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The effect of grapefruit juice and seville orange juice on the pharmacokinetics of dextromethorphan: The role of gut CYP3A and P-glycoprotein

Marika Pasternyk Di Marco[a,d], David J. Edwards[b], Irving W. Wainer[a,c], Murray P. Ducharme[a,d,*]

[a]Faculté de Pharmacie, University of Montreal, Montreal, Canada
[b]Wayne State University, Detroit, MI, USA
[c]Georgetown University Medical Center, Washington DC, USA
[d]MDS Pharma Services, Montreal, Canada

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Abstract

The objective of this study was to determine the effects of grapefruit juice and seville orange juice on dextromethorphan (DM) pharmacokinetics. Eleven healthy volunteers were studied over a 3-week period consisting of 5 study days each separated by a three-day washout. All subjects refrained from drinking caffeine containing beverages (coffee, soda, etc.) 8 h before orally taking DM (30 mg) with 200 ml water, 200 ml grapefruit juice, 200 ml water, 200 ml seville orange juice, and 200 ml water on Study Days 1 to 5. Aliquots of urine samples were assayed and analysed for DM, and the DM metabolites dextrorphan, 3-methoxymorphinan and 3-hydroxymorphinan using a validated HPLC method employing a phenyl column and a fluorescence detection. Results suggests that DM could provide some useful information on P-glycoprotein or related membrane efflux protein activity in the human gastro-intestinal tract. Bioavailability (F) of DM increased significantly with grapefruit and seville orange juice, but only returned to half the baseline value after three days of washout. This confirms that grapefruit and seville orange juice are long-lasting and perhaps irreversible inhibitors of gut CYP3A/P-glycoprotein. Grapefruit and seville orange juice appeared to have the same overall effect on DM pharmacokinetics. In addition, this paper presents a novel

* Corresponding author. Senior Director, Pharmacokinetics and Pharmacodynamics, MDS Pharma Services, 2350 Cohen, St-Laurent, Canada, H4R 2N6. Tel.: +1-514-333-0042x4520; fax: +1-514-333-7666.

E-mail address: Murray.Ducharme@MDSPS.com (M.P. Ducharme).

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method of phenotyping for CYP2D6, CYP3A and P-glycoprotein using DM as a probe. © 2002 Elsevier Science Inc. All rights reserved.

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Introduction

The major phase I drug metabolizing enzyme in humans, CYP3A, and the membrane ATPase transporter protein, P-glycoprotein, are found in high concentrations in the enterocytes of the small intestine [1,2]. Intestinal CYP3A enzymes have been found to alter the bioavailability of many drugs while P-glycoprotein is responsible for the multidrug resistance of many chemotherapeutic agents [3,4,5]. In addition, a significant overlap in substrate specificity exists between CYP3A and P-glycoprotein [6].

Many commonly used medications are substrates for the CYP3A enzymes. One of these medications is the over-the-counter antitussive agent, dextromethorphan (DM) hydrobromide. DM is also a substrate for CYP2D6 and it has been extensively used to probe drug phenotype subjects relative to the activity of CYP2D6 [7]. While the use of DM for CYP2D6 phenotyping is clear, a controversy exists regarding the usefulness of this agent for the determination of expressed CYP3A activity [8].

The metabolic disposition of DM and its metabolites has been studied in several species [9]. The studies have demonstrated that DM is predominately metabolized via two major pathways: O-demethylation by CYP2D6 enzymes to dextrorphan (DX) and N-demethylation by CYP3A enzymes to 3-methoxymorphinan (3-MM). There is then further demethylation of 3-MM by CYP2D6 enzymes and DX by CYP3A enzymes to a third metabolite, 3-hydroxymorphinan (3-OH) (Fig. 1).

It has been reported that the intake of certain food groups such as citrus juices can change the bioavailability of drugs [10]. One of these citrus juices is grapefruit juice. Its effect on drug metabolism has been extensively studied because it is often taken at breakfast concomitantly with many drugs. Since there is an overlap between CYP3A and P-glycoprotein substrate specificity [6], Grapefruit juice also affects drug transport in the intestinal wall. It has recently been established that certain components (such as citrus psoralens) in grapefruit juice are responsible for the inhibition of p-glycoprotein [12].Grapefruit juice has been shown to increase the oral bioavailability of many drugs, such as cyclosporine or saquinavir, by inhibiting intestinal metabolism [11,12]. This was proven because no interaction was seen when saquinavir or cyclosporine are administered intravenously and grapefruit juice was given orally [11,12]. This effect appears to be specific to CYP3A enzymes and P-glycoprotein transporters that are found in the gut wall of the intestine since grapefruit juice does not appear to significantly affect enzymatic liver activity [11,12].

Another citrus juice that appears to affect drug bioavailability is the juice derived from seville oranges (SOJ). Seville orange juice is not usually consumed as a juice because of its sour taste, but it is found in marmalade and other jams. Seville orange juice has been reported to be a possible inhibitor of CYP3A enzymes without affecting P-glycoprotein when taken concomitantly with cyclosporine [13].

