydroxy-morphinan). Apparent volumes of the distribution of the parent compound and metabolite were $V_{DM}$ and $V_{DOR}$, respectively. We made the following assumptions:

1) An absorption lag-time $t_{lag}$ was included, in line with earlier observation (21).

2) Since O-demethylation of DM to DOR represents about 82% of its total metabolic clearance (22) and...
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DM renal clearance is small (23), DM total clearance was supposed to be equal to the DM to DOR formation clearance. Hence, $\text{CL}_{\text{DM}} = k_{12, i} \cdot V_{\text{DM}}$ and $k_{10} = 0$.

3) DM systemic availability $F$ was considered to be the product of the fraction absorbed $F_a$, the fraction escaping metabolism in the gut wall $F_G$, and the fraction escaping hepatic first-pass metabolism $F_H$. First-pass production of DOR was modeled through a fraction $E_H = 1 - F_H$, with $E_H = \text{CL}_{\text{DM}}/Q_H$ and hepatic blood flow $Q_H$ set to a constant value (90 L/h). First-pass production of other metabolites was neglected.

4) Inhibition by quinidine was modeled so that the DM to DOR biotransformation rate was multiplied by a constant $C_{\text{inh}} < 1$ in the presence of quinidine ($Q = 1$):

$$k_{12, i} \cdot \left[ k_{12} \cdot \left( 1 - Q \right) + C_{\text{inh}} \cdot k_{12} \cdot Q \right] \exp(\eta_i) \cdot \left( \text{eq. 1) } \cdot \right)$$

where $k_{12, i}$ is the biotransformation rate in subject $i$ and $k_{12}$ is the average population value in the absence of quinidine ($Q = 0$). Inter-individual variability was described by the exponential term in eq. 1 where $\eta_i$ was assumed to be normally distributed with mean 0 and variance $\omega^2$. Inhibition by quinidine also affected first-pass production of DOR through the liver extraction ratio $E_H$ (see above). Possible effects of quinidine on other model parameters were tested according to models similar to eq. 1 without any significant improvement of goodness of fit (differences between objective functions < 6.6, see below).

5) Between-subject variability of parameters $k_a$, $t_{\text{lag}}$, $k_{20}$, $V_{\text{DM}}$, and $V_{\text{DOR}}$ was modeled with exponential random effect terms, as described for $k_{12}$ in eq. 1.

6) Residual variability of DM and DOR concentrations was similarly described with exponential models.

Clearances were derived from base parameters as $\text{CL}_{\text{DM}} = k_{12} \cdot V_{\text{DM}}$ and $\text{CL}_{\text{DOR}} = k_{20} \cdot V_{\text{DOR}}$. Apparent elimination half-lives of the parent compound and metabolite were calculated as $t_{1/2, \text{DM}} = t_{1/2, \text{DOR}} = \ln(2)/k_{12}$, with DOR being formation rate-limited ($k_{12} < k_{30}$).

The model was implemented in the NONMEM software (version VI, University of California, San Francisco, CA) and estimated using the first-order conditional estimation method with interaction (FOCE INTER option). Statistical comparison of models of increasing complexity was based on the likelihood ratio test, with $P < 0.01$ considered to represent a significantly better fit (i.e., decrease of objective function > 6.6 for one additional parameter). Model evaluation also considered relative standard errors of parameter estimates (RSE, calculated as standard error of the estimate / mean population estimate) and residual plots. Mean bias and 95% CI were calculated on the basis of differences between observed and model-predicted concentrations in individual subjects.

Statistical Methods

The Fisher's exact test and the Mann-Whitney U-test were used for group comparisons of categorical and continuous variables, respectively. An odds ratio (OR) with 95% CI was estimated for comparing NSAID consumption in placebo and quinidine groups. Statistical significance was set at 0.05 (two-tailed tests). Data analysis was performed using SPSS version 17 (SPSS Inc., Chicago, IL) and R statistical software (R: A Language and Environment for Statistical Computing - R Foundation for Statistical Computing, Vienna, Austria).

Genetic analyses for quantitative traits was carried out using the PLINK analysis toolset (24) and Haploview (25). A Wald test was used for testing for an association between the quantitative phenotypes and genotypes assuming an additive model.

