An Attempt to Evaluate Diagnostic and Prognostic Significance of Blood Endogenous Ethanol in Alcoholics and Their Relatives

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OSTROVSKY, YU. M., P. S. PRONKO, S. N. SHISHKIN, V. B. KOLESIKOV AND S. I. VOLYNETS. An attempt to evaluate diagnostic and prognostic significance of blood endogenous ethanol in alcoholics and their relatives. ALCOHOL 6(2) 97–102, 1989.—Endogenous ethanol in the blood of human subjects was measured by gas chromatography. In healthy males, 12–13-year-old boys (sons of alcoholic and nonalcoholic fathers), and alcoholic inpatients (after cessation of all drugs), the endogenous ethanol levels ranged from 0 to 4.3 mg/l. The results showed no significant differences between the groups. At the period of alcohol withdrawal reactions the concentrations of endogenous ethanol were minimal in patients with delirium tremens and maximal in patients with mild alcohol withdrawal syndrome, the dynamics of this parameter being dependent on the severity of the alcohol withdrawal syndrome and the nature of the drugs prescribed.

THE recent data obtained by the use of highly sensitive and specific methods provide unequivocal evidence for the presence of endogenous ethanol (EE) in samples of blood from healthy abstaining subjects and tissues from laboratory animals. The concentration of EE in blood varies within the range from values below detection limits to 39 mg/l (16–18, 23, 25, 45).

The sources of EE have been a topic of much debate (21, 27, 45). The results of experiments on conventionally reared and germ-free animals (18,21) show that the only immediate precursor of EE, acetaldehyde, can be produced by microflora of the gastrointestinal tract and by a number of enzymes which constantly occur in tissues. Pyruvate dehydrogenase (EC 1.2.4.1) (27), phosphoethanolamine phosphatase (EC 4.2.99.7) (12), 5-deoxypentose-phosphate aldolase (EC 4.1.2.4) (40) and a number of other enzymes (7, 30, 37, 40, 44) found in animal tissues play a decisive role in producing endogenous acetaldehyde. Acetaldehyde is reduced to EE by alcohol dehydrogenase (EC 1.1.1.1), and this is, in our opinion, one of the main physiological functions of the enzyme (35).

Experiments on ethanol- and water-prefering rats have demonstrated that ethanol intake by animals is inversely proportional to the EE content in their blood and tissues (33,34).

The relationships between alcohol preference and EE levels were confirmed by other authors and examined in another series of investigations (1,9). Various manipulations which influence ethanol intake by animals or methods used in treatment of patients with alcoholism changed blood and liver EE levels. It was noted that all the factors (stress, starvation, vitamin B, deficiency, administration of hydroythiamine, ioprimidox or tetrahydroisoquinolines) which enhance alcohol preference decreased EE levels, while all the manipulations (administration of thiamine, thiamine pyrophosphate, riboflavin, glutamine, lithium chloride, diethyldithiocarbamate, cyanamide) which reduce alcohol consumption increased EE concentrations (31, 32, 42, 46).

The information about EE levels in human alcoholics has been obtained from studies on small groups of patients and is rather contradictory (9, 22, 23, 45). The clinical significance of this index is also unclear, although there is evidence for ethanol concentrations in blood of alcoholic patients depending on the severity of their state and the presence of delirium tremens (39).

The most important factors that determine severity of the

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Withdrawal syndrome (AWS) are high concentrations of ethanol in blood over drinking periods as well as the rate and rate of the decrease in alcohol concentrations: the lower these concentrations reduce, the more pronounced is withdrawal (6,29). Within 15-18 hours after cessation of alcohol intoxication, blood ethanol reaches "zero" (28) i.e., its concentration approaches the endogenous level. However, no information is available about relationship between EE concentrations in blood of alcoholic patients and severity of withdrawal during periods of their abstinence from alcohol. This stimulated us to study the level of EE in blood of alcoholic patients in the course of treatment of withdrawal and subsequent antialcoholic therapy.

