Correlation Between Platelet Monoamine Oxidase Activity and Serotonin Content in Alcoholism Subtypes

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Summary
Since early-onset (Type II) alcoholics who exhibit antisocial behavior have been suggested to possess serotonergic defects, the present study was undertaken to compare alcohol subtypes (Type I versus Type II) with regard to their platelet monoamine oxidase (MAO) activities and platelet and blood serotonin (5-HT) contents in order to clarify the possible determinative role of these biochemical markers in subtyping of alcoholics. Seventeen Type I and 16 Type II chronic alcoholic patients and 17 healthy volunteers were included in the study. Alcohol intake variables and the severity of drinking problems of the subjects were assessed by Schedules for Clinical Assessment in Neuropsychiatry (SCAN) and the Michigan Alcoholism Screening Test (MAST), respectively. When compared to the healthy subjects, platelet MAO activity and platelet 5-HT content were found to be decreased whereas plasma 5-HT content was increased in both alcoholic groups. Platelet MAO activity and 5-HT level of the Type II group were significantly lower and plasma 5-HT content significantly higher than those of Type I patients, suggesting that the alteration is more predominant in early-onset alcoholism. Since a positive correlation was found between platelet MAO activity and 5-HT content and a negative correlation between platelet MAO activity and plasma 5-HT level, particularly in Type II alcoholics, decreased platelet 5-HT content in Type II alcoholics has been suggested to not result from the increased serotonin metabolism by platelet MAO but rather to be possibly caused by some defects in central 5-HT synthesis or its reuptake mechanisms by platelets. However, the results suggested that platelet 5-HT content and MAO activity still appear to be useful biochemical measures for the subtyping of alcoholics.

Key Words: Alcoholism subtypes, platelet, monoamine oxidase, serotonin (5-HT).

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INTRODUCTION
Alcoholism appears as a complex syndrome charac-

terized by a high morbidity, comorbidity, treatment resistance, chronic course and a high suicidal rate, which is suggested to be related to alterations in ne-
Serotonin (5-HT) is a biogenic monoamine, synthesized in the central nervous system, where it modulates a variety of behavioral functions, including the regulation of sleep, appetite, nociception, mood, stress and sexual behavior. Serotonergic dysfunction is implicated in various types of psychopathological conditions, such as antisocial personality disorder, alcoholism, depression with suicidality, antisocial behavior with aggression, obsessive-compulsive syndromes, psychosis, eating disorders, substance abuse and schizophrenia. It has been suggested that male alcoholics who start drinking early and exhibit antisocial behavior have a serotonergic defect. Decreased serotonergic function is thought to be related to impulse control disorders, with an inverse relationship between platelet 5-HT content and antisocial behavior and aggression. Since direct measurement of brain 5-HT is not possible, and uptake, storage and release of platelet 5-HT resemble the corresponding processes in the central serotonergic neurons, the platelet is proposed as a peripheral model for the central nervous system neuron. Previous studies indicated that platelet 5-HT content was found to be decreased in drinking alcoholics, which might result from the lowered 5-HT synthesis, decreased platelet reuptake, increased platelet release or increased metabolism by monoamine oxidase (MAO).

The main metabolic pathway of 5-HT is oxidative deamination by MAO, a flavoenzyme which plays an essential role in the metabolism of biogenic amines. MAO is found in two different forms designated as MAO-A and MAO-B. MAO-A was reported to catalyze the breakdown of 5-HT and play an important role in the regulation of intracellular 5-HT levels in the central nervous system, whereas MAO-B was reported to be the only isoform in human platelets which is thought to be responsible for the deamination of 5-HT. Since platelets share similar biochemical processes with neurons and platelet MAO activity was found to be related to central monoamine turnover, platelet MAO has been suggested as a possible marker for alcoholism. There have been multiple reports that platelet MAO activity is decreased in alcohol-dependent subjects and in relatives of alcoholics compared to controls. Lower platelet MAO activity is claimed to be a characteristic of the early-onset (Type II) group, which is associated with a positive family history of alcoholism and personality traits, such as suicidal and criminal behaviors, impulsiveness and sensation-seeking personality.

Since we previously have reported that platelet MAO activity, a marker of serotonin turnover, was decreased in Type II alcoholics in the same research sample and since the platelet 5-HT content was shown to be diminished in alcoholics by some other authors, the present study focused more directly on the association of platelet serotonin level and MAO activity in alcoholic subtypes. With this aim, we have examined a possible differentiated pattern of platelet MAO activity and platelet and plasma serotonin content in patients with Types I and II alcoholism classified on the basis of the age of onset of alcoholism.