The objective of this study was to investigate the effect of grapefruit and seville orange juice on the activities of CYP2D6, CYP3A and P-glycoprotein and the resulting effect on the pharmacokinetics of DM. The results of this study present differential effects of grapefruit and seville orange juice on DM

pharmacokinetics. DM could provide some useful information on P-glycoprotein or related membrane efflux protein activity in the human gastro-intestinal tract.

Methods and Materials

Clinical Methods

This study was conducted over a three-week period and included 11 healthy volunteers (5 females and 6 males). Subjects ranged from 14 to 43 years of age (median of 32 years). Each patient's demographic, medical and sample data were recorded on a case report sheet. Grapefruit juice was prepared by diluting 1 part 100% pure concentrated frozen (Minute Maid™) juice with 3 parts of water. Seville orange juice was prepared by squeezing fresh fruit. For each individual volunteer, 200 ml of water and juice was measured in a plastic container. There were 5 study days, each separated by 3 days of washout. A dose of 30 mg of DM hydrobromide (contained in 10 ml of the over-the-counter cough medicine Balminil DM®) was taken on each study day before bedtime. Volunteers took the DM followed by 200 ml of water (Study Day 1), by 200 ml of grapefruit juice (Study Day 2), by 200 ml of water (Study Day 3), by 200 ml seville orange juice (Study Day 4) and by 200 ml of water (Study Day 5). On each of the 5 days, a spot urine sample was obtained before administration of DM and the 8-h complete urine output was collected in the morning. Consumption of any caffeine containing beverages (such as coffee, soda, etc.. . .) was prohibited for at least 8 h before taking any dose of DM.

Analytical Method

Aliquots of the hydrolysed urine samples were assayed according to the method developed by Wenk et al. [14]. Briefly, urine blanks were spiked with a concentration (38 ug/ml) of the internal standard, levalorphan, and concentrations of DM, DX, 3-MM, 3-OH to form a calibrated standard curve. The urine samples were incubated at 37 °C for 18 h with 20 ul of β-glucuronidase and 0.75 ml 0.1 M sodium acetate (pH 5.0). Bond-Elut 1 ml C_{18} solid-phase extraction columns (Analytichem Int., Harbor City, Ca., USA) were pre-conditioned with (6 ml) methanol and (6 ml) water and 4 ml 0.1 M sodium carbonate (pH 9.2). Deconjugated urine samples (0.25 ml) and 2 ml of 0.1 M sodium carbonate buffer (pH 9.2) were deposited onto the columns. Volumes were aspirated under a light pressure and columns were sequentially washed with 2 ml of water and 2 ml of acetonitrile. DM and metabolites were then eluted with a mixture of 3 ml of methanol-acetonitrile-2% phosphoric acid (5:3:2, v/v/v) and evaporated to dryness using a Speed Vac. The resulting residues were reconstituted in 0.01 M hydrochloric acid and an aliquot was injected onto the HPLC system for analysis. The HPLC column used in the analysis was a Rexchrome S5μ, 100 A° phenyl (15 cm × 4.6 mm i.d.) column, coupled with a phenyl guard column (Regis Technologies, IL, USA). The chromatographic system was composed of a Thermo Separations (SP) p4000 quaternary pump, a SP automatic injector and a SP (FL 3000) fluorescence detector. The optimum wavelength for detection was λ = 238 nm with no cut off for emission. The mobile phase consisted of acetonitrile:potassium phosphate buffer [10 mM, pH 3.5] (35:65, v/v) applied as an isocratic gradient with a flow rate of 0.8 ml/min for 5 minutes and then 1.0 ml/min for the rest of the 25 minutes run.

Validation of the analytical assay

The analytical assay was validated for linearity and precision. Blank urine samples were spiked with all four analytes at a concentration range of 0.5 to 49.0 ug/ml. Linearity was assessed by calculating the linear regression coefficient (R) of the parent compound (DM) and the metabolites DX, 3-OH and 3-MM. Calibration curves were produced using peak area of the analyte over peak area of the internal standard (levalorphan) versus the analyte concentration. The linear regression coefficient (R) for the following compounds tested were: DM: R = 0.945, DX: R = 0.978, 3-OH: R = 0.864, 3-MM: R = 0.959.

Precision of the analytical method was measured for repeatability and reproducibility of the analyte concentrations within assays (intra-day) and between days (inter-day). The intra-day assay variabilities (n = 5) were determined to be (CV%; coefficient of variation): DM = 14.5 and 7.4% (3.9 and 1.3 ug/ml); 3-MM = 14.2 and 6.2% (5.2 and 1.7 ug/ml); 3-OH = 15.5 and 22% (3.1 to 1.0 ug/ml); DX =15 and 10.5% (6.5 and 2.2 ug/ml). The inter-day assay variabilities over a three day period for high and low concentrations were determined to be: DM = 5.1 and 4%; 3-MM = 6.9 and 2.2%; 3-OH = 7 and 10% and DX = 9.4 and 5%.The limit of quantification of the analytical method (LOQ) was at 0.1 μg/ml for the four analytes.

