Acta Pediatr Mex 2008;29(2):57-60

INP

Artículo original

Characterization of oxidative phenotype in a pediatric population of Tlaltizapan, Morelos, Mexico

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RESUMEN

Antecedentes: El polimorfismo genético tiene un papel importante en la respuesta a los medicamentos basada en la capacidad metabólica, la cual determina la variabilidad interindividual. Las diferencias cuantitativas en la expresión del sistema del citocromo P450 y del *CYP2D6*, son evaluados a través del metabolismo del dextrometorfán

Objetivo: Caracterizar el fenotipo oxidativo de una población pediátrica de Tlaltizapán, Morelos usando como fármaco sonda el dextrometorfán.

Material y métodos: Estudio prospectivo, transversal, descriptivo y experimental, en el Centro Rural de Investigación de Tlaltizapán, Morelos, dependiente del Instituto Nacional de Pediatría, de una población pediátrica sana. Se realizó un estudio de fenotipificación usando la excreción urinaria de dextrometorfán y su metabolito.

Resultados: Se estudiaron 17 niños. Cinco niños (29%) fueron metabolizadores lentos y 12 niños (71%) metabolizadores no lentos. Clínicamente conocer el tipo de metabolismo, sea lento o no lento, indica que los grupos ubicados en los extremos del histograma de frecuencias están en riesgo de tener reacciones adversas o falta de respuesta terapéutica, cuando reciben fármacos metabolizados por esta vía metabólica del CYP2D6.

Palabras clave: farmacogenética, metabolismo, fenotipo, CYP2D6, población rural.

ABSTRACT

Background: Genetic polymorphisms play an important role in the response to a drug regimen, based on the metabolic rate, which determines the interindividual variability. Quantitative differences in the cytochrome P450 expression, specially *CYP2D6*, can be assayed by means of a drug metabolism such as that of dextromethorphan.

Aim: To characterize the oxidative phenotype of a pediatric population in Tlaltizapan, Morelos, using dextromethorphan as a metabolic probe.

Materials and methods: This prospective, transversal, descriptive and experimental study was performed in a Mexican Rural Research Centre, located in Tlaltizapan, Morelos, a dependence of the National Institute of Pediatrics. It includes the phenotyping of a healthy pediatric population by evaluating their urinary excretion of dextromethorphan and its metabolites.

Results: Five children (29%) of the 17-subject population evaluated, were slow metabolizers, while the remaining 12 children (71%) were non-slow or fast metabolizers.

Conclusion: Clinically the type of metabolism within a population, whether slow or fast, indicates that individuals in both extremes of the histogram are in high risk of having adverse reactions to drugs, or poor developing or failed therapeutic response receiving when *CYP2D6*-metabolized drugs.

Key words: Pharmacogenetics, metabolism, phenotyping, CYP2D6, rural population.

Cytochrome P450 is a group of hemoproteins which catalize the biotransformation of endogenous and exogenous compounds; 56% of the liver-transformed drugs correspond to the CYP3A4 activity, 20% to the CYP2D6 and 15% to CYP2C9 and CYP2C19[1-3]. Phenotype is the physical expression of an individual's genetic information. In this study, the expression of the CYP proteins within a population can be evaluated as the rate of their metabolic processes such as oxidation [4].

CYP2D6 is an important protein, which catalizes the metabolism of endogenous compounds as well as a number of different drugs, such as tricyclic antidepressants, opioids, antipsycotic drugs, beta blockers and antiarrythmic drugs [5]. This cytochrome is coded on chromosome 22 and, due to its polymorphism, it is expressed in multiple metabolic processes [6]. Substances such as dextromethorphan can be used to assay the metabolic rate of CYP2D6, which may be different in certain populations, since biotransformation of the drug is a process which depends on individual's phenotype.Dextromethorphan is a central-acting opioid anticoughing agent, which prevents the coughing reflex acting directly in the medulla oblongata. It is absorbed through the gut, and plasmatic concentrations

can be detected within 15 to 30 minutes, and its effect lasts from 8 to 12 hours; it is metabolised by the CYP2D6 in the liver and its half life within the body is 11 hours. It is cleared through urine and feces. Adverse effects include nausea, vomiting, dizziness and somnolence, which are enhanced when combined with monoaminooxidase inhibitors. Dextromethorphan does not induce tolerance nor pharmacological dependence [7,8].