MATERIALS AND METHODS

Subjects

The patient group consisted of 33 voluntary male al-
cohol patients who satisfied the diagnostic criteria of the International Criteria of Diseases (ICD-10) for alcohol dependence (WHO, 1992). All the patients were receiving treatment for their withdrawal symptoms at an in-patient treatment center during the time of the investigation. Alcohol and Drug Modules of Schedules for Clinical Assessment in Neuropsychiatry (SCAN) were administered to each patient in order to gather data on alcohol intake variables and drinking related behavior. The lifetime severity of drinking problems was assessed by the Michigan Alcoholism Screening Test (MAST).

Seventeen Type I and 16 Type II male chronic alcoholic patients were included in the study. The patients whose subjective alcohol problems had started before reaching 21 years of age (≤20) were categorized as Type II. For inclusion in the Type II group, the reporting of at least two instances of social complications related to alcoholism before reaching 21 was required (i.e. job loss, alcohol-related absence from school or work, arrest for intoxicated behavior, or violence while intoxicated). Those who did not meet these criteria were categorized as Type I dependent. The time when alcoholic patients satisfied the rule for the social complications of alcoholism was considered the beginning of their alcohol misuse. In the Type II group, ages ranged from 27 to 62 years with an average of 41.6 ± 9.6. In the Type I group, ages ranged from 34 to 56 years with an average of 45.1 ± 6.3. Blood samples were obtained on the fifteenth abstinent day of the alcoholic patients. Seventeen healthy males selected on the basis of the Michigan Alcoholism Screening Test (MAST) were used for the determination of platelet MAO activity while the rest was used for the determination of platelet 5-HT level. The samples were kept frozen at -80°C until used.

All subjects provided written informed consent for participation in the study, which was approved by the local ethics committee (99-7).

Reagents and equipment

All chemicals were obtained from Sigma Chemical Co. (Germany). Spectrophotometric measurements were performed using Shimadzu 1601 PC spectrophotometer and high performance liquid chromatography (HPLC) measurements were performed using the HPLC system of Dionex, USA.

Determination of plasma and platelet 5-HT levels

Blood samples were drawn by venipuncture after an overnight fast into tubes containing EDTA as anticoagulant. 10 ml of blood was divided into two portions. One portion was centrifuged for 5 min. at 1,000 x g and the supernatant was kept as plasma. The next portion was centrifuged for 5 min. at 10,000 x g and 4°C, to obtain platelet rich plasma (PRP). Platelet counts were determined on aliquots of pooled PRP diluted in Isoton II and counted twice on a thrombocounter (Coulter Electronics, STKS). After the platelet count, 2 ml PRP was centrifuged for 10 min. at 2,000 x g. The supernatant was discarded and the pellet was suspended in 1 ml of a mixture containing 4% perchloric acid and 0.15% EDTA. The mixture was centrifuged for 10 min. at 2,000 x g and 4°C. The resulting supernatant was filtered through 0.45 µm Millipore filters by centrifugation for 5 min. at 2,000 x g (Sigma, microcentrifuge filter ultrafree-CL, Durapore PVDF membrane) and 4°C. The eluate was divided into two portions: one portion was used for the determination of platelet MAO activity while the rest was used for the determination of platelet 5-HT level. The samples were kept frozen at -80°C until used.

The portions kept for 5-HT determination in plasma and PRP were thawed and centrifuged for 3 min. at 2,000 x g. Plasma and platelet 5-HT contents were determined according to a previous method. Aliquots of the supernatants were applied to the HPLC system equipped with a 5 µm C18 column. The elution buffer consisted of 50 mM potassium phosphate (KP), pH 5.0 and 12% methanol, with flow rate of 1 ml.min⁻¹ under isocratic conditions. The fluorescence detector (Dionex, RF 2000) was set at 230 nm.
excitation and 338 nm emission. Plasma and platelet 5-HT contents were expressed as nmol/L and nmol/10⁹ platelets, respectively.

**Determination of platelet MAO activity**

Platelet MAO-B activity was measured in PRP samples by the method of Holt. A chromogenic solution, consisting of 1 mM vanillic acid, 500 µM 4-aminoantipyrine, 4 U.ml⁻¹ peroxidase in 0.2 M KP buffer, pH 7.6, was prepared daily. Assay mixture contained 167 µl chromogenic solution, 667 µl 500 µM benzylamine, and 133 µl KP buffer, pH 7.6. The mixture was preincubated at 37°C for 10 min. before the addition of enzyme. Reaction was initiated by the addition of the PRP (100 µl) and absorbance increase was monitored at 498 nm at 37°C for 60 min. Molar absorption coefficient of 4654 M⁻¹.cm⁻¹ was used to calculate the initial velocity of the reaction. Results were expressed as nmol/10⁹ platelets.