Analysis of the Data

Excreted amounts of DM and metabolites in the urine

The complete elimination of DM and its 3 metabolites was determined in each volunteer by multiplying the urinary concentrations by the total urine volume. Since all excretion pathways were accounted for, the amount of DM and its excreted metabolites in the urine indicated how much of the oral dose was absorbed.

Bioavailability (F) or the fraction of the ingested dose of a drug that is available to the systemic circulation in the human body after oral administration of a drug [15,16] is the product of the fraction that is absorbed (F_A); the fraction of drug that is absorbed and not metabolized in the gut wall (F_G), and the fraction of the absorbed drug that escapes metabolism in the gastrointestinal tract and liver (F_H). Hence, [$F = F_A \times F_G \times F_H$].

Since systemically available DM is almost exclusively metabolized by the liver, the total clearance approximates the hepatic clearance ($CL_H = Cl_T$). F_H can therefore, be estimated by the following equation: $F_H = 1 - (CL_H/Q_H)$, assuming a normal hepatic blood flow of 1.5 L/min. In rats, the bioavailability of DM has been reported to be 10% on average [17]. We set F to that value because no human data is available on the bioavailability of DM and it is therefore the most proper value to use. It was then possible to calculate F_A for all the study days since we had the total percent of DM and metabolites excreted in the urine in each volunteer for every study day. We assumed for simplicity purposes, that grapefruit juice on Study day 2 completely inhibited P-glycoprotein activity in the gut wall allowing 100% absorption of DM ($F_A = 1$). This assumption is reasonable since the total excretion of DM increased by more than 2.5 fold when taken with grapefruit juice. Regardless of the value that one starts at, the results and interpretation would be the same since they are based on the observation that the absorption is increased by 2.5 fold with GJ. F_A on Study Day 1 was then easily calculated by dividing the fraction absorbed (1.0) by the ratio of the total % excreted on Study day 2 (ie., $1.0/(14.1/4.04) = F_A$). The bioavailability parameters for the other study days may then be

calculated by multiplying 1 by the ratio of total % excreted on that study day by the total % excreted on study day 2 (eg., Study day 3: $F_A = 1/(9.2/14.1)$).

Since, F, F_H and F_A were each estimated on Study Day 1, F_G was simply calculated by the formula $F_G = F/F_A * F_H$. Because CYP3A enzymes are present in the gut wall and are the most important contributors to gut wall metabolism of DM, the F_G parameter for all the other study days were calculated using the relative CYP3A enzymatic activity (see below) for that study day compared to the Study Day 1 : $F_{G\ Day\ i} = F_{G\ (Day\ 1)}$ * CYP3A activity (Day i)/CYP3A activity (Day 1) (e.g., $F_{G\ (Day\ 2)} = 0.349 * 0.14/0.09$).

Determination of partial metabolic clearances

The phenotypic activity of CYP2D6 and CYP3A for each volunteer were determined by using two approaches. It is well known that poor metabolizers (PM) of CYP2D6 have an abundance of unmetabolized DM with very little of its metabolites present in the urine after several hours following the administration of a single oral dose [17]. On the other hand, individuals classified as EM have the ability to metabolize DM to its respective metabolite (DX) by CYP2D6 enzymes with very little trace of DM excreted unchanged in the urine. It was therefore, directly evident from the amount of DM/DX excreted in urine, which subject was a poor or extensive metabolizer of CYP2D6. This status was merely qualitative and did not provide a "quantitative" measure of how active CYP2D6 enzymes were in each of these subjects. It was therefore, important to derive and provide a robust method to calculate the relative activity of CYP2D6 and CYP3A in these subjects. This was done using the pharmacokinetic information hidden in the urinary excretion of DM and of its 3 metabolites over an 8-h period.

Partial metabolic clearances, which in the case of DM are reflective of CYP3A and CYP2D6 activities, were therefore calculated according to a modified version of the equations proposed by Lessard et al. [18]. The partial metabolic clearance of DM to the O-demethylated metabolites is an index of CYP2D6 enzyme activity while the partial metabolic clearance of DM to the N-demethylated metabolites is an index of CYP3A activity (Fig. 1).

