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Cytochrome P-450IID6 phenotyping in cancer patients: debrisoquin and dextromethorphan as probes

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Abstract The usefulness of substituting dextromethorphan for debrisoquin as a probe for cytochrome P-450IID6 deficiency was investigated in 20 male cancer patients. Each patient was studied on two occasions. An oral dose of dextromethorphan (60 mg) was administered to 13 patients and are week later an oral dose of debrisoquin (10 mg) was administered to each patient. The order was reversed for the other 7 patients. An 8-h urine sample was collected after administration of each test drug and assayed for parent drug and metabolites. Five poor metabolizers (PMs) and 15 extensive metabolizers (EMs) of debrisoquin were tested. The debrisoquin metabolic ratio (DMR), calculated as [parent drug]/[metabolite], correlated with the metabolic ratio of dextromethorphan ($R^2 = 0.58$, $P = 0.0001$). All PMs of debrisoquin (metabolic ratio > 12.0) were easily identified as being PMs of dextromethorphan (metabolic ratio > 0.30). Within the EM group, there was a significant correlation between the metabolic ratios of debrisoquin and dextromethorphan ($R^2 = 0.82$, $P < 0.0001$). There was not as clear a correlation in the PM group ($R^2 = 0.32$, $P = 0.32$). These findings suggest that dextromethorphan can be substituted for debrisoquin in establishing the debrisoquin phenotype in a patient population with metastatic cancer.

Key words Cytochrome P-450IID6 · Debrisoquine · Dextromethorphan

Introduction

Genetic polymorphisms are important in identifying subjects at risk not only for adverse drug reactions [1,3,5,6,7,11,14,19,20,23,25], but possibly also for certain diseases [2,3,8,9,15,22]. Debrisoquin is a guanethidine-like antihypertensive whose metabolism is polymorphically controlled [15,23,27]. A subject population can be divided into extensive and poor debrisoquin metabolizers depending on whether a wild-type or mutant allele is expressed on chromosome 22 [12,22]. The oxidative metabolic routes of numerous other drugs cosegregate with debrisoquin, which serves as a model substrate to predict the other drug's disposition.

Debrisoquin phenotyping is complicated by difficulties in drug assay methodology and by the investigational status of debrisquin in the US. The relative insolubility of the 4-OH debrisoquin metabolite and the requirement for parent and metabolite quantitation by gas chromatography/mass spectrometry limit the widespread use of this phenotyping method. Debrisoquin's investigational status makes it not only difficult to obtain, but complicated to explain to potential subjects. Using debrisoquin to determine the cytochrome P-450IID6 phenotype may also result in adverse drug reactions (hypotension) in poor metabolizers.

The demethylation of dextromethorphan to dextrorphan is accomplished by cytochrome P-450IID6. Dextromethorphan is available as an over-the-counter agent and widely available HPLC analytical methodology allows for easier metabolite phenotype determination [17]. Previous work has shown that dextromethorphan can be substituted for debrisoquin in normal subjects [21]. However, the ability of dextromethorphan to substitute for debrisoquin in a patient population has not been tested. Within a patient population, the presence of confounding factors including illness, alcohol, tobacco, and medications may influence any correlation between dextromethorphan and debrisoquin. The objective of this study was to determine whether the more readily available dextromethorphan can be substituted for debrisoquin in newly diagnosed, previously untreated cancer patients.

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Methods

Subjects

The study involved 20 male patients (weight 72 ± 9 kg) ranging in age from 47 to 72 years with the characteristics shown in Table 1. Informed consent and previously untreated neoplastic disease with normal hepatic and renal functions were prerequisites for inclusion in the study. Drugs known to affect microsomal metabolism, such as phenobarbital or cimetidine, were not used for 1 week prior to the study. Cancer chemotherapy drugs were withheld until completion of the initial phenotyping.

Study design

Subjects were studied on two occasions approximately 1 week apart. Part 1 consisted of 60 mg of dextromethorphan (Robitussin; A.H. Robins, Richmond, Va.) administered orally followed by an 8-h urine collection. Part 2 consisted of 10 mg of debrisoquin (Declinax; Hoffman-La Roche, Nutley, N.J.) administered orally followed by a 8-h urine collection. Part 1 was completed first by 13 patients and part 2 firstly by 7 patients.

