ISSN 0025-7680

ORIGINAL ARTICLE

MEDICINA (Buenos Aires) 1996; 56: 378-382

ENZYME POLYMORPHISM ON THE METABOLIC O-DEMETHYLATION OF DEXTROMETHORPHAN IN A SOUTHAMERICAN POPULATION

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Summary The polymorphic oxidative metabolism of debrisoquine and sparteine were discovered in the seventies by Mahgoub and Eichelbaum. Since then, many other therapeutic substances were added and one of these drugs is dextromethorphan. The object of this investigation was to ascertain the distribution of the oxidative phenotype of dextromethorphan in the Uruguayan population. The drug and its metabolite, dextrorphan, were quantified in the urine of 165 healthy volunteers by a modificacion of an HPLC method by Chen et al. The metabolic ratio was calculated and frequency distribution histograms were drawn. By inspection of the histogram two antimodes can be assigned which determine three sub-populations: on one side the fast extensive metabolizers (n = 30, 18.2%), in the middle the extensive metabolizers (n = 123, 74.5%) and on the other extreme of the histogram the slow metabolizers (n = 12, 7.3%). No other studies have confirmed thus far this trimodal distribution. This research will be continued by genotyping the populations studied in order to confirm these findings and to elucidate the underlying genetic mechanisms of the polymorphism.

Key words: P450 2D6, genetics, polymorphism, Southamerican population.

The polymorphic oxidative metabolisms of debrisoquine and of sparteine were discovered independently[1, 2]. During routine pharmacokinetic studies of the two drugs exaggerated pharmacological responses were observed in certain individuals. Further studies indicated that the individuals affected were not able to metabolize the probe drug in the same manner as others. Genealogical studies demonstrated that the impaired ability to metabolize these drugs was inherited as a Mendelian autosomal recessive trait[3]. Defective drug metabolism is due to the absence or marked reduction of cytochrome P450IID6 in the liver of most poor metabolizers[4].

Important individual differences in dextromethorpan O-demethylation were reported and a genetic basis was postulated. With the phenotype panel approach, a significant relationship between dextromethorphan O-demethylation and debrisoquine 4-hydroxylation could be established. Dextromethorphan provides a new probe drug for the detection of debrisoquine/sparteine type extensive (EM) and poor metabolizer (PM)[5].

Five to 10% of individuals in Caucasian populations are of the PM phenotype and show deficient metabolism of debrisoquine and of over 30 other drugs[3, 6, 7]. Arias et al.[8] found that Central American Cuna Amerindians from eastern Panama lack PM of sparteine. However, the same research group studying the Ngawbé Guaymí of Western Panama found 5.2% of PM.

Uruguay is nailed like a wedge between Brasil and Argentina, facing the Atlantic coast of South America at 35° latitude. Natives (Charrúas, Chanás, Guaraníes, Guayanás) inhabited its territory, part of the Rio de la Plata basin, until the second half of the XIX century when they were apparently «exterminated». However, anthropologists claim that there are still some population clusters

Received: 6-II-1996 Accepted: 17-IV-1996

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phylogenetically related to ancient local Amerindians.

Nowadays the Uruguayan population is composed mostly of the old Spanish descent, the immigrants (Spanish, Italians, and Central Europeans) who arrived during the first half of this century and of a small cluster of blacks brought as slaves from Africa during the XVIII/XIX century. Racial admixture has not been as intense as in other countries of the region although crossing between Caucasians (white Spanish) and black has been relatively common so that mulattos are a significant portion of the population.

The present communication describes the polymorphic distribution of the oxidative metabolism of dextromethorphan in a sample of healthy Uruguayan individuals.

Subjects and Methods

Subjects

A total of 165 unrelated, normal, healthy subjects were recruited among medical students from the university hospital and outpatients demanding medical control at suburb health units in Montevideo city. Subjects selected were non-smokers and were not taking any drug during and for at least two weeks before the study. The racial origin was confirmed by investigating their ancestry back to the third generation and by employing anthropometric criteria. Physical medical examination and blood biochemistry were performed to rule out an unrecognized liver, metabolic or renal disease. All volunteers were informed of their rights as participants under the Helsinki declaration, as well as the objectives and risks of the study and informed consent forms were signed.

The subjects received a capsule containing 30 mg of dextromethorphan hydrobromide and urine was collected for 8 hours.

Phenotyping procedure

The drug and its metabolite, dextrorphan, were quantified in urine by a modification of the method by Chen et al.[9] In short, a conventional solvent-solvent extraction procedure was used for the isolation of the analytes from urine samples after beta-glucuronidase hydrolysis. The yield was 75 and 83% for dexthromethorphan and dextrorphan respectively. The compounds were separated on a cyano column using a mobil phase of acetonitrile: triethylamine: distilled water (pH 3) and then were measured by fluorescense detection. Calibration curves in the range of 50-10000 ng/mL were linear and passed through the origin. The precision and accuracy were > 98% for both products, inter-day variability was between 1% and 2% and the lowest detectable concentration was 10 ng/ml. Eight subjects were phenotyped 5 times along 5 months in order to ascertain intra-individual reproducibility of the method during a long period (Table 2).

