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Sparteine oxidation rate in alcohol dependent patients

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Summary

Aim of the study*: Assessment of the CYP2D6 isoenzyme activity in alcohol dependent patients based on sparteine oxidation rate.*

Material and methods*: Oxidation rate of sparteine was examined in a group of 102 patients with diagnosis of alcohol dependence and has been compared with a control group consisting of 160 volunteers. The assessment of oxidation rate of sparteine was carried out during the withdrawal syndrome.*

Results*: Among the patients, 97.1% were classified as extensive metabolisers (phenotype EM) and 2.9% as poor metabolisers (phenotype PM). There was no statistically significant difference in proportions of EM's and PM's subgroups between patients and the controls. Significant differences were observed in the distributions of sparteine metabolic oxidation ratio (MR); a higher oxidation rate was noted in the studied group, in comparison with the controls. The length of the dependence period does not have any effect on the MR values.*

Conclusion*: Patients who are in the midst of the alcohol withdrawal syndrome do not have a defected process of oxidation in the presence of CYP2D6 and as a result of this parameter, there is no need to reduce the dosage of drugs metabolized by this enzyme.*

Key words: sparteine oxidation, CYP2D6, alcohol dependence, alcohol withdrawal syndrome

Introduction

Dependence and harmful use of alcohol is a serious medical, psychological, and social problem. Health complications, conflicts with the law, psycho-degradation and family relation disturbance are the reasons for searching for effective methods of preventing and treating alcohol dependence together with its consequences. Alcohol abuse contributes to a large number of health impairments, including internal body damage, infections and oncological, dermatological, post-traumatic, neurological, psychiatric disorders – all requiring expensive pharmacological treatment [1]. Stress associated with psychophysical suffering is one of the factors activating the mechanism of dependence, resulting in a relapse of drinking alcohol [2, 3].

The optimization of pharmacotherapy of post-alcoholic damages is very important in the comprehensive care of alcohol dependent patients. In the pharmacotherapy of complications due to alcoholism, a number of drugs metabolized in the presence of CYP2D6 (sparteine-debrisoquine hydroxylase) are used. For example medicines used in cardiac-blood vessel disorders such as β-adrenoreceptor blockers, H_2 receptor antagonists, antitussic, antidiabetic, analgetics, antidepressive and antipsychotic drugs [4, 5, 6].

The oxidation process of endogenic substances and xenobiotics are genetically determined and take place in the presence of enzymes from the cytochrome P-450 group. One of them is the isoenzyme CYP2D6 that is involved in the oxidative metabolism of many different drugs used in the treatment of patients addicted to alcohol. According to the CYP2D6 activity two classes of phenotypes may be distinguished: extensive metabolisers (phenotype EM) and poor metabolisers (phenotype PM). PM subjects have an impaired metabolism of CYP2D6, while in EM's the CYP2D6 activity is normal. The individual variability of the CYP2D6 capacity may affect the efficiency of pharmacotherapy in the case when standard doses of drugs metabolized by that enzyme are used. It can also be a cause of severe side effects in patients with a PM phenotype [6, 7, 8]. The frequency of PM's among Caucasians of North America, Western Europe and Poland range from 6 to 10% [5, 7, 8]. The phenotype may be identified by a phenotyping approach, assessing sparteine oxidation rate (sparteine is a test drug whose metabolism is solely dependent on the function of the CYP2D6 enzyme) [7, 8].

The aim of study

The aim of this study is to assess the possible differences in the CYP2D6 metabolic capacity based on the sparteine oxidation rate between groups of patients with a diagnosis of alcohol dependence during the withdrawal period and a group of healthy volunteers. The results of the study could be helpful in clinical practice, in optimizing the treatment of health impairments caused by alcohol.

Material and methods

The study included 102 patients, with a diagnosis of alcohol dependence, according to DSM-IV and ICD-10 criteria. They were hospitalized in the Rehabilitation and Treatment Center of Drug Dependence of the Psychiatric Hospital in Wrocław. Men and women above 18 years of age, in a psychic state allowing them to be able to give their consent were eligible for this study. Pregnant and breast-feeding women, persons with liver and renal impairment and those who underwent treatment within the last three months using drugs metabolised by CYP2D6 and drugs which have an effect on the oxidation rate – inductors and inhibitors of CYP2D6, were excluded.

A test of sparteine oxidation was carried out in 102 subjects, on day 3 (±2) of abstinence. Among them were 10 women (9.8%) and 92 men (90.2%). The examined patients were in the age between 26 and 59 years (mean age 41.4 years), with an ac-

tive period of dependence from 1 to 32 years (average 12.3). A control group of 160 healthy individuals were tested for their oxidation phenotypes. This group comprised of 74 females (46%) and 86 males (54%), from the Wrocław region, aged 18 to 73 years (mean age 40.8 years).