Results

Baseline Characteristics

Forty patients were included in the study (Fig. 1). Baseline characteristics, including CYP2D6 genotype and phenotype distribution in the quinidine and placebo groups, are provided in Table 1. The 2 groups had a similar distribution of the major CYP2D6 genotype frequencies: homozygous full function genotype *1/*1 (15 in the placebo group vs. 10 in the quinidine group), heterozygous (5 vs. 5), homozygous loss of function genotype *4/*4 (1 vs. 10) and gene duplication carriers *1/*1xN (1 vs. 2).

The results from phenotyping using MR were concordant with the genetic phenotype prediction in 12 of 17 patients (70.6%) in the placebo group, as compared to 2 of 16 patients (12.5%) in the quinidine group (Fisher’s exact test, $P = 0.001$). In the group pretreated by quinidine, CYP2D6 activity was switched to a slower metabolizing phenotype than predicted by genotype in 14 of 16 (87.5%) patients, one genotypic EM was switched to a complete blockade of CYP2D6 activity (PM).

The use of concomitant medications in this mostly healthy patient group was low (13 participants were treated with NSAID or acetaminophen, one patient with benzodiazepines), most notably there was no patient with
risk factors for serotonin syndrome that DM has been associated with. Among the other potential adverse drug responses to DM are psychomimetic effects, bromism, cardiac arrhythmia, and abuse potential. With our single dose of DM no such side effects were observed.

**DM Pharmacokinetic Parameters and Effect of CYP2D6 Inhibition**

Pharmacokinetic data were available on 35 patients. Parameter estimates from a population pharmacokinetic model are provided in Table 2. The relative standard errors of the estimates were < 25% for all parameters with the exception of the absorption rate, indicating that the data were adequate to estimate the model. The inter-individual variability was large (> 50%) for both the DM to DOR biotransformation rate and the DOR elimination rate, with individual ranges of 9.4 and 12.9-fold, respectively. Predicted individual and population concentration versus time profiles are illustrated in Fig. 2, panel A and B. The correlations between measured and predicted individual concentrations are provided in panels C and D. No significant bias was observed between the observations and predicted individual concentrations (DM mean bias 0.9 ng/ml, 95% CI -0.8 - 2.7; DOR mean bias 0.2 ng/ml, 95% CI -0.4 - 0.9). The relative standard errors of the estimates were < 25% for all parameters with the exception of the absorption rate, indicating that the data were adequate

<table>
<thead>
<tr>
<th></th>
<th>DM+quinidine group (n = 18)</th>
<th>DM+placebo group (n=22)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years) median (range)</td>
<td>28 (16 - 46)</td>
<td>32 (17 - 48)</td>
<td>0.50</td>
</tr>
<tr>
<td>Gender male, n (%)</td>
<td>15 (83.3)</td>
<td>15 (68.2)</td>
<td>0.46</td>
</tr>
<tr>
<td>female, n (%)</td>
<td>3 (16.7)</td>
<td>7 (31.8)</td>
<td></td>
</tr>
<tr>
<td>Weight (kg) median (range)</td>
<td>75 (55 - 92)</td>
<td>70 (50 - 110)</td>
<td>0.50</td>
</tr>
<tr>
<td>Height (cm) median (range)</td>
<td>176 (153 - 192)</td>
<td>175 (161 - 199)</td>
<td>0.47</td>
</tr>
<tr>
<td>Etiology of ligament lesion sport, n (%)</td>
<td>17 (94.4)</td>
<td>16 (72.7)</td>
<td>0.11</td>
</tr>
<tr>
<td>other, n (%)</td>
<td>1 (5.6)</td>
<td>6 (27.3)</td>
<td></td>
</tr>
<tr>
<td>Pain intensity when the accident occurred (10-point VAS, n = 38) median (range)</td>
<td>4 (0 - 10)</td>
<td>7 (2 - 10)</td>
<td>0.34</td>
</tr>
<tr>
<td>2D6 genotypes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*1/*1xN: n (%)</td>
<td>2 (11.1)</td>
<td>1 (4.5)</td>
<td>0.82</td>
</tr>
<tr>
<td>*1/*1: n (%)</td>
<td>10 (55.6)</td>
<td>15 (68.2)</td>
<td></td>
</tr>
<tr>
<td>*1/*4: n (%)</td>
<td>4 (22.2)</td>
<td>4 (18.1)</td>
<td></td>
</tr>
<tr>
<td>*1/*5: n (%)</td>
<td>0 (0)</td>
<td>1 (4.5)</td>
<td></td>
</tr>
<tr>
<td>*1/*6: n (%)</td>
<td>1 (5.6)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>*4/*4: n (%)</td>
<td>1 (5.6)</td>
<td>1 (4.5)</td>
<td></td>
</tr>
<tr>
<td>2D6 predicted phenotype (genotype)2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UM: n (%)</td>
<td>2 (11.1)</td>
<td>1 (4.5)</td>
<td>0.79</td>
</tr>
<tr>
<td>EM: n (%)</td>
<td>15 (83.3)</td>
<td>20 (90.9)</td>
<td></td>
</tr>
<tr>
<td>PM: n (%)</td>
<td>1 (5.6)</td>
<td>1 (4.5)</td>
<td></td>
</tr>
<tr>
<td>2D6 phenotype (DM/DOR urinary ratio, n=33)</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>UM: n (%)</td>
<td>0 (0)</td>
<td>1 (5.9)</td>
<td></td>
</tr>
<tr>
<td>EM: n (%)</td>
<td>1 (6.3)</td>
<td>11 (64.7)</td>
<td></td>
</tr>
<tr>
<td>IM: n (%)</td>
<td>13 (81.3)</td>
<td>3 (17.6)</td>
<td></td>
</tr>
<tr>
<td>PM: n (%)</td>
<td>2 (12.5)</td>
<td>2 (11.8)</td>
<td></td>
</tr>
</tbody>
</table>

