OXIDATIVE PHENOTYPE STATUS IN RELATED SUBJECTS

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Abstract: Human population varies with regard to the rate of drug metabolism. Differences in pharmacological activity of a drug in patients who belong to the same population result from a different enzymatic activity and different genotypes of those subjects. The aim of the study was to assess the incidence of extensive (EM) and poor (PM) oxidative phenotypes in related persons and to establish whether any associations exist between an individual, genetically conditioned drug oxidation capacity and a family relationship. The study comprised 61 healthy subjects including 39 females and 22 males aged between 18 and 77 years (mean age 39.02 ± 16.55 years). The persons belonged to 20 families and were first degree relatives. The oxidative phenotype status was established based on the metabolic ratio (MR); the amounts of urinary output of dextromethorphan and dextrorphan in the 10 h urine were determined using the HPLC method. Prevalence of poor metabolizers in the group of relatives reached 16.4%. The percentage of poor metabolizers was higher in the group of relatives than in the control group (9.6%), however, the difference was not statistically significant. Inheritance of the oxidative phenotype among relatives is mainly associated with mothers and their daughters.

Keywords: dextromethorphan, drug oxidation, family, genetic polymorphism

Human population varies with respect to the rate of drug metabolism. Diversity of pharmacological activity of the same drug in patients from one population results from a different enzyme activity and is due to their different genotypes. Genetic polymorphism in different populations can be determined using methods for phenotyping or genotyping.

Apart from affecting drug effectiveness and occurrence of side effects, the oxidative phenotype status can predispose an individual to a large number of diseases. In the studies on disease etiopathogenesis the role of genetically determined disturbances in xenobiotic metabolism has been emphasized. Some of the diseases including psychiatric, neoplastic, autoimmune or cardiovascular diseases are of a family nature. The risk of developing these increases according to genetic relationship and is the highest in first degree relatives. Therefore, establishing the oxidative polymorphisms which would allow to predict the phenotype status in the individual’s relatives (parents, children), seems justified.

The aim of the study was to assess the incidence of an extensive (EM) and poor (PM) oxidative phenotype among relatives and to investigate whether an association exists between a genetically determined, individual drug oxidation capacity and a family relationship.

EXPERIMENTAL

The study comprised 61 healthy subjects including 39 females and 22 males aged between 18 and 77 years (mean age 39.02 ± 16.55 years). The persons belonged to 20 families and were first degree relatives. Parents and 1 or 2 children were examined in 9 families. In the remaining 11 families mother and her children participated in the study. Each subject was informed as to the aim and methods of the research and agreed to participate. The design of the study was approved by the Local Ethics Committee for Research at the Medical University of Łódź, Poland.

In order to assign the oxidative phenotype, the volunteers were administered a single oral dose of 40 mg of dextromethorphan hydrobromide, DHBr (capsule prepared in our department with substance supplied by Sanofi-Biocom, Rzeszów, Poland). The amounts of the urine output of dextromethorphan (DM) and its metabolite – dextrorphan (D) were determined in the 10 h urine using high performance liquid chromatography (HPLC) according to Hou et al. (1). The liquid chromatographic system consisted of a Model 600 pump (Waters, USA), UV-VIS detector (Waters, Model 2487). Separation was performed on a Nova Pack Phenyl (3.9 × 150 mm, Waters). The mobile phase consisted of acetonitrile/10 mM phosphate buffer, pH 4.0 (55:45 v/v) with a flow rate of 1 mL/min and pressure of 1200 psi. The assay was performed with the UV detector operating at 280 nm. Under the chromatographic conditions used in this study, the retention times of dextrorphan and dextromethorphan were 3.16 min and 6.3 min, respectively.
The samples were vortexed and then titrated with 1 M NaOH to pH of 11.0 to 11.5. The sample was eluted sequentially with 10 mL and 5 mL volumes of 10% n-butanol in hexane. To the combined eluent was added 300 µL of 0.01 M HCl. The sample was then shaken for 20 min and centrifuged at 2000 rpm for 15 min. The aqueous phase of each sample was injected for HPLC for quantification of dextromethorphan and dextrorphan. Typical standard curves for dextromethorphan and dextrorphan were linear and had a correlation coefficient of 0.996 and 0.999, respectively. The oxidative phenotype was assigned based on the metabolic ratio (MR) calculated as follows:

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MR = \frac{0-10 \text{ h urinary output of unchanged dextromethorphan (DM)}}{0-10 \text{ h urinary output of dextrorphan (D)}}
\]

Subjects with dextromethorphan/dextrorphan ratios over 0.3 (MR > 0.3) were classified as poor metabolizers (PM) and a group of extensive metabolizers (EM) included subjects with metabolic ratios below or equal 0.3 (MR < 0.3). The obtained results were a subject of statistical analysis. The age of the studied individuals was characterized regarding the number of subjects in a group and the arithmetical mean was calculated. The 95% confidence interval was admitted to assess the incidence of the studied phenotypes. Statistical significance of differences found in the occurrence of oxidative phenotypes in the groups of family members and the controls was assessed using the independence test \( \chi^2 \). The analysis of variance was applied to compare mean values of dextromethorphan (DHBr) and dextrorphan (D) output in the groups of mothers, fathers, sons and daughters.

RESULTS

Among the relatives, poor oxidative phenotype was found in 10 subjects (16.4%), whereas the extensive one – in 51 (83.6%) ones. In the control group, 10 persons (9.6%) were found to be poor metabolizers and extensive oxidative phenotype was recognized in 94 subjects (90.4%). The percentage of poor metabolizers was higher among family members than among controls but the differences were not statistically significant (\( p = 0.1978 \) (Table 1)).

The examination of family members showed a bimodal distribution of the occurrence of the particular values of the metabolic ratio (MR). (Table 2) In the group of poor metabolizers the metabolic ratio values ranged between 0.366 and 13.760 (mean 3.166 ± 4.476), whereas its values ranged between 0.001 and 0.178 (mean 0.049 ± 0.046) in the group of extensive metabolizers. Mean urine levels of dextromethorphan and dextrorphan were significantly different in poor and extensive metabolizers. Dextromethorphan urinary output in samples from extensive metabolizers was < 2.116 µmol, while in poor metabolizers it was > 0.595 µmol. Percentage of dose recovery in urine as dextromethorphan was found < 5.29% in extensive metabolizers and >1.488% in the group of poor metabolizers. Dextrorphan urinary output was > 2.204 µmol in extensive metabolizers and < 11.303 µmol in poor metabolizers. Dose recovery in urine as dextrorphan was > 5.51% of the dose taken in extensive metabolizers and < 28.257% in the group of poor metabolizers. The mean value of log MR was 1.558 (± 0.555) in the group of extensive metabolizers and 0.14 (± 0.575) in poor metabolizers. The value of log MR = -0.5 (MR = 0.3) was not found in any of the examined subjects.

Table 1. The incidence of poor and extensive oxidative phenotype in the related subjects and in the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of patients</th>
<th>Poor metabolizers</th>
<th>Extensive metabolizers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Related subjects</td>
<td>61</td>
<td>10</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16.4%</td>
<td>83.6%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C.I. 7.1%-25.7%</td>
<td>C.I. 74.3%-92.9%</td>
</tr>
<tr>
<td>Control group</td>
<td>104</td>
<td>10</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.6%</td>
<td>90.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C.I. 3.9%-15.3%</td>
<td>C.I. 84.7%-96.1%</td>
</tr>
</tbody>
</table>

\( p = 0.1978, \chi^2 = 1.66 \)

\( p \) – statistical significance (\( p < 0.05 \) for statistically significant differences)
\( \chi^2 \) – the Chi2 test used to compare two calculated values
C.I. – 95% confidence interval
Table 3 presents values of correlation coefficients for urinary output of dextromethorphan and its metabolite – dextrorphan among the particular groups of the examined families.