Equations to phenotype for CYP2D6 and CYP3A enzymatic activities

$$CL(CYP2D6) = [(Ae_{DX}*2) + Ae_{3-OH}]/4)/Total\ excreted \tag{1}$$

$$CL(CYP3A) = [(Ae_{3-OH}*2) + Ae_{3-MM}]/4)/Total\ excreted \tag{2}$$

Where Ae_{DX} represents the excreted amount (umoles) of DX arising from the metabolism of DM by CYP2D6 enzymes (O-demethylation) while Ae_{3OH} is the excreted amount (umoles) of 3-OH arising from the metabolism of 3-MM by CYP2D6 enzymes. In Eq. (2), $Ae_{3\text{-}MM}$ represents the excreted amount (umoles) of 3-MM arising from the metabolism of DM by CYP3A enzymes, while Ae_{3OH} represents the excreted amount (umoles) of 3-OH arising from the metabolism of DX by CYP3A enzymes. Since CYP3A enzymes are present in the gut wall and in the liver, contrary to CYP2D6 enzymes which are located primarily in the liver, an adjustment had to be incorporated in the formula to account for the fact that DM and its metabolites are "in contact" with CYP3A enzymes twice as often as with CYP2D6. In

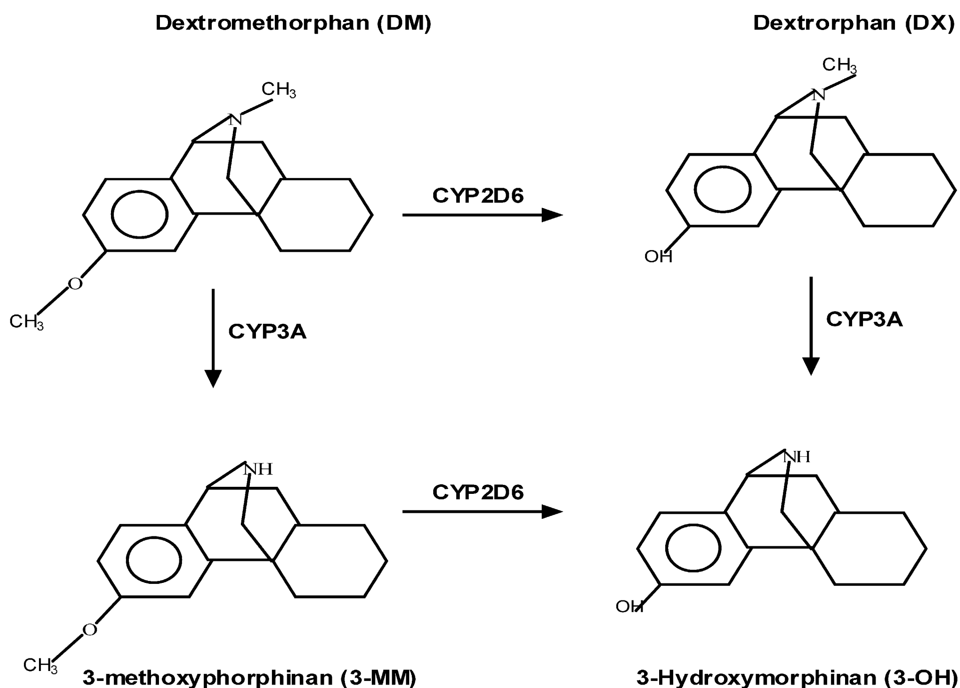

Fig. 1. Chemical structures of DM and of its O- and N-demethylated metabolites.

the formulas, total excreted represents the total amount of DM, 3-OH, DX and 3-MM excreted in the urine during the 8-h period.

Statistical Analyses

Statistical analysis was performed with SYSTAT for Windows (V5.03, SYSTAT, Evanston IL,1993). Differences between treatment groups in the amount excreted of DM and its metabolites were

Table 1
PK Parameters of DM when administered with H_2O, GJ and SOJ in volunteers who are EM

PK parameters	CTL Day 1 (Mean)	GJ Day 2 (Mean)	CTL Day 3 (Mean)	SOJ Day 4 (Mean)	CTL Day 5 (Mean)
Total % Excreted (±SD)	4.04 ± 5.0	14.1 ± 6.3*	9.2 ± 3.6	13.3 ± 8.1*	10 ± 2.57
F	0.10	0.54*	0.29	0.46*	0.29
F_H	0.99	0.99	0.99	0.99	0.99
F_A	0.29	1.0	0.65	0.94	0.71
F_G	0.35	0.54	0.44	0.49	0.41

Note: F: bioavailability [$F = F_H \times F_G \times F_A$]; F_H: (CYP3A/CYP2D6) escaping metabolism by the liver where $F_H = 1 - (CL_H/Q_H)$ and $CL_H = CL_T$ and Q_H assumed 1.5 L/min; F_G: (CYP3A) fraction escaping metabolism in the intestinal gut wall; F_A: (P-glycoprotein) fraction that is absorbed; CTL: control days when DM was taken with water.
P value <0.05 when all 5 treatments are compared between each other. * Day 2 and Day 4 are not significantly different from each other.