Pharmacogenetic layout.

The metabolic ratio (MR) was calculated and frequency distribution histograms were drawn. The cumulative frequency distribution was presented as a probit plot[10] (Fig. 1). Means and coefficient of variation (CV) were utilized to describe data dispersion and Student t Test for statistical analysis.

In their work, Schmid et al.[5] described an antimode for the Swiss population. Any subject with a molar metabolite ratio > 0.3 was categorized as PM, and any subject with a dextromethorphan: dextrorphan ratio < 0.3 was classified as EM. In our study, the antimodes were established by determining gaps in the frequency distribution and by the inflection points in the probit plot.

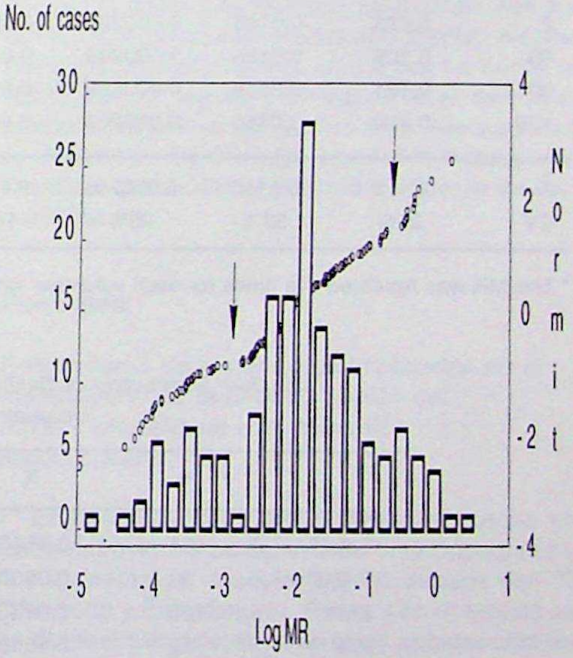

Fig. 1.— Frequency distribution of MR (Dextromethorphan/Dextrorphan) in a sample of Uruguayans and probit regression on the Log. MR. Two antimodes are indicated by the arrows.

Results

Table 1 shows the anthropometric data of the population and Table 2 shows the intraindividual variability of the MR repeated chronically.

The CV of the population is around tenfold higher than the intra-individual variation (Table 3). By inspection of the histogram, two antimodes can be tentatively assigned. The subpopulation on the extreme EM side is well defined and separated from the principal mode by an antimode placed somewhere between values of MR of 0.001920 and 0.000880. An apparent subpopulation on the PM side seems to have the antimode somewhere around the values of MR of 0.1950 and 0.3186; in between these two modes a third one can be distinguished. (Fig. 1).

Twelve subjects clustered between MR values of 0.1950 and 1.534 (MR > 0.3). These 12 subjects (6.7%) were assigned to the PM group. The

Table 1.— *Anthropometric Data of the Population*

Male	Female	Age years	Weight kg	Height m
106 (64%)*	59 (36%)*	35±12**	77±15**	1.73±0.11**

* Number of individuals and percentage of the population.
** Mean ± Standard Deviation.

Table 2.— *Reproducibility of the MR in eight individuals*

Day*	Vol 1	Vol 2	Vol 3	Vol 4	Vol 5	Vol 6	Vol 7	Vol 8
1	0.158	0.0152	0.000176	0.0142	0.0149	0.102	0.0344	0.0347
2	0.073	0.0146	0.000329	0.0136	0.0143	0.375	0.0422	0.0292
60	0.106	0.0166	0.000239	0.0166	0.0101	0.207	0.0307	0.0375
90	0.121	0.0056	0.000289	0.0136	0.0103	0.081	0.0443	0.0279
120	0.102	0.0160	0.000205	0.0169	0.0154	0.250	0.0496	0.0258
$\bar{X}$	0.112	0.0136	0.000248	0.0140	0.0130	0.203	0.0379	0.0310
CV	28%	33%	25%	14%	20%	59%	17%	16%

* The MR was repeated five times for each volunteer with one month in between.