*1Mann-Whitney U-test for continuous variables; Fisher’s exact test for categorical variables. *2The phenotype predicted from genotype was obtained as follows (29): UM if *1/*1xN; EM if *1/*1, *1/*4, *1/*5, or *1/*6; PM if *4/*4.
Impact of CYP2D6 Phenotype on Pre-emptive Analgesia by Dextromethorphan

Table 2. Population pharmacokinetic parameters of DM and DOR.

<table>
<thead>
<tr>
<th>Model parameter</th>
<th>Mean population estimate</th>
<th>RSE (%) a</th>
<th>Interindividual variability (CV, %)</th>
<th>Range b</th>
<th>Literature values mean (range) c</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$ (h⁻¹)</td>
<td>2.8</td>
<td>50</td>
<td>124</td>
<td>0.13 – 37</td>
<td>2.6 (0.8 – 3.9)</td>
</tr>
<tr>
<td>$t_{lag}$ (h)</td>
<td>0.45</td>
<td>2</td>
<td>5</td>
<td>0.44 – 0.49</td>
<td>0.8 (0.5 – 1.2)</td>
</tr>
<tr>
<td>$k_{12}$ (h⁻¹)</td>
<td>0.074</td>
<td>21</td>
<td>53</td>
<td>0.014 – 0.13</td>
<td>0.041 (0.029 - 0.069)</td>
</tr>
<tr>
<td>$C_{inhib}$</td>
<td>0.52</td>
<td>22</td>
<td>NA</td>
<td>NA</td>
<td>0.27 (NA)</td>
</tr>
<tr>
<td>$V_{DM}$ (l)</td>
<td>627</td>
<td>8</td>
<td>70</td>
<td>0.04 – 0.54</td>
<td>0.51 (0.21 – 1.07)</td>
</tr>
<tr>
<td>$V_{DOR}$ (l)</td>
<td>1700</td>
<td>14</td>
<td>63</td>
<td>463 - 990</td>
<td>961 (585 - 1292)</td>
</tr>
<tr>
<td>Residual variability DM</td>
<td>30%</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residual variability DOR</td>
<td>24%</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Relative standard error calculated as (standard error of the estimate) / (mean population estimate)
b Conditional estimates of individual values (samples 1 and 2, see text)
c Values taken from (21) for a 30 mg DM dose, with $k_{12}$ and $C_{inhib}$ calculated as $k_{12} = \ln(2)/t_{1/2}$ and $C_{inhib} = CL_{total \ with \ quinidine} / CL_{total \ without \ quinidine}$.