**Clinical chemistry**

Biochemical parameters were measured in the plasma samples of the subjects in autoanalyzer (Roche Modular System) and the hematological parameters were determined using an electronic cell counter (Coulter STKS).

**Statistical analysis**

The results were expressed as the mean ± SD. Kruskall-Wallis analysis and Mann-Whitney U test of variance were used for comparison of the groups of the variables. Chi-square test was used for comparison of some demographic characteristics of the groups. Possible relationships between biochemical measures and some clinical variables, such as the lifetime severity of alcohol consumption, were assessed by the Pearson product-moment correlation analysis. A value of p< 0.05 was considered as statistically significant.

**RESULTS**

No statistically significant difference was found between the study groups in age and years of educati-
Mean platelet MAO activity of alcoholics (25.53 ± 2.80 nmol/10^9 platelets) was found to be significantly lower than that of the healthy controls (36.28 ± 4.90 nmol/10^9 platelets) (p< 0.01) (Table 2). Additionally, Type II alcoholics had lower platelet MAO activity than that of Type I alcoholics (p< 0.01) and controls (p< 0.001). A significant positive correlation was detected between the platelet MAO activity and 5-HT content (p< 0.001) (Figure 2), whereas a strong negative correlation was found between the platelet MAO activity and the plasma 5-HT content (p< 0.001) (Figure 3) in all study groups.
Relationship between the plasma serotonin (5-HT) content and platelet monoamine oxidase (MAO) activity in the study subjects. Platelet 5-HT content and MAO activity were both expressed as nmol/10^9 platelets.

Table 3 represents the biochemical parameters measured in the blood of the study subjects. Transaminase and α-glutamyltransferase (GGT) activities, total lipid, cholesterol, and low density lipoprotein (LDL-C) contents were found to be increased, while high density lipoprotein (HDL-C), total protein, albumin and hemoglobin contents, packed cell volume (PCV), mean corpuscular volume (MCV) and red blood cell (RBC) count were found to be decreased in alcoholics when compared to control subjects (p<0.01). Alanine aminotransferase (ALT), aspartate aminotransferase (AST) and GGT activities, total lipid, total cholesterol and LDL-C levels were negatively correlated with platelet 5-HT content in Type I and II alcoholics (r= -52, r= -0.50, r= -54, r= -53, r= -51 and r= -55 in Type I; r= -60, r= -61, r= -59, r= -60, r= -62 and r= -63 in Type II alcoholics, respectively) (p< 0.01). These parameters were positively correlated with plasma 5-HT content both in Type I and II alcoholics (r= 49, r= 0.50, r= 49, r= 51, r= 52 and r= 50 in Type I; r= 55, r= 57, r= 61, r= 60, r=63 and r=59 in Type II alcoholics, respectively) (p< 0.05). HDL-C level, hemoglobin content, RBC count and PCV were positively correlated with platelet MAO activity only in Type II alcoholics (r= 53, r= 55, r= 58, and r= 56, respectively) (p< 0.05).

ALT, AST and GGT activities, total lipid, total cholesterol and LDL-C levels were also found to be negatively correlated with platelet MAO activity in Type I and II alcoholics (r= -57, r= -0.56, r= -58, r= -53, r= -55 and r= -54 in Type I; r= -61, r= -61, r= -66, r= -65, r= -68 and r=-66 in Type II alcoholics, respectively) (p< 0.01), while HDL-C level, hemoglobin content, RBC count and PCV were positively correlated with platelet MAO activity only in Type II alcoholics (r= 53, r= 55, r= 58, and r= 56, respectively) (p< 0.05).

**DISCUSSION**

In the present study, no statistically significant difference was found between the study groups in age and years of education. The average age of onset of alcohol abuse symptoms was significantly lower in Type II alcoholics (p<0.01). Duration of alcohol dependence was found to be significantly higher in alcohol or drug abuse Type II alcoholics (p<0.05). The lifetime severity of alcohol abuse symptoms, as measured by MAST, however, was not statistically different between the alcoholic groups.