Phenotyping (sparteine test)

In the morning, on day 3 (±2) of the withdrawal period, patients were given 100 mg of sparteine sulphate on an empty stomach. Following this, urine samples were collected over the next 6 hours. The sparteine contents and its metabolites (2- and 5-dehydrosparteine) present in urine were defined with the help of gas chromatography according to Eichelbauma's method, using 17-ethylsparteine as an internal standard [9].

The metabolic ratio (MR) of sparteine oxidation was calculated using the formula:

$$M = \frac{\text{Amount of sparteine excreted with urine}}{\text{Amount of 2-,5- dehydrosparteine excreted with urine}}$$

Examined individuals whose MR were lower than 20 were defined as extensive metabolisers (EM), while those with MR value equals or greater than 20 were defined as poor metabolisers (PM).

Statistical analysis

For the statistical analysis of data, a computer program "Statistica" (6th version), from the StatSoft company® was used.

Two-sided tests were performed, at the statistic significance level of 0.05. Apart from this, the value of the critical level p (so-called p-value) was also given. This is the lowest level of significance at which the verified hypothesis ought to be rejected. To assess the hypothesis, that two samples were drawn from different populations, the Wald-Wolfowitz Runs test and the Kolmogorov-Smirnov Two-Sample test were used. The Mann-Whitney U-test was used to evaluate the differences in rank sum between two groups. The test used for a dichotomic type of features was a test of the differences between two proportions. The Kruskal-Wallis ANOVA and Median tests assessed the hypothesis that the different samples in the comparison were drawn from the same distribution or from distributions with the same median. For comparison of the same group in two different phases the Wilcoxon's Matched Pairs test and Signs test was carried out.

Results

In the group of patients dependent on alcohol, ninety-nine persons (97.1%) were classified as extensive metabolisers and 3 persons (2.9%) as poor metabolisers. In the control group 146 persons (91.2%) were assigned as extensive metabolisers and 14 (8.8%) as poor ones. The percentages of EM's and PM's among the controls did not

deviate from the Caucasian population standard [5, 10, 11].

There was no statistically significant difference in the distribution of MR values between the male and female group. The p-values in the Wald-Wolfowitz Runs test and Mann-Whitney U-test amounted p=0.261 and p=0.356 respectively. Also, in the control group, the distribution of the MR values were the same in both groups (p=0.64). As a result of this, differences of sex structure in the examined and control group should not have any effect on verification of the hypothesis concerning the distribution equality of MR values in both of the above groups. No significantly statistical differences were shown in the distribution of MR values between groups from the 1st, 2nd, 3rd, 4th, 5th day of abstinence from alcohol (p= 0.56 and p=0.146, Kruskal-Wallis ANOVA and Median test respectively).

Hypothesis of an equality of the MR value distributions in the examined subgroups divided according to the length of dependence period was verified. The first group included persons with the dependence period range from 1 to 5 years, the second group from 6 to 10 years, the third – from 11 to 19 years, the fourth group – above 20 years. There were no statistically significant differences in the distributions of MR values between the above subgroups ($p > 0.05$). Therefore, it can be ascertained that the length of the dependence period does not have effect on MR values.

There was no statistically significant difference in the frequency of PM and EM phenotypes between patients and the controls (p=0.0587, alternative two-sided hypothesis).

However, we found a statistically significant difference in the distribution of MR values between the studied groups and controls (p=0.000001 in Mann-Whitney U-test

Table 1

Descriptive statistics of MR values in the control group and the examined group

MR value	Valid N	Mean	Median	Minimum	Maximum	Range	Standard deviation
Control group	[illegible]	14.34	1.11	[illegible].14	449.45	449.11	[illegible]
Examined group	[illegible]	1.56	[illegible].65	[illegible].76	42.11	42[illegible].1	[illegible].45

and Kolmogorov-Smirnov Two-Sample test, respectively) (Table 1).

Also a difference in the distribution, between EM of the studied group and the control group is statistically significant (p=0.000001). In the studied group the mean MR value was lower than in the control group, thus the sparteine oxidation is faster in the group of alcohol dependent patients. Distribution of MR value in the control group is shown in Fig.1 and in Fig.2 for the studied group.