Table 2
Partial metabolic clearances of DM to O- and N-demethylated metabolites as indexes of CYP2D6 and CYP3A activities obtained after oral administrations of DM with H_2O, GJ and SOJ in individuals who are EM

DAYS	EM (n=9)	
	O-DEM CYP2D6 (L/h)	N-DEM CYP3A (L/h)
1 (H_2O)	0.43 ± 0.02	0.14 ± 0.04
2 (GJ)	0.46 ± 0.01	0.09 ± 0.03
3 (H_2O)	0.45 ± 0.01	0.11 ± 0.04
4 (SOJ)	0.42 ± 0.04	0.10 ± 0.01
5 (H_2O)	0.44 ± 0.02	0.12 ± 0.01

determined using a one way analysis of variance (ANOVA) for repeated measures. If a statistical difference was found between groups ($P < 0.05$), contrast testing was performed to see where the differences between the study days occurred.

Results

Pharmacokinetic Results

Bioavailability (F) calculations are presented numerically in Table 1. Computations of F_H (an index of CYP3A4/CYP2D6 activity in the liver), F_A (an index of P-glycoprotein activity in the

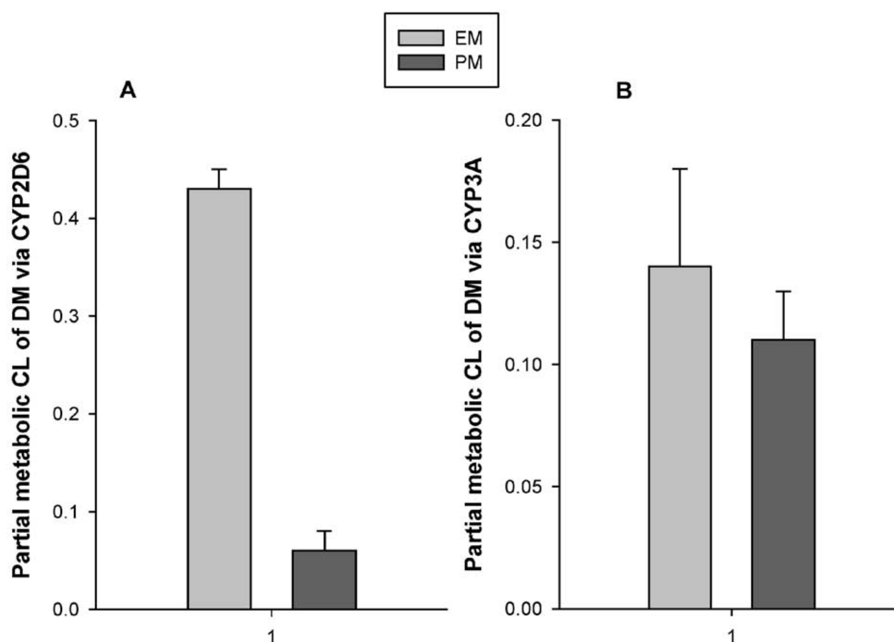

Fig. 2. Comparison of partial metabolic CL of A) CYP2D6 and B) CYP3A activities for EM and PM individuals that were calculated on Study Day 1.

intestinal gut wall) and F_G (an index of CYP3A activity in the intestinal gut wall) are also presented in Table 1. The P-glycoprotein nomenclature in this study does not necessarily mean that it is "the P-glycoprotein" that transports DM and/or its metabolites, since it may be the other analogous transport proteins yet to be named. We are referring to it as P-glycoprotein for readability purposes, and because most substrates of CYP3A are also substrates of P-glycoprotein. The results in Table 1 show that F_H remained constant (0.99) throughout the 5 study days, suggesting that grapefruit and seville orange juice had no influence on the enzymatic activity of CYP2D6/CYP3A enzymes found in the liver. The fraction of the administered dose of DM that escaped first pass metabolism (F_G and F_A) were found to increase significantly when grapefruit or seville orange juice were taken with DM on study days 2 and 4, respectively. As Table 1 demonstrates, grapefruit and seville orange juice significantly increase the excretion of DM and its metabolites ($P < 0.05$). In comparison, no differences were noted between the different control days when DM was administered with water ($P = 0.79$).

After analysis of the urine samples, it was evident that 2 out of the 11 volunteers in this study had a CYP2D6 PM phenotype while the remaining 9 had EM phenotypes. The results obtained from the proposed phenotyping calculations are presented numerically in Table 2.

Fig. 2 graphically demonstrates the comparison of EM vs. PM calculated phenotyping results that were obtained for study Day 1 using the previously proposed equations. The average calculated metabolic CL via CYP2D6 was 0.43 ± 0.02 for EM and 0.06 ± 0.02 L/h for PM. The average calculated metabolic CL via CYP3A was 0.14 ± 0.04 for EM and 0.11 ± 0.02 L/h for PM.

Discussion

DM has been considered by many to be a suitable probe for phenotyping subjects as either EM or PM relative to CYP2D6 activity [19,20]. It has been reported that unmetabolized DM is the major component excreted in urine by individuals that are PM [17]. In this study, 2 out of the 11 volunteers were determined to be PM of CYP2D6 enzymes after an 8-h urine collection where the excretion of DM and its 3 metabolites were quantified. In addition, since PM subjects do not metabolize DM, they have larger circulating plasma concentrations of DM which makes them drowsy. This side effect was observed in the 2 PM subjects.