Sample analysis

A sensitive and specific HPLC dextromethorphan and dextrorphan assay was used [16]. The urine volume was recorded and a 1-ml sample incubated at 65°C for 6 h after thebaine (an internal standard), acetate buffer and beta-glucuronidase had been added. Following incubation, NaOH and a 0.05 *M* phosphate buffer were added to a pH of 11. Extraction with butanol/hexane (10:90 v/v) was followed by back extraction using HCl prior to injecting the sample onto the HPLC system. The chromatographic conditions included the use of a Waters system with a 25-cm Waters phenyl column, 51:49 acetonitrile/10 m*M* KH_2PO_4 (pH 4) containing 10 m*M* hexane sulfonic acid mobile phase at a flow rate of 1.2 ml/min, and a Perkin-Elmer fluorescent detector at an excitation wavelength of 228 nm with no emission cutoff filter.

Debrisoquin and its chief metabolite 4-OH debrisoquin were determined in an aliquot of the urine according to the method of Idle et al. [18] except for the addition of a water wash step for the toluene extract after derivatization. The addition of this step allowed the analysis to be performed on a capillary column without degrading the column performance.

Analysis was performed on a capillary gas chromatograph (model 6000, Varian, Walnut Creek, Calif.) fitted with a nickel-63 electron capture detector and a splitting capillary injector with a split ratio of 12:1. The column used was a 30-m DB-1 fused-silica capillary column (J&W Scientific, Folsum, Calif.) with an inside diameter of 0.32 mm and a film thickness of 0.25 μm. The carrier used was helium at a linear velocity of 24 cm/s. The oven temperature was held at 150°C for 1 min. The injector and detector temperatures were set at 250°C and 275°C respectively. The sample size injected was 1 μl. Quantitation was performed on a Chromatopac (model CR4A, Shimadzu Instruments, Columbia, Md.). Internal standardization and single point calibration were used. The retention times determined for debrisoquin, 4-OH debrisoquin and 7-methoxy-guanoxan (internal standard) were 8.0, 10.7, and 15.2 min, respectively.

Table 1 Characteristics of patients phenotyped with dextromethorphan and debrisoquin

Patient characteristic	*n*
Habits	
Ethanol use	12
Tobacco use	17
Liver function	
Total bilirubin < 1.5 mg/d	20
Kidney function	
Creatinine < 1.5 mg/d	17
Creatinine 1.5–2.1 mg/d	3
Tumor type	
Lung	12
Colorectal	2
Renal Cell	1
Prostate	1
Lymphoma	2
Unknown primary	1
Sarcoma	1

Data analysis

The percentage of parent drug and metabolite in each patient's urine was determined and the metabolic ratio was calculated from molar drug concentrations according to the expression:

metabolic ratio = [parent drug]/[metabolite]

From the metabolic ratio, each patient was classified as an extensive metabolizer (EM) or poor metabolizer (PM). A PM was defined as having an altered drug clearance evidenced by a metabolic ratio > 12.0 for debrisoquin and > 0.3 for dextromethorphan [24, 27].

Pearson's coefficient of correlation (*r*) was determined by plotting the dextromethorphan metabolic ratio (continuous, dependent variable) against that of debrisoquin (continuous, independent variable) using least-squares linear regression analysis. Correlations below 0.25 were chosen to indicate no relationship, 0.25–0.50 as indicating a fair degree of relationship, 0.50–0.75 as a good relationship and those above 0.75 as an excellent relationship [10]. In addition, for all analyses, a $P < 0.05$ criterion for significance was adopted.

Results

Figure 1A shows the correlation between the debrisoquin metabolic ratio and the dextromethorphan metabolic ratio in all patients ($n = 20$). The metabolic ratio of debrisoquin correlated with that of dextromethorphan ($R^2 = 0.58$, $P < 0.0001$). All PMs of debrisoquin (metabolic ratio > 12.0) were easily identified as being PMs of dextromethorphan (metabolic ratio > 0.30). The study included 15 EMs and 5 PMs.

Figure 1B shows the correlation between the debrisoquin metabolic ratio and the dextromethrophan metabolic ratio in the extensive metabolizer subpopulation ($n = 15$). Within the extensive metabolizer group there was a close correlation between the debrisoquin and the dextromethorphan metabolic ratio ($R^2 = 0.82$, $P = 0.0001$).

Figure 1C shows the correlation between the debrisoquin metabolic ratio and the dextromethorphan metabolic ratio in the PM subpopulation ($n = 5$). Within the PM group, the significant correlation found in the total population and in the EM subgroup was not apparent ($R^2 = 0.32$, $P = 0.32$).