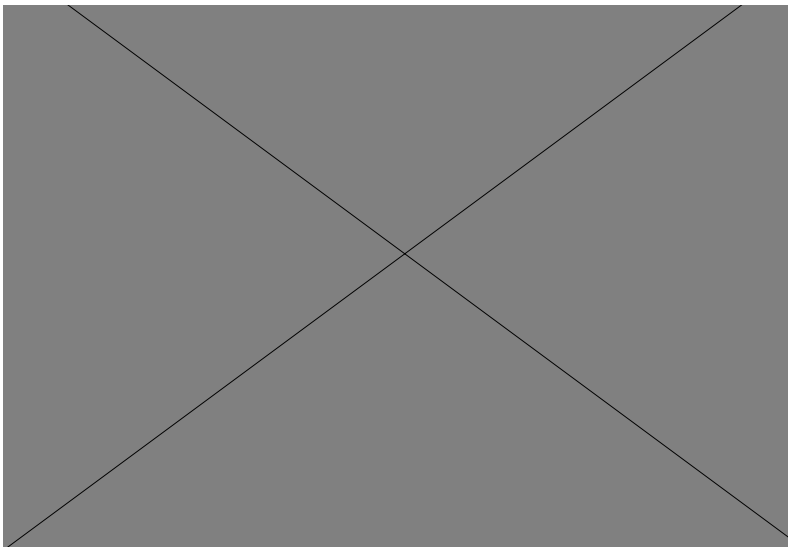

Fig. 1. **Distribution of MR in the control group**

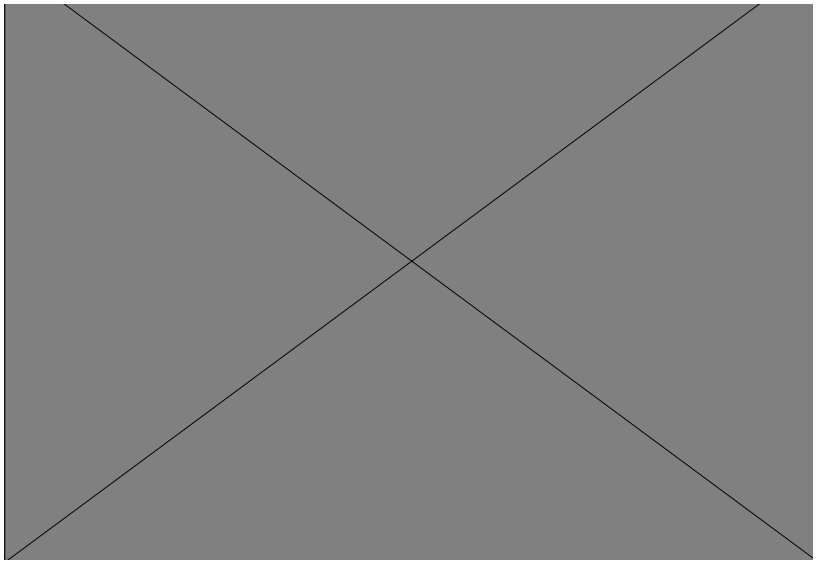

Fig. 2. **Distribution of MR in the studied group**

Discussion

In the available literature only a few research papers relating to the influence of alcohol dependence on the sparteine-debrisoquine oxidation rate can be found. The effect of alcohol abuse on the oxidation rate was examined by Vincent-Viry [12]. In her research, she was unable to affirm a statistically significant effect of alcohol

abuse on the frequency of persons with a PM phenotype. Similarly, in our research, no statistically significant effect of the dependence on the percentage of PM phenotypes was shown. Llarena examined the effect of alcohol on the oxidation rate use on debrisoquine metabolic ratio (MR), in healthy volunteers. He didn't affirm any statistically significant effect of ethanol on this rate [13]. In our research, we examined the influence of the alcohol withdrawal syndrome on the oxidation rate (MR in the sparteine test). The results obtained showed a statistically significant higher oxidation rate during the period of the withdrawal syndrome as compared to the control group. The difference in results between our research and that of Llerena could be caused by incomparability in methodology of both studies. Based on his study Loriot suggests the inducible effect of alcohol on the CYP2D6 activity in chronic alcoholic patients [14]. The inducible effect of alcohol on the CYP2D6 was shown also in the study of cellular expression of CYP2D6 in the brains of alcoholic patients [15].

Conclusion

Comparing to the control group, a statistically significant faster sparteine oxidation was affirmed in the studied group during the period of alcohol withdrawal syndrome. A statistical difference was also observed in the distribution of MR values in the subgroups of people with an EM phenotype. There was no significant influence on the MR distribution depending on the dependence length period and number of days (1-5) of abstinence.

The results of the study suggest that patients who are in the period of the alcohol withdrawal syndrome do not have a defected process of oxidation in the presence of CYP2D6 and as a result of this parameter, there is no need to reduce the dosage of drugs metabolised by this enzyme. Obviously, the dosage used should be adjusted according to the clinical state of the patient.

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