Fig. 2: Concentration versus time profiles for DM (panel A) and DOR (panel B) in 35 patients. Observed concentrations for patients pretreated with 50 mg quinidine (blue triangles) and placebo (black circles) are displayed together with model-predicted curves at the population level (bold lines) and individual level (thin lines). Panels C and D show observed versus individual model-predicted concentrations of DM and DOR, respectively. The diagonal line represents a perfect fit of the model to the data.
to estimate the model. The inter-individual variability was large (> 50%) for both the DM to DOR biotransformation rate and the DOR elimination rate, with individual ranges of 9.4 and 12.9-fold, respectively.

Individual parameter estimates in patients randomized to quinidine and placebo are summarized in Table 3. Two patients with CYP2D6 *4/*4 genotype were excluded from the analysis because no further inhibition of CYP2D6 by quinidine was expected. The inhibition of the DM to DOR biotransformation rate by quinidine was associated with a prolongation of the apparent DM and DOR half-lives, an increased DM systemic availability, and a reduced first-pass production of DOR. Quinidine was estimated to decrease the DM to DOR biotransformation rate 1.9-fold. Clearance estimates varied 3- to 4-fold within each group, indicating a large unexplained variability. They were also consistent with data from the urine collection (Table 3). Median DM clearance was 1.7-fold higher in EM than IM patients (Mann-Whitney U-test, $P = 0.028$) and 3.4-fold higher in EM than PM patients ($P = 0.073$).

**Analgesic Requirements in the 0 - 48 hour Time Interval After Surgery**

Table 4 describes NSAID, acetaminophen, and morphine consumption in the 0 - 48 hour time interval after surgery.

### Table 3. Pharmacokinetic parameters according to quinidine treatment and CYP2D6 urinary phenotype.

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>$Cl_{DM}$ (l/h)</th>
<th>$P$-value</th>
<th>$t_{1/2 DM}$ (h)</th>
<th>$F_{in}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo (n = 16)</td>
<td>59 (22 - 83)</td>
<td>0.35 (0.08 - 0.75)</td>
<td>7.8 (5.6 - 20.0)</td>
<td>0.08 - 0.75</td>
</tr>
<tr>
<td>Quinidine (n = 17)</td>
<td>31 (14 - 48)</td>
<td>&lt; 0.001</td>
<td>13.2 (9.5 - 28.8)</td>
<td>0.66 (0.46 - 0.85)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urinary phenotype</th>
<th>$Cl_{DM}$ (l/h)</th>
<th>$P$-value</th>
<th>$t_{1/2 DM}$ (h)</th>
<th>$F_{in}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>UM (n = 1)</td>
<td>44</td>
<td>0.51</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>EM (n = 8)</td>
<td>58 (22 - 83)</td>
<td>0.036 (0.08 - 0.75)</td>
<td>8.1 (5.7 - 20.0)</td>
<td>0.62 (0.08 - 0.85)</td>
</tr>
<tr>
<td>IM (n = 16)</td>
<td>34 (14 - 83)</td>
<td>0.073 (0.18 - 0.93)</td>
<td>13.2 (5.7 - 27.8)</td>
<td>0.62 (0.08 - 0.85)</td>
</tr>
<tr>
<td>PM (n = 4)</td>
<td>17 (6 - 74)</td>
<td>0.073 (0.18 - 0.93)</td>
<td>0.073</td>
<td>0.6 (0.46 - 0.85)</td>
</tr>
</tbody>
</table>

A Conditional individual estimates from population pharmacokinetic model (see text).

b Mann-Whitney U-test for comparing treatment groups and phenotype groups (IM vs. EM, PM vs. EM).

c Of 35 patients with pharmacokinetic data, 2 patients with CYP2D6 *4/*4 genotype were excluded.

d Of 35 patients with pharmacokinetic data, 29 provided urine data.