The above data on the relationships between alcohol preference and EE levels make it interesting to investigate concentrations of EE in healthy sons of alcoholic and nonalcoholic fathers and to test the hypothesis that blood EE may be a biological marker to identify individuals at-risk for the development of alcohol addiction.

METHOD

Subjects

Alcoholic patients, 95 males aged 26-62 (mean 40 years), admitted to the clinic of the Grodno Regional Psychoneurologic Dispensary were studied. The patients met the criteria of the second and the third stages of alcoholism according to the classification of Portnow and Pyatinskaya (38): excessive alcohol intake, craving for alcohol of both obsessive and compulsive characters with loss of quantitative and decrease of situational control, withdrawal syndrome, changed forms of intoxication (amnesia, psychopathologic disorders), personality disorders of alcoholic type, changed tolerance to alcohol and the first signs of organic brain damage. The duration of alcohol abuse ranged from 4 to 23 years with a mean of 10.5 years. The last drink before admission was less that 24 hours in 15 of our patients, from 24 to 72 hours in 77 of them and more than 72 hours in 3 subjects.

We grouped the patients into three categories according to the severity of alcohol withdrawal reactions. The first group included 42 subjects with mild AWS manifested in craving for alcohol and somato-autonomic disorders (tremor, asthenia, sweating, thirst, tachycardia, labile blood pressure and dyspepsia). Twenty-eight alcoholics with severe AWS, characterized by pronounced somato-neurologic and psychopathologic disorders, belonged to the second group. Apart from the above symptoms, these patients had sleep disturbances, perception disorders, anxiety, depression. One of the patients had seizures, and two alcoholics showed the symptoms of acute hallucinosis. Twenty-five patients manifested delirium tremens during hospitalization, and they were included into the third group.

Detoxification. Treatment of withdrawal and vitamin therapy were continued for a period of 10 to 20 days and thereafter a specific antialcoholic therapy was given. During the first period alcoholic patients were treated with polyionic mixtures, sodium thiosulphate or unithiol, magnesium sulphate, glucose solutions. B group vitamins and ascorbic acid. Four of the patients with mild AWS received sedative drugs. 3 patients were treated with hypnotic drugs and 4 with antidepressants. In addition to detoxification and symptomatic therapy, alcoholics with severe AWS and delirium tremens were prescribed a combination of neuroleptics and tranquillizers. Drugs and dosage were chosen to attain rapid and marked sedative effect and control of withdrawal or psychotic symptomatology. The patients were given: haloperidol, 5-30 mg/day for 2-14 days (n=35); levoxamazine, 100-150 mg/day for 5-15 days (n=5); other neuroleptics (n=5); diazepam, 20-80 mg/day for 3-14 days (n=21); nitrazepam, 10-40 mg/day for 5-10 days (n=5); and other tranquillizers (n=13). Twelve patients showed symptoms of depression and they were treated with amitriptyline, 50-150 mg/day for 14-24 days.

The aversion therapy was given either with apomorphine (n=5), or metronidazole (n=21), or disulfiram (n=16), or with their combination (n=41). Following 10 days the treatment with either apomorphine-alcohol reactions (6-10 reactions over 12-20 days) or with metronidazole (1-2 g/day over 10-30 days) was started, while that with disulfiram (0.5 g/day over 20-25 days)—20 days after admission, following discontinuation of apomorphine or metronidazole. The complete course of treatment in the clinic was continued for 40 to 50 days.

Toxic doses of ethanol are usually eliminated within 24-48 hours (48). The measurement of EE concentrations in blood was commenced on the 2nd day after admission, when most of the patients (n=80) had abstained from alcohol for more than 48-72 hours, and was continued every other day up to the 10-12th days. During the first week some of the patients were tested daily. The examination was subsequently repeated every 8-10 days until discharge from the clinic. Blood sampling was not performed on days of apomorphine-alcohol reactions.