In our study, platelet 5-HT concentration was markedly decreased in alcoholic subjects when compared to healthy controls, with significantly lower platelet 5-HT content in Type II versus Type I alcoholics. Since it is generally accepted that low platelet 5-HT levels might be the result of decreased 5-HT synthesis, decreased platelet uptake, increased platelet release or increased 5-HT deamination by MAO, decreased platelet 5-HT levels are mostly attributed to the lower neuronal 5-HT levels in the central nervous system (CNS). In alcoholics, it was reported that plasma tryptophan availability was decreased and the affinity of 5-HT for its carriers or receptors was increased, providing other evidences of a diminished availability of 5-HT in the synapse and confirming the hypothesis of 5-HT deficiency in alcoholism.

The alcohol withdrawal syndrome has been considered to be a manifestation of neuroadaptive responses to chronic alcohol use and a central feature of alcohol dependence. This period, which overlaps with early abstinence, has been linked to increased
metabolism, and reduced function of platelet 5-HT and selective serotonin reuptake inhibitors (SSRI) have been reported to reduce craving for alcohol. Our finding demonstrating a significant reduction in platelet 5-HT levels in the alcoholic subjects is in good agreement with a previous report suggesting a decrease in the 5-HT levels of alcoholics during withdrawal. Although it is not clear whether platelet or plasma 5-HT levels reflect corresponding serotonergic changes in the human brain, it seems possible that central serotonergic function may be decreased in alcoholics since it was previously suggested that alcohol consumption has been known to stimulate serotonin turnover, and the withdrawal process may decrease 5-HT release from serotonergic neurons. It has been previously postulated that the effect of alcohol on 5-HT neurotransmission and metabolism may be modified by the presence of tolerance or dependence and comorbidities. However, we excluded these variables by measuring the blood 5-HT levels only in alcoholics who are alcohol dependent, but sober, during withdrawal.

Our data showing that Type I and II alcoholics differed regarding platelet and plasma 5-HT levels supported the previous investigations reporting that Type I and II alcoholics may differ in 5-HT measures, and there is a significant difference between these two subtypes in platelet tritiated imipramine binding. In this regard, platelet 5-HT content appears to be a useful biochemical measure for the subtyping of alcoholics.

The platelet and plasma 5-HT concentrations were found to be negatively correlated in all study groups, supporting the idea that there is neither a relevant 5-HT synthesis nor a marked 5-HT turnover in platelets and that platelets may have a role as a scavenger for free extracellular 5-HT uptake. Since the correlation between the platelet and plasma 5-HT concentrations in Type II alcoholics was found to be much stronger than that of the controls, it was suggested that 5-HT reuptake by platelets may be disordered in early-onset alcoholics.

The present study also confirmed the previous investigations indicating that alcoholics had lower platelet MAO activity when compared to the healthy controls. The platelet MAO activity of Type II alcoholics was found to be significantly lower than that of Type I alcoholics, in accordance with the earlier reports. In order to explain the association of lower platelet MAO activity and alcoholism, particularly the early-onset type, it was suggested that platelet MAO is a genetic marker for the size or functional capacity of the central monoamine systems, and the serotonergic system in particular. The significant positive correlation found between platelet MAO activity and 5-HT content and the strong negative correlation found between platelet MAO activity and plasma 5-HT content in alcoholics are not in accordance with some early reports suggesting that increased platelet MAO activity in alcoholics may lead to an increase in 5-HT catabolism and cause a decrease in platelet serotonin levels. However, our finding was supported by a report suggesting that a low level of platelet MAO seems to be constitutional rather than an effect of alcohol consumption and that platelet MAO acts as a genetic marker for the central serotonin system.

Transaminase and GGT activities, total lipid, cholesterol and LDL-C contents were found to be increased, while HDL-C, total protein, albumin and hemoglobin contents, PCV, MCV and RBC counts were found to be decreased in alcoholics when compared to control subjects, indicating the disordered liver functions and anaemia in alcoholics, in accordance with the previous reports. ALT, AST and GGT activities, total lipid, total cholesterol and LDL-C levels were found to be negatively correlated with platelet 5-HT and MAO content and positively correlated with plasma 5-HT content, whereas HDL-C level, hemoglobin content, RBC count and PCV were positively correlated with platelet 5-HT and MAO content and negatively correlated with plasma 5-HT content in Type II alcoholics. Collectively these data suggest that even though its nature is still unclear, there are important correlations between some platelet monoaminergic parameters and hematological and biochemical variables.
In summary, the present study confirmed the notion that platelet 5-HT content and MAO activity are altered in alcoholic subgroups and that these measures can be regarded as useful discriminative markers for subtyping of alcoholism.

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