### Table 4. Analgesic consumption in the 0 - 48 hour time interval after surgery.

<table>
<thead>
<tr>
<th></th>
<th>Quinidine group (n = 17)</th>
<th>Placebo group (n = 20)</th>
<th>$P$-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose (mg): mean (range)</td>
<td>57.2 (0 - 100)</td>
<td>47.5 (1 - 107)</td>
<td>0.33</td>
</tr>
<tr>
<td>Number (% of participants without morphine use)</td>
<td>1 (5.9%)</td>
<td>0 (0%)</td>
<td>--</td>
</tr>
<tr>
<td>NSAID</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose (mg): mean (range)</td>
<td>27.8 (0 - 180)</td>
<td>66.8 (0 - 165)</td>
<td>0.018</td>
</tr>
<tr>
<td>Number (% of participants without NSAID use)</td>
<td>11 (64.7%)</td>
<td>5 (25.0%)</td>
<td>0.022</td>
</tr>
<tr>
<td>Acetaminophen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dose (mg): mean (range)</td>
<td>1118 (0 - 7000)</td>
<td>1175 (0 - 7000)</td>
<td>0.94</td>
</tr>
<tr>
<td>Number (% of participants without acetaminophen use)</td>
<td>12 (70.6%)</td>
<td>14 (70.0%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

a Two patients with CYP2D6 *4/*4 genotype and one patient who had a peripheral bloc were excluded.

b Fisher's exact test for proportions; Mann-Whitney U-test for dose.

c In “intravenous ketorolac equivalents” (see Methods).

d In “oral acetaminophen equivalents” (see Methods).
Impact of CYP2D6 Phenotype on Pre-emptive Analgesia by Dextromethorphan

groups. Two patients with CYP2D6 *4/*4 genotype and one patient on which a peripheral block was performed were excluded from this analysis. A majority of patients in the placebo group with normal CYP2D6 activity required NSAIDs (15 of 20, 75.0%), but only 6 of 17 patients (35.3%) required NSAIDs in the quinidine group (Fisher’s exact test, \( P = 0.022 \)). The odds ratio for NSAID consumption in the placebo vs. quinidine group was 5.5 (95% CI 1.3 - 22.7) at 48 hours after surgery. We observed no difference between groups with respect to acetaminophen or morphine consumption. Patients who required NSAIDs had higher DM clearances and shorter DM half-lives than those not requiring NSAIDs, but the differences did not reach statistical significance (median clearances 47 vs. 36 l/hours, \( P = 0.20 \); median half-lives 9.3 vs. 12.6 hours, \( P = 0.14 \)).

Pain Intensity and Adverse Drug Effects in the 0 - 48 Hour Time Interval After Surgery

Pain scores on a visual analogue scale did not differ significantly between the placebo and quinidine groups (median 1.7 vs. 2.0, \( P = 0.38 \) at 24 hours; 1.0 vs. 1.2, \( P = 0.53 \) at 48 hours). No significant difference was observed with respect to maximum scores for somnolence (median 4.5 vs. 3.4, \( P = 0.85 \)), nausea (median 0.6 vs. 0.7, \( P = 0.58 \)), and dizziness (median 0.2 vs. 0.4, \( P = 0.53 \)). We also investigated if chronic pain levels post-intervention differed between the treatment groups, but given the very low average pain levels of the largely healthy study participants (0.95 on a 0 - 10 scale for 28 patients at one month post intervention) we were unable to carry out meaningful analyses.

Genotyping of 2 Functional Variants in ABCB1

We tested whether the C3435T and G2677T/A variants of the ABCB1 gene were associated with NSAID consumption in a quantitative trait analysis and the results did not reach significance (not shown).

Discussion

The efficacy of DM as a pre-emptive neuromodulatory analgesic in post-operative pain has been investigated in many clinical settings and the results have been equivocal (8). We report the first prospective, randomized, double-blind clinical trial comparing the neuromodulatory efficacy of DM with and without a CYP2D6 phenotype switch by quinidine, using post-operative acute pain as a model. Pharmacologic inhibition of CYP2D6 by quinidine can mimic CYP2D6 deficiency (phenotypic switch) and increase systemic and central nervous system exposure to DM (26). Our results suggest that a single oral dose of quinidine (50 mg) leads to sufficient CYP2D6 inhibition and more favorable DM pharmacokinetics allowing significant sparing of NSAID consumption in the 48 hour time interval post-surgery, confirming the influence of CYP2D6 activity and the concept of drug combination to improve the NMDA neuromodulatory antagonist efficacy of dextromethorphan (27). Additional studies will be necessary in order to confirm the NSAID sparing effect of DM.