To date, there are reports in an interethnic phenotype prevalence based on dextromethorphan metabolism: 5-10% European Caucasians [9,10], 1-2% Afroamericans [11], 7.3% Uruguayans [12] and 3.2% Mexico Americans are slow metabolizers [13].

In a recent study conducted in Mexico, the phenotyping of 58 adult Tepehuans and 88 adult mestizos was performed; 6.8% of mestizos were slow metabolizers, but none of the Tepehuans showed this type of metabolism [14].

In our country, this drug is widely prescribed, mostly in rural communities. In a study by Chico and colleagues in 2003 in the community of Tlaltizapan, Morelos, dextromethorphan alone or combined was prescribed in 18.9% of the prescription notes released from local health centres; it was prescribed to less than 18-year old patients [15].

However, the use of dextromethorphan as a metabolic probe to assay the activity of CYP2D6 has not been reported in pediatric populations. It is a pharmacological tool to predict therapeutic success or an index of toxicity of related drugs.

Considering this background, the purpose of the present study was to characterize the oxidative phenotype of a pediatric population from Tlaltizapan, Morelos, Mexico, by using dextromethorphan as a pharmacological probe.

MATERIALS AND METHODS

A prospective, transversal, descriptive and experimental study was conducted in a Mexican Rural Research Centre, located in Tlaltizapan, Morelos, dependent of the National Institute of Pediatrics. Voluntarily participating healthy children, were included in the study. Children aged between 3 and 15 years, weighing over 15 kg, with adequate sphincter control and no history of enuresis. Their parents were born in Tlaltizapan. Children who received pharmacological treatment during the last two weeks, presenting with allergy or hypersensitivity to dextromethorphan: girls with evident or suspected pregnancy, drug use or alcohol and tobacco use, were excluded from the study.

This study was considered as a low-risk. Parents or responsible relative signed an informed consent letter.

A single oral dose 0.5 mg of dextromethorphan solution, per kilogram, was administered the night before, first-day urine was obtained the following morning.

The urine samples had their pH and total volume measured, and 5 mL aliquots were prepared to facilitate their transportation, in an ice-cold container, to the Laboratory of Pharmacology of our institution. Samples were stored at –70°C until analyzed.

Samples were analyzed by high performance liquid chromatography (HPLC) combined with fluorescence, through a special technique previously validated by our group [16].

The samples were hydrolyzed through an overnight incubation with beta-glucuronidase in a water bath at constant 37°C; 5 mL of hexane-butanol (95:5, v/v) were added to each 0.5 mL urine aliquot, then vigorously vortexed for one minute and centrifuged at 800 *g* for 5 minutes.

The organic phase was separated by evaporation at 37°C under a nitrogen stream. The remaining material was dissolved with 200 µL of mobile phase and 100 µL were injected to the chromatographic system.

Reagents. Dextrometorphan hydrobromide, dextrorphan-D-tartrate and verapamil hydrochloride were analytical grade from ICN Biomedical Inc. (Aurora, Ohio 44202, USA). Chromatographic solvents were HPLC grade. Beta-glucuronidase was from *Helix pomatia* 20,000 units (ICN

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Recibido: septiembre, 2007. Aceptado: diciembre, 2007.