Individuals who were determined to have an EM phenotype excreted DX as the major metabolite in the urine. This finding is in agreement with other published studies for individuals who are EM [17]. As the schematic diagram of the metabolism of DM in Fig. 3 illustrates, DM is metabolized in the liver by CYP2D6 enzymes to form the DX metabolite. This metabolite is active and has been found to be the one that is responsible for a large proportion of the antitussive effect of DM [20].

In previous papers, phenotypic determinations of CYP2D6 or CYP3A were usually carried out with the administration of DM and measuring the concentrations of DM, DX and 3-MM in a "spot" urine sample to derive a simple metabolic ratio. However, since DX and 3-MM are further metabolized to 3-OH by another enzyme, this method of calculating the metabolic ratio may not be robust [20,21]. We are proposing another method to calculate the activity of CYP2D6 and CYP3A in subjects by taking into account further demethylation to 3-OH by DX and 3-MM in the equation presented in this paper (Data Analysis section in "Determination of partial metabolic clearances"). Very little change was observed (Table 2) on all 5 study days when DM was O-demethylated to DX via CYP2D6 enzymes found in the

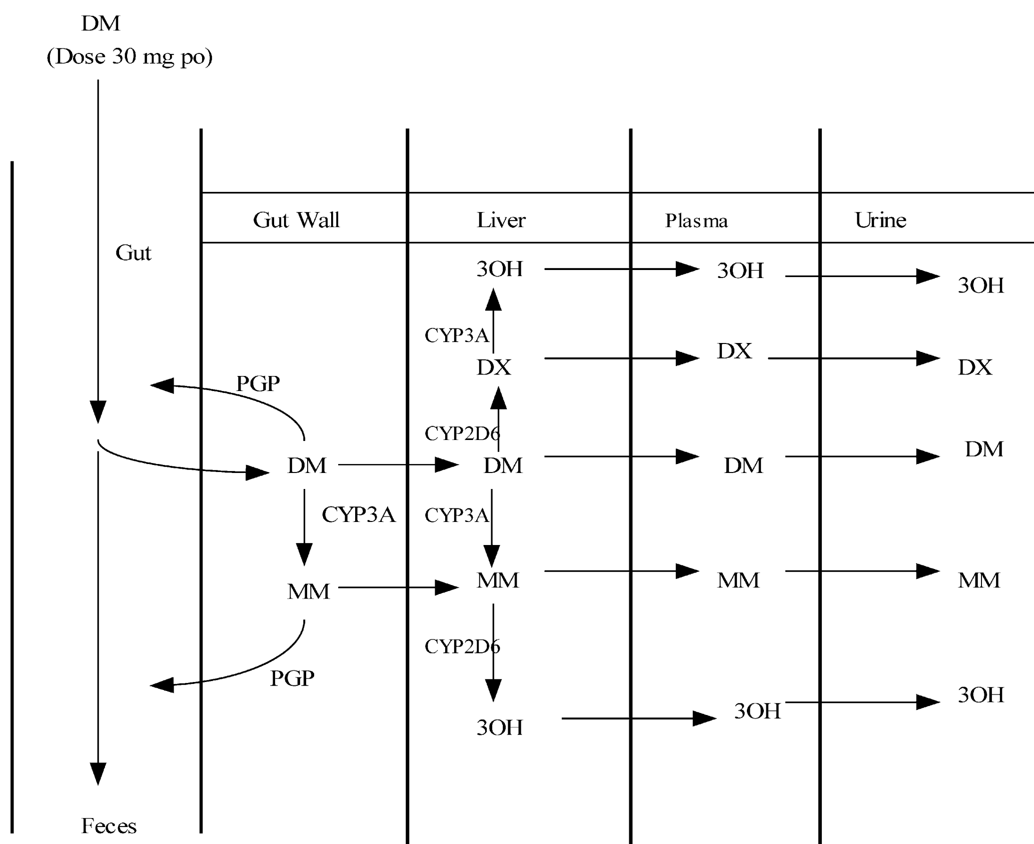

Fig. 3. Model depicting first pass metabolism of DM and elimination of DM and its metabolites from the circulatory system (plasma) into urine.

liver in volunteers who were deemed EM. This confirms the lack of inhibition of CYP2D6 enzymes by GJ and SOJ. PM were also found to have a much lower CYP2D6 activity than EM (eg., 0.43 L/h for EM vs. 0.06 L/h for PM on Study day 1, a finding supporting the proposed formulas.