The equivocal results obtained in different post-operative conditions after using DM alone might be related to the different expected pharmacokinetic profiles in poor and EMs of CYP2D6. In effect, CYP2D6 function is highly variable in the general population, about 10% of individuals have absent CYP2D6 activity (6) and drug-drug pharmacokinetic interactions might increase this percentage considerably (28).

A multitude of functional polymorphisms of the CYP2D6 gene have been described. We limited our genetic analysis in our population to the 4 major functional variants (*3, *4, *5, *6) and to analysis of gene duplication, allowing the detection of ~ 80% of genetic CYP2D6 variability (29). The 2 groups had a similar distribution of the major CYP2D6 genotype frequencies with only one predicted PM in each group. CYP2D6 inhibition by quinidine was quantified in 2 ways: First we conducted urinary phenotyping by measuring metabolic DM/DOR ratios, demonstrating that a great majority of genotype-predicted EMs were switched to lower CYP2D6 activity after pretreatment with quinidine. Second, a population pharmacokinetic model of DM and DOR was developed. The mean population parameters and ranges of conditional individual estimates were comparable to published values (21). The model permitted us to estimate that in the presence of quinidine the DM to DOR biotransformation clearance is reduced 1.9-fold. These results are compatible with several previous studies showing that a single dose of quinidine allows increasing exposure to DM (4). Our study is also in agreement with earlier data indicating that CYP2D6 inhibition by quinidine is less than complete (30). Higher or multiple doses of quinidine might be necessary to inhibit CYP2D6 activity completely.

Our key finding is a significant sparing of NSAID consumption in patients receiving the quinidine + DM combination compared to DM alone. The frequency and dose of NSAID use was significantly reduced when CYP2D6 was inhibited by quinidine, with an odds ratio of 5.5 for NSAID consumption in the placebo vs. quini-
The published data are equivocal for the effect of DM as monotherapy on acute pain, but at least 2 studies demonstrate an NSAID sparing effect with oral DM, although CYP2D6 genotype was not evaluated (31,32).

CYP2D6 inhibition by quinidine had no influence on the morphine and acetaminophen consumption in the present study. NSAIDs are particularly efficacious in orthopedic acute pain, even more than opioids (33), although the dose dependence of the analgesic effect makes a direct comparison difficult. This might be why we observed NSAID sparing, but no significant morphine and acetaminophen sparing in patients pre-treated with quinidine compared to placebo. However, using a higher DM dose (150 mg), a significant reduction in morphine requirement was demonstrated in a double-blind randomized study in 50 patients undergoing elective abdominal hysterectomy (34). In our trial, morphine consumption was fairly low, possibly related to the type of surgical intervention, known to cause low intensity pain, and free access to NSAIDs and/or morphine as soon as pain levels were intense (5/10 on the VAS).

In addition to its inhibitory effect on CYP2D6, quinidine is also a potent inhibitor of p-glycoprotein (the product of the ABCB1 “MDR-1” gene) (35). P-glycoprotein inhibition might increase the central nervous system availability of DM and its efficacy in the nociceptive processes. DM was indeed suggested to be a p-glycoprotein substrate (36), even though more recent evidence in human cells argues against it (37). We did not observe any significant association between the 2 putative major functional ABCB1 polymorphisms (C3435T and G2677T/A) and NSAID consumption in this study, but statistical power was low and the genetic survey was not exhaustive.

**Conclusion**

In summary, we show that CYP2D6 phenotype modulates the pre-emptive analgesic efficacy of DM. Even though inhibition of CYP2D6-mediated DM metabolism by a single dose of 50 mg quinidine was only partial, significant NSAID sparing was observed in the group with reduced CYP2D6 activity. Because patients potentially treated with DM for pre-emptive analgesia are a heterogeneous group with respect to CYP2D6 activity and thus analgesic sparing potential through enzyme inhibition, use of CYP2D6 inhibitors constitutes a potential means to increase DM neuromodulatory effect.

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**Authorship Contributions**

Study design: Ehret, Dayer, Desmeule. Conducted the study: Ehret, Chabert, Rebsame. Performed data analysis: Ehret, Daali, Gex-Fabr. Manuscript prepared by: Ehret, Daali, Gex-Fabry, Desmeule. Revisions of the manuscript: Rebsamen, Wolff, Forster, Moursli, Piguet, Fritschy, Rossier

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