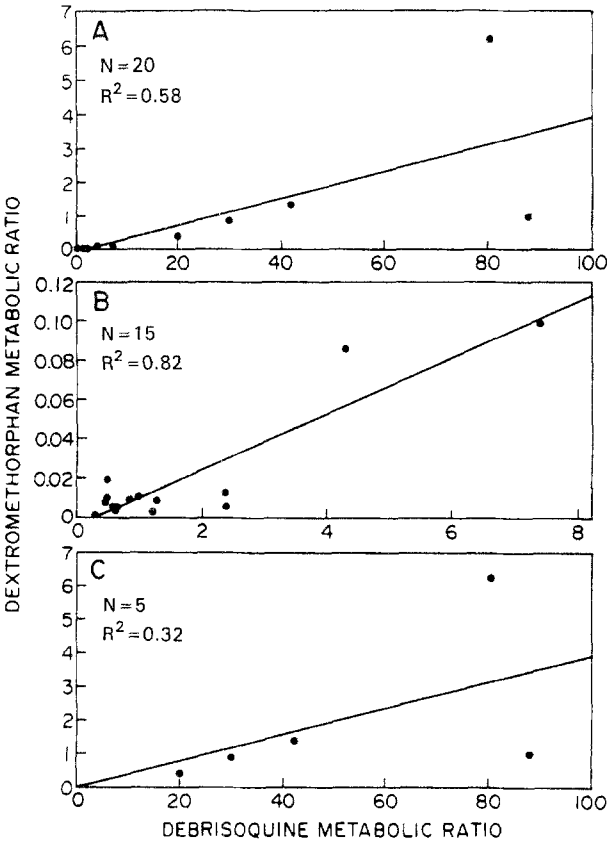

Fig. 1A–C Correlation of urinary dextromethorphan and debrisoquin metabolic ratios in cancer patients. **A** Data from all patients completing parts 1 and 2 of the study, **B** Data from the extensive metabolizer subgroup and **C** from the poor metabolizer subgroup

Discussion

In 1985, Schmid et al. [26] studied 268 healthy subjects with debrisoquin and dextromethorphan and found complete concordance of the two phenotypic assignments. They concluded that for practical purposes, the phenotyping protocol is the same for debrisoquin and dextromethorphan, except that the analysis of dextromethorphan is faster and simpler than that for debrisoquin. However, these were healthy subjects with minimal confounding variables present.

In this study, 20 male cancer patients were phenotyped using both debrisoquin and dextromethorphan as probes. This patient group was elderly, had a history of tobacco and ethanol use and was often receiving concomitant comedications. Six additional subjects refused debrisoquin phenotyping. The primary concern of these individuals was the investigational status of debrisoquin. Dextromethorphan was readily accepted by all patients since its administration as cough syrup was familiar. We found that the metabolic ratio of debrisoquin significantly correlated with that of dextromethorphan. All PMs of debrisoquin were easily identified as being PMs of dextromethorphan. Within the EM subgroup, there was also a good correlation between the metabolic ratios of debrisoquin and dextromethorphan. These findings suggest that the presence of cancer, smoking and/or ethanol history and concomitant medication use did not interfere with the phenotype classification. Secondly, in a patient population, the substitution of dextromethorphan for debrisoquin accurately classfied each subject as either an EM or a PM. A similar study in female cancer patients is needed to establish any gender differences.

Debrisoquin has been the standard pharmacologic probe for determining whether a subject is an EM or a PM. Restriction fragment length polymorphism (RFLP) and allele-specific polymerase chain reaction (PCR)-based DNA amplification techniques are available for determining cytochrome P450IID6 genotype. Genotyping is required to distinguish between a heterozygous or homozygous trait [13]. However, as suggested by Schmid et al. in 1985, and confirmed by this study, dextromethorphan is a more practical probe because of patients' familiarity with cough syrup, the ease of the assay for parent and metabolite concentrations, the lack of adverse drug reaction risk in PMs, and the ability to accurately assign debrisoquin phenotype. Cancer patients, among others, are exposed to many confounding events both intrinsically and extrinsically. However, this study revealed that, despite the presence of these variables, dextromethorphan remains an acceptable substitute and is preferred by patients.

Screening subjects for their ability to hydroxylate debrisoquin extends beyond the prediction of adverse drug reactions. Cytochrome P-450IID6 phenotype may also be useful in determining susceptibility to certain diseases. The debrisoquin EM phenotype correlated with an increased risk of lung cancer [8, 9] and bladder cancer [4]. The PM debrisoquin phenotype may be a risk factor for post-menopausal breast cancer [22]. Since cytochrome P-450IID6 activity appears to be a risk factor for some diseases, dextromethorphan is a more practical marker in assigning debrisoquin phenotype while screening patients for disease susceptibility. Even though genotyping may become the preferred method of identifying IID6 status, phenotyping remains widely available and dependable as a screening procedure.

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