Previous papers have reported that GJ is an inhibitor of intestinal CYP3A enzymes found in the gut but not CYP3A enzymes found in the liver [11]. In that study the administration of GJ with cyclosporine, was shown to affect F without affecting F_H. Meaning that GJ affects gut wall metabolism but not liver CYP3A activity. In the present study, N-demethylated metabolites (DM to 3-MM) formed via CYP3A were affected by the consumption of grapefruit or seville orange juice. On the other hand, no change was seen on the formation of 3-OH from DX, further demonstrating that these citrus juices only affect CYP3A enzymes and/or transporters in the intestinal gut wall and not liver CYP2D6 activity (Table 2). When CYP2D6 and CYP3A activity were calculated for volunteers who were determined to be PM, a remarkable difference was observed compared to volunteers determined to be EM. Subjects that were deemed as PM cleared very little of the drug (DM and metabolites) as compared to EM on all 5 days of the study.

The total per cent excretion of the drug was calculated using the urinary concentrations of DM and all of the DM metabolites after administration of DM with GJ, SOJ and water. The only source of an increase in

the total per cent excreted was an increase in the absorption process (F_A) of the drug through the gut wall. When the volunteers with an EM phenotype were concomitantly administered DM and grapefruit juice (Study Day 2) there was an increase in F (54%) as compared to the value of F (10%) observed after the concomitant administration of DM and water (Study Day 1). The increase in the absorption process (F_A) suggests inhibition of P-glycoprotein activity in the intestinal gut wall by GJ or SOJ.

After the concomitant administration of DM and grapefruit juice (Study Day 2), there was a 3-day washout period. The subjects were then concomitantly administered DM and water (Study Day 3). However, the observed F_A (65%) did not completely decrease to the baseline value (29%) observed on Study Day 1. This indicates that grapefruit juice may have produced an irreversible or quasi-irreversible inhibition of P-glycoprotein and CYP3A activity in the gut wall. The administration of grapefruit and seville orange juice in each volunteer were separated by 7 days. We believe that this "washout " was sufficient to ensure the return to baseline of P-glycoprotein and CYP3A activity before seville orange juice was administered. This is in agreement with another study that reported that the inhibitory effect of grapefruit juice disappears after 3 to 7 days [22]. The administration of seville orange juice with DM (Study Day 4) produced an identical effect on the DM pharmacokinetic profile as was observed with the concomitant administration of DM and grapefruit juice. This suggests that grapefruit and seville orange juice contain the same type of inhibitors. This study also demonstrates that grapefruit and seville orange juice have long lasting inhibitory properties requiring at least a seven-day washout period.

In this study, grapefruit and seville orange juice have been found to contribute to the increase in bioavailability and absorption of DM by affecting first pass metabolism and absorption through the intestinal gut wall. This increase suggests inhibition of CYP3A (F_G) and P-glycoprotein or related membrane efflux protein (F_A) activities. Other reports have demonstrated that DM undergoes a marked first pass elimination due to the enzymatic activity of CYP3A and CYP2D6, but not due to the influence of P-glycoprotein proteins [16]. This paper contradicts this finding and demonstrates that an increase in the absorption of DM is seen with grapefruit and seville orange juice. The only possible way that the total excreted amounts of DM and its metabolites increased is that the activity of the P-glycoprotein transport protein is affected by grapefruit and seville orange juice [23]. These results suggest that DM appears to be a substrate of P-glycoprotein or related membrane efflux protein activities in the human gastro-intestinal tract.

In conclusion, we have demonstrated the inhibitory effect of grapefruit and seville orange juice on the pharmacokinetics of DM by showing an inhibition of CYP3A activity as well as P-glycoprotein. The increase in the fraction absorbed (F_A) when GJ or SOJ was taken with DM caused a simultaneous irreversible or quasi-irreversible inhibition of P-glycoprotein and CYP3A activity in the gut. We propose to phenotype subjects using partial metabolic clearances as indexes of CYP2D6 and CYP3A activity, while the total amount of DM and metabolites excreted will be a relative activity index between subjects for the P-glycoprotein activity. The equations proposed in this paper may therefore, be a more robust way of calculating the activities of CYP2D6, CYP3A and P-glycoprotein simultaneously in individuals using DM as the probe drug.

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References

[1] Lown KS, Mayo RR, Leichtman AB, Hsiao HL, Turgeon DK, Schmiedlin-Ren P, Brown MB, Guo W, Rossi SJ, Benet LZ, Watkins PB. Role of intestinal P-glycoprotein (mdr 1) in interpatient variation in the oral bioavailability of cyclosporine. Clinical Pharmacology and Therapeutics 1997;62(3):248–60.

[2] Wacher VJ, Silverman JA, Zhang Y, Benet LZ. Role of P-glycoprotein and cytochrome P450 3A in limiting oral absorption of peptides and peptidomimetics. Journal of Pharmaceutical Sciences 1998;87(11):1322–30.

[3] Robert J. Structure and function of P-glycoprotein. Bulletin du Cancer, vol. 81 (5). France: Fondation Bergonie et universite de Bourdeaux II, 1994;87. p. 400–8.