The control group consisted of 37 men aging from 18 to 57 years. They were admitted to the surgical department of the city hospital and examined during the period of convalescence following insignificant operations and fractures of bones. The hospital authorities and the patients themselves gave their consent to the examination. The control subjects showed no signs of psychiatric illness and alcoholism in an exploratory interview. They had abstained from alcoholic beverages for 10 to 20 days before the tests.

The subjects also included 369 randomly selected schoolboys aging from 12 to 13 years. They were examined after obtaining a written permission at the Public Health Department and the Educational Department of the Grodno Regional Soviet and the Grodno City Soviet of People's Deputies. Blood samples were obtained at the periods of annual medical examination. A group of 25 sons of alcoholic fathers were selected from the population of the schoolboys tested. Such boys were shown to be at high risk for the development of alcoholism in future (4,14).

To determine endogenous ethanol, we used samples of capillary blood (0.2 ml) obtained by a puncture of a fingertip. In the schoolchildren, blood sampling was performed from 8 till 9 a.m. after light meal. Blood was obtained from the adult individuals at 8 a.m. after they had fasted overnight.

Ethanol Assay

Ethanol concentrations in blood were determined by a gas chromatographic technique (47) with a slight modification. Blood (0.2 ml) was transferred into 14 ml vials, each containing 0.2 ml solutions of sodium fluoride (40.0 mg/ml), sodium nitrite (6.9 mg/ml) and n-propanol (3.2 mg/ml) as internal standard and stirred. To obtain a greater yield of ethanol from the water phase, 0.4 g of sodium chloride was added into the vials. The vials were made air tight with rubber septa and pressure sealed with aluminium caps.
TABLE 1
ENDOGENOUS ETHANOL IN BLOOD OF HUMAN SUBJECTS

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of Samples (n)</th>
<th>Range (mg/l)</th>
<th>Mean ± SEM (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sons of nonalcoholic fathers</td>
<td>344</td>
<td>0–3.42</td>
<td>0.36 ± 0.02</td>
</tr>
<tr>
<td>Sons of alcoholic fathers</td>
<td>25</td>
<td>0–0.90</td>
<td>0.29 ± 0.05</td>
</tr>
<tr>
<td>Adult control subjects</td>
<td>37</td>
<td>0–1.94</td>
<td>0.35 ± 0.06</td>
</tr>
<tr>
<td>Alcoholics after discontinuation of all drugs</td>
<td>41</td>
<td>0.05–1.00</td>
<td>0.37 ± 0.03</td>
</tr>
</tbody>
</table>

Sometimes blood samples were stored in a refrigerator at −20°C up to 2 weeks until used. The concentrations of ethanol in blood samples remained unchanged after a month's storage of that kind. An LChM-8 MD, model (Chromatograph, USSR) gas chromatograph fitted with hydrogen flame ionization detectors was used for analysis. One 1.5 metre, 3 mm i.d. stainless steel column packed with 0.25–0.5 mm polymer of styrol and divinyl benzol (Polysorb 1, NPO Biolar, Olayne, USSR) was conditioned at 200°C for 48 hours.

The operation conditions were: column temperature, 125°C; injector temperature, 150°C; detector temperature, 130°C; nitrogen (carrier gas) flow, 30 cm³/min; air flow, 300 cm³/min; and hydrogen flow, 30 cm³/min.

The vials were placed in a water bath maintained at 65°C and allowed to equilibrate for 15 minutes prior to injection of the headspace sample (1.0 or 2.0 ml) into the gas chromatograph.

The lower limit of sensitivity of the method with our modification was 0.1 mg/l of ethanol.

The statistical significances were calculated by Student's t-test. In some cases the nonparametric sign test of Fisher was used. Pearson's correlation was used in the calculation of correlations. All the results are given as means ± SEM.

RESULTS

Although the levels of blood EE in sons of nonalcoholic fathers varied over a wide range (Table 1), they were lower than 0.80 mg/l in 95% of the boys examined. Due to the predominance of lower concentrations, the distribution curve had a pronounced positive asymmetry (Fig. 1). The average values for EE concentrations in sons of alcoholic fathers did not differ significantly from those found in children of nonalcoholic parents. The distribution graphs of EE concentrations were essentially the same for both these groups.