Este artículo debe citarse como: Flores PJ, Juárez OH, Flores PC, Barranco GM, y cols. Characterization of oxidative phenotype in a pediatric population of Tlaltizapan, Mexico. Acta Pediatr Mex 2008;29(2):57-60.
La versión completa de este artículo también está disponible en: **www.revistasmedicasmexicanas.com.mx**

Biomedical Inc). Membrane filter Acrodiscs PVDF 25 mm 0.2 µm were from Waters Assoc., (Milford, MA, USA).

Instrumentation. A chromatographic system consisting of a solvent delivery pump Model 510 (Waters Assoc., Milford, MA, USA), Rheodyne 7125 six-way valve (Waters), fluorescence detector Model 2475 (Waters), reverse-phase column Spherisorb 5µm phenyl (25 x 0.46 cm id. Waters), and Millennium Software version 32 were used.

From the ratio between urinary concentration of dextromethorphan and dextrorphan (mean metabolite), the metabolic rate (MR) was determined and the oxidative phenotype was assigned. An MR less than 0.3 indicates non-slow metabolizers, while a MR over this value indicates slow metabolizers [17].

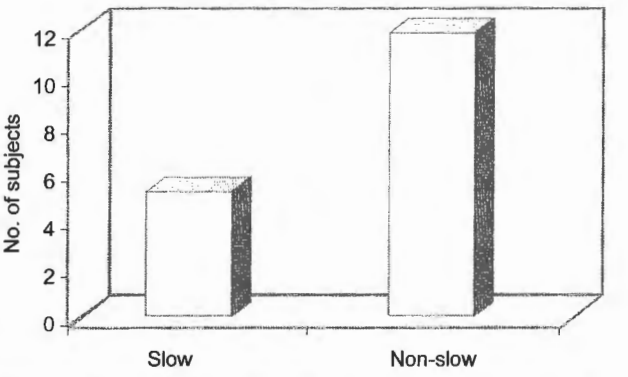

Figure 1. CYP2D6 phenotype from the pediatric population, Tlatizapan, Morelos.

RESULTS AND DISCUSSION

Seventeen healthy volunteers were included in the study, 9 females and 8 males, 3 to 15 years old, with a median of 9.3 years. The children weighed an average of 20.30 kg; height ranged from 0.98 to 1.69 m, median 1.36 m (see Table 1).

Analysis of the data according to the CYP2D6 phenotype found among the community, showed that 5 children (29%) were slow metabolizers; the remaining 12 children (71%) were phenotyped as non-slow metabolizers, as seen in Figure 1.

Phenotype results were loaded on ID cards which included general data of the children, the date of the study and metabolic characterization, including also a brief description of what it means to have either one or the other phenotype and, furthermore, a table containing CYP2D6-metabolized drugs as well.

These preliminary results suggest that further studies should be carried out in order to better understand the metabolic phenotype of our Mexican population, thus avoiding dependence on foreign literature which may orient us on the type of metabolic behaviour, but do not show specific information from our real phenotypes.

Clinically, the type of metabolism within a population, whether it is slow or not, indicates that individuals in both extremes of the histogram are in high risk of adverse reactions against the drugs, or of not responding to therapeutic measures, when *CYP2D6*-metabolized drugs are administered to them.

Table 1. Demographic and phenotypic data from the studied children

Total number of analyzed volunteers	*17 subjects median (range)*
Gender	9 females 8 males
Age (years)	9.35 (3-15)
Weight (kg)	20.30 (17.5-70)
Height (m)	1.36 (0.98-1.69)
Urinary pH	5.1 (4-6)
Dose interval (mg)	8.7-30
Metabolic rate for CYP2D6	
Slow metabolizers	54.52 (0.71-211.17)
Non-slow metabolizers	0.06 (0.01-0.1)
Metabolic characterization	
No. of subjects slow metabolizers	5 (29.41%)
No. of subjects non-slow metabolizers	12 (70.59%)

Acknowledgements

We thank José Luis Montesinos Merlos for the technical support and the community of Tlaltizapán, Morelos in Mexico for facilitating our study.

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