Based on the obtained results, a statistically significant correlation was shown with regard to dextromethorphan output between mothers and their daughters ($r = 0.99; p < 0.05$) and between mothers and their sons ($r = 0.56; p < 0.05$). Similarly, correlation of dextrorphan (metabolite of dextromethorphan) output between mothers and their children ($r = 0.46; p < 0.05$). No correlation with regard to dextromethorphan and dextrorphan output between fathers and their children was found in this study.

**DISCUSSION AND CONCLUSION**

In the present study an attempt was made to establish an association between genetically determined polymorphism of drug oxidation and the family relationship (parents – children). The percentage of poor metabolizers among relatives was higher than among controls, however, the differences were not statistically significant ($p = 0.1978$). Oxidative polymorphism in families has also been the subject of the study by Brosen et al. The authors showed the prevalence of poor metabolizers among 64 first degree relatives (Danish population) as high as 27%, whereas phenotype of poor metabolism was identified in 9% of subjects from the control group. (2)

Family occurrence of the oxidative phenotype has also been studied by Vincent-Viry et al. The authors demonstrated correlation of dextromethorphan and dextrorphan output among particular family members. The excretion of the metabolite (dextrorphan, D) in boys was found to be associated more strongly with the output in their mothers than in fathers (3).
Our own studies revealed that there is a higher correlation in regard to dextromethorphan output between mothers and their daughters than between mothers and their sons. A correlation was found also concerning dextrorphan output between mothers and their children.

Interindividual polymorphism of cytochrome P450 enzyme activity can predispose to the occurrence of some diseases including neoplastic and neurological ones. (4-10). Family studies on genetic relationships revealed an increased risk of developing numerous diseases among first degree relatives.

The relevance of pharmacogenetic studies in neurological and mental diseases has been emphasized with a particular role of CYP2D6 isozyme determination. Brosen et al. observed that the risk of mental diseases (particularly depression) occurrence is by 10-15% higher in first degree relatives than in the whole population. Polymorphism of oxidation plays a significant role in the metabolism of tricyclic antidepressants, thus phenotyping in all the members of a family where the disease has been recognized, seems justified (2). Administration of standard doses of antidepressants in poor metabolizers can result in the occurrence of side effects which can be misinterpreted as intensification of depression symptoms and/or somatic symptoms such as mucosal dryness, blurred vision, heart rate disturbances and even delirium (11, 12).

Similarly, most of β-adrenolytic and antiarrythmic drugs applied in cardiovascular diseases are metabolized through oxidative processes. There is a tendency to family occurrence of cardiovascular diseases. Clinical consequences of impaired metabolism (PM) is the intensified β-adrenolytic action and, in case of metoprolol – loss of its selective effect on β1-adrenergic receptors (loss of the cardioselective effect). Applying antiarrythmic drugs in patients with poor metabolizer phenotype increases danger of occurrence of side effects, and particularly the proarrhythmic effect. Cooccurrence of poor drug metabolism and cardiovascular insufficiency, which can additionally impair functioning of the organs responsible for xenobiotic metabolism i.e. the liver and kidneys, seems the greatest risk factor for the occurrence of side effects (12).

In conclusion, genetically determined oxidative polymorphism can affect not only drug effectiveness but it can be a factor predisposing to disease development in related persons, as well. Our own studies revealed that percentage of poor metabolizers was higher in the group of related persons than in the control, inheritance of oxidative phenotype among related persons concerns mainly mothers and their daughters. Assigning oxidative phenotype in the mother allows to predict with high probability the phenotype in her daughters and all the consequences associated with genetically determined oxidative polymorphism.

Acknowledgments

This study was supported by a research grant 502-18-665 from the Medical University, Łódź, Poland.

REFERENCES

4. Ingelman-Sundberg M.: Pharmacogenomics J. 5, 6 (2005).