On the second day of treatment the EE concentrations in alcoholic patients varied from 0.12 to about 19.10 mg/l.

On the third day after admission to the hospital the mean level of EE in the overall group of alcoholics was similar to that determined on the second day, but by the sixth day the EE concentration was below the initial level (Fig. 2). However, the direction of changes and the range of variations in the EE concentrations were different in individual patients; in addition to the decrease in the EE concentrations (n=17) we noticed an increase (n=9) or fluctuations in them (n=49),

FIG. 1. Distribution curve of endogenous ethanol concentrations in blood of sons of alcoholic and nonalcoholic fathers. Open columns: sons of nonalcoholic fathers; solid columns: sons of alcoholic fathers.

FIG. 2. Mean levels of blood EE in different groups of patients with alcoholism during complete course of treatment. Patients with mild AWS (○-○); severe AWS (Δ—Δ); delirium tremens (□-□); total group of patients (○-○). Each point is the mean from 94–50 subjects in the total group of patients, 42–20 subjects with mild AWS, 28–18 subjects with severe AWS, 25–13 subjects with delirium tremens. Filled symbols indicate statistically significant differences from the initial values. The last 3 points on the graphs indicate the levels of EE obtained on the 19th–22nd, 29th–32nd and 39th–41st days of treatment. Detoxification, vitamin therapy, treatment of withdrawal were continued for a period of 10 to 20 days. Metronidazole was started from the 10th to the 24th days of treatment, whereas disulfiram—from the 20th to the 25th days.
whereas some patients did not show appreciable changes in the levels of blood EE (n=20).

The grouping of patients according to the severity of their AWS reactions elicited significant differences in the blood EE concentrations. In the patients with mild AWS blood EE concentrations were much higher as compared with the levels found in the two other groups on the second and the third days after admission (Fig. 2), p<0.001 in all the cases.

On the second and the third days the levels of blood EE in alcoholics correlated negatively with the occurrence of delirium tremens (r = -0.7, p<0.001) and with the severity of withdrawal (r = -0.3, p<0.001) on assuming mild AWS to be equal to 1 point, severe AWS—to 2 points and delirium tremens—to 3 points. The application of χ² method also indicated a correlation between the concentration of EE and the occurrence of delirium tremens, χ² (1) = 14.3, p<0.001, and the severity of AWS, χ² (2) = 39.5, p<0.001, in our patients.

As seen from Fig. 2, in the group of patients with mild AWS blood EE tended to decrease from the fourth day, and it was significantly lower than the initial levels on subsequent days. On the contrary, the patients with severe AWS and delirium tremens showed an elevation in the EE concentrations. From the 10th day the differences between the groups were negligible.

The intensive drug therapy seemed to affect the EE concentrations in our patients. Therefore a comparison was drawn between 3 homogeneous in treatment and character of withdrawal groups of patients (n=25 in all cases) at different stages of AWS therapy (Fig. 3). It is interesting to note that FE generally reached a maximum by the time of disappearance of withdrawal symptoms in the course of treatment, and in some of the patients EE values were in the range from 10 to 25 mg/l. Mean concentrations of EE were higher when compared with the values obtained earlier (during withdrawal) in the group with severe AWS (r = 2.55, p<0.01) and in the group with delirium tremens (r = 4.02, p<0.001). The same test also indicated statistical significance of these differences in the group with severe AWS (B = 22, p = 0.0001) and in that with delirium tremens (B = 23, p<0.0001). In the patients with mild AWS, EE was only somewhat lowered after disappearance of withdrawal symptoms.

As seen from Fig. 3, a few days after the discontinuation of alcohol withdrawal therapy, the EE concentrations considerably decreased in all the groups of patients (for mild AWS r = 2.90, p<0.005; B = 16, p = 0.114; for severe AWS r = 2.99, p<0.004; B = 22, p<0.0001; and for delirium tremens r = 1.25, p<0.002; B = 20, p<0.002).