Table 3.— *Distribution of Dextromethorphan/Dextrorphan MR in the Uruguayan Population*

	n	$\bar{X}$	CV (%)	Skew.	Kurt.
Total*	165	0.0585	279	5.8	43.8
F-EM**	30	0.0003	83	1.2	1.4
EM***	123	0.0233	149	2.5	5.9
PM****	12	0.5513	67	2.5	7.0

* Normality Test: $p > 0.05$.
** Fast Extensive Metabolizers (Extreme right mode)
*** Extensive Metabolizers (Central mode)
**** Poor Metabolizers (Extreme left mode)

latter frequency of PM is similar to that found by European authors[1, 2, 5, 9]

Discussion

The interdisciplinary study of interethnic or population differences in response to, or in disposition of, exogenous chemicals has been termed pharmacoanthropology. To the extent that such differences are inborn, pharmacoanthropology is a branch of pharmacogenetics.

However, it can be difficult to determine whether a given inter-ethnic or inter-racial difference has an environmental or a genetic cause: person-to-person variation within each of two populations may be mostly genetic, while the difference between the two populations may be due to customary intake of different foods[10].

In the study of Schmid et al.[5], in which dextomethorphan was used as probe drug, the frequency distribution histogram shows a clear separation between EM and PM. However, in the present work a definite separation between these sub-populations was not confirmed.

Nevertheless, a distinct mode was found in the extreme Fast EM (F-EM) domain with 30 subjects clustering between values of MR of 0.000880 and 0.000011, a mean MR of 0.000303 ± 0.00024 and a CV of 79% (Table 3). Blacks and mulattos were 6.4% in the F-EM subpopulation, 8.9% in the 123 subjects comprising the main mode (EM) and 0.0% in the 12 PM.

In a review paper, Kalow[10] draws a comparison between bebrisoquine metabolic ratio in Spain and Sweden. There clearly were more «very fast extensive metabolizers» (F-EM) in the Spanish than in the Swedish population. It therefore would not be surprising to find a relatively high proportion of F-EM in our country, since we have a strong Spanish descent.

Very high activity of CYP2D6 has been explained by gene duplication in Northern-Europeans. It is the object of our ongoing research to find out the genetic basis of this unique feature of the MR distribution in our population.

Cultural and peculiar gastronomic habits that facilitate exposure to particular xenobiotics, as well as other environmental features, would also provide a basis to explain the differences found between this population transplanted to Southamerica and their ancestors in Europe. The environment acts most strongly through food intake.

It should be stressed that the xenobiotics found in Southamerican and European diets are qualitatively different. A much higher intake of beef, the widespread habit of drinking the infusion of Ilex paraguayensis (*yerba mate*), the use of folk medicines (plant alkaloids, flavonoids, etc) even among inhabitants of the city, etc, might have played a role in determining the unique frequency distribution of the MR found in the present study.

Further pharmacogenetic studies, including restriction fragment length polymorphism combined with allele-specific PCR amplification[11] and comparison with other South American Amerindian populations, are in progress. Our studies are made possible through a collaborative program established between the Department of Pharmacology and Therapeutics in Montevideo, the Department of Clinical Pharmacology of the Karolinska Institute at Stockholm and the Department of Pharmacology and Toxicology of the University of Antioquia at Medellín, Colombia.

Acknowledgements: The authors gratefully acknowledge Professor Folke Sjozqvist from the Karolinska Institute at Stockholm and Professor Eliott Vesell from the University of Pennsylvania for scientific support. We also thank the Scientific Service of Hoffman La-Roche at Nutley, NJ, for providing the standards of dextromethorphan and its metabolites. This study was supported by a grant from the Comisión Sectorial de Investigación Científica, Universidad de la República Oriental del Uruguay/1992.

Resumen

Polimorfismo de las enzimas implicadas en el metabolismo de la O-demetilación del dextrometorfano en una población sudamericana

El polimorfismo de las enzimas implicadas en los metabolismos oxidativos de la debrisoquina y la esparteína se descubrió en la década del 70 (Mahgoub y Eichelbaum). Desde ese entonces se ha demostrado que muchas otras substancias terapéuticas son metabolizadas como los mencionados fármacos. Una de ellas es el dextrometorfano. El objetivo de la presente investigación es evaluar la distribución del fenotipo oxidativo del dextrometorfano en la población uruguaya.

Se midió el fármaco y su metabolito oxidado, el dextrorfano, en la orina de 165 voluntarios sanos mediante una modificación de un método analítico por HPLC descripto por Chen y col. Se calcularon los cocientes metabólicos y se graficaron en histogramas de frecuencia. La inspección de los histogramas permite asignar dos antímodos que a su vez limitan 3 subpoblaciones en la distribución: a) metabolizadores extensos rápidos, b) metabolizadores extensos medios y c) metabolizadores lentos. Esta distribución trimodal no había sido comunicada hasta el presente.

Se están implementando estudios de genotipificación de la población bajo exámen a efectos de confirmar estos hallazgos y dilucidar el mecanismo genético subyacente.

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There is nothing so powerful as truth - and often nothing so strange.

No hay nada más poderoso que la verdad - y a menudo nada más extraño.

Daniel Webster (1782-1852)

Argument on the murder of Captain White, 1830