[4] Silvasti M, Karttunen P, Tukiainen H, Kokkonen P, Hanninen U, Nykanen S. Pharmacokinetics of dextromethorphan and dextrorphan: a single dose comparison of three preparations in human volunteers. International Journal of Clinical Pharmacology and Therapeutics Toxicology 1987;25(9):493–7.

[5] Van Asperen J, Van Tellingen O, Beijnen JH. The pharmacological role of P-glycoprotein in the intestinal epithelium. Pharmacology Research 1998;37(6):429–35.

[6] Wacher VJ, Wu CY, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochrome P450 3A and P-glycoprotein: implications for drug delivery and activity in cancer chemotherapy. Molecular Carcinogenesis 1995;13(3): 129–34.

[7] Ducharme J, Abddullah S, Wainer IW. Dextromethorphan as an in vivo probe for the simultaneous determination of CYP2D6 and CYP3A activity. Journal of Chromatography B, Biomed Applications 1996;678(1):113–28.

[8] Jones DR, Gorski JC, Haehner BD, O'Mara Jr EM, Hall SD. Determination of cytochrome P450 3A4/5 activity in vivo with dextromethorphan N-demethylation. Clinical Pharmacology and Therapeutics 1996;60(4):374–84.

[9] Kamm JJ, Taddeo AB, Van Loon EJ. Metabolism and excretion of tritiated dextromethorphan by the rat. Journal of Pharmacology and Experimental Therapeutics 1967;158(3):437–44.

[10] Bailey DG, Arnold JM, Bend JR, Tran LT, Spence JD. Grapefruit juice-felodipine interaction: reproducibility and characterization with the extended release drug formulation. British Journal of Clinical Pharmacology 1995;40(2): 135–40.

[11] Ducharme MP, Warbasse LH, Edwards DJ. Disposition of intravenous and oral cyclosprine after administration with grapefruit juice. Clinical Pharmacology and Therapeutics 1995;57(5):485–91.

[12] Kupferschmidt HH, Fattinger KE, Ha HR, Follath F, Krahenbuhl S. Grapefruit juice enhances the bioavailability of the HIV protease inhibitor saquinavir in man. British Journal of Clinical Pharmacology 1998; 45(4):355–9.

[13] Edwards DJ, Fitzsimmons ME, Schuetz EG, Yasuda K, Ducharme MP, Warbasse LH, Woster PM, Schuetz JD, Watkins P. 6′,7′ -Dihydroxybergamottin in grapefruit juice and Seville orange juice:effects on cyclosporine disposition, enterocyte CYP3A4, and P-glycoprotein. Clinical Pharmacology and Therapeutics 1999;65(3):237–44.

[14] Wenk M, Todsco L, Keller B, Follath F. Determination of dextromethorphan and dextrorphan in urine by high-performance liquid chromatography after solid-phase extraction. Journal of Pharmaceutical and Biomedical Analysis 1991;9(4):341–4.

[15] Shargel L, Yu ABC. Hepatic elimination of drugs. In: Mehalik CL, editor. Applied Biopharmaceutics and Pharmacokinetics. Appleton and Lange. Stamford, Conneticut; 1999. pp. 353–89.

[16] Navia MA, Chaturvedi PR. Design principles for orally bioavailable drugs. D.D.T. 1996;1(5):179–89.

[17] Wu D, Otton SV, Kalow W, Sellers EM. Effects of route of administration on dextromethorphan pharmacokinetics and behavioral response in the rat. Journal of Pharmacology and Experimental Therapeutics 1995;274(3): 1431–7.

[18] Schadel M, Wu D, Otton SV, Kalow W, Sellers EM. Pharmacokinetics of dextromethorphan and metabolites in humans:influence of the CYP2D6 phenotype and quinidine inhibition. Journal of Clinical Psychopharmacology 1995;15(4):263–9.

[19] Lessard E, Yessine MA, Hamelin BA, O'Hara G, LeBlanc J, Turgeon J. Influence of CYP2D6 activity on the disposition and cardiovascular toxicity of the antidepressant agent venlafaxine in humans. Pharmacogenetics 1999;9(4):435–43.

[20] Price Evans DA. Genetic factors in drug therapy. Clinical and molecular pharmacogenetics. New York: Cambridge University Press, 1993.

[21] Kashuba AD, Nafziger AN, Kearns GL, Leeder JS, Gotschall R, Gaedigk A, Bertino Jr JS. Limitations of dextromethorphan N-demethylation as a measure of CYP3A activity. Pharmacogenetics 1999;9(4):453–62.
[22] Bochner F, Somogyi AA, Chen ZR. Dextromethorphan metabolism in rat:interstrain differences and the fate of individually administered oxidative metabolites. Xenobiotica 1994;24(6):543–52.
[23] Lilja JJ, Kivisto KT, Neuvonen PJ. Duration of effect on grapefruit juice on the pharmacokinetics of the CYP3A4 substrate simvastatin. Clinical Pharmacology and Therapeutics 2000;68(4):384–90.