From the 20th up to the 40th days of antialcoholic therapy we observed a rise in the EE levels in all the groups of patients (Fig. 2). We believe this to be disulfiram effect. Metronidazole did not alter the blood EE concentrations (before the treatment and after it 2.37±0.60 and 2.22±0.46 mg/l, respectively; n=26, B=14, p=0.345), whereas disulfiram appreciably increased them (1.48±0.27 and 5.02±0.92 mg/l, 1.69, p<0.002; n=25, B=22, p=0.00001).

DISCUSSION

The levels of EE in healthy adults and children were essentially the same and approximated to the results of other investigators (17,45). A somewhat wider range of variations in EE concentrations was noted (25), but the distribution of the concentrations was similar to that obtained in the present study. On admission the concentrations of EE in patients with alcoholism, especially in those with mild AWS, were higher than the levels in healthy individuals. Some uncertainty of the time of the last alcohol intake may suggest a shorter withdrawal period in patients with mild AWS as compared to those with severe AWS and delirium tremens, and the trace amounts of exogenous ethanol in blood can explain the difference in blood ethanol levels found on the 2nd day. However, the decrease in the ethanol concentration from 3.5 to 1 mg/l in patients with mild AWS over 96 hours could hardly be considered as the final part of the elimination curve. Experiments on healthy volunteers showed that at low concentrations (100 mg/l) ethanol eliminated very rapidly and the endogenous level was attained within 1.5-2 hours (19). We may also assume that over a long period of time blood contained ethanol reversely bound to albumin and hemoglobin during intoxication (36). However, the values for the binding constants indicate that at the concentrations found in our patients on the second day of hospitalization only 5.5-7% of ethanol could be in a bound state and might have exogenous origin.

The relatively high levels of EE in alcoholics at all stages of treatment and the heterogeneous dynamics of this index in different patients (decrease, increase and fluctuations) cannot be explained only by elimination of residual exogenous ethanol. This may be a result of the intensive drug therapy. Our data show that after the discontinuation of all drugs the EE concentrations in alcoholics abstaining for more than 3 weeks did not differ from those in controls (Table 1). This coincides with the literature data. No differences were found in the levels of EE between patients with alcoholism and healthy individuals (23,45) and an insignificant shift to lower concentrations was observed in abstaining drug-free patients with alcoholism compared to controls (10).

We found considerable variations in the levels of EE among the groups of patients with a different severity of the...
ENDOGENOUS ETHANOL IN BLOOD OF HUMAN SUBJECTS

AWS. There was a negative correlation between the concentrations of blood EE and the severity of withdrawal and between EE and the presence of delirium tremens during the observation. The correlation may reflect an effect of metabolic disturbances arising in the AWS on the EE level. In particular, this may be related to the activation of catecholaminergic processes in the central and peripheral regions of the sympathetic nervous system observed during severe withdrawal and delirium tremens (3,26). Such changes are similar to those considered as factors accelerating the ethanol metabolism in humans (20,24) and can reduce the level of EE in blood. Our suggestion is in line with the data on the decrease of the blood EE concentrations after adrenalin administration to rats (5). Starvation in delirium could also contribute to the decrease in the EE level because of its reduced production in the intestine or tissues.

Interesting results were obtained in experiments on animals. Alcohol withdrawal caused a significant decrease of EE concentrations in rats with chronic ethanol intoxication, whereas apomorphine prevented this decline. The apomorphine action is thought to be materialized through the effect on the dopamine metabolism (2).

The relationship between the level of EE and the severity of the AWS and the data on more frequent delirium tremens in alcoholics with low concentrations of EE (39) also enables us to suggest that EE can play a certain role in the development of the states discussed.

During the drug therapy, the withdrawal symptoms were abated in patients with severe AWS from the 4th to the 8th days and in patients with delirium tremens—from the 5th to the 12th days. It was within this period that the EE concentrations were increased (Fig. 2). The elevation of EE in these groups of patients might be due to their intake of neuroleptics and sedatives since the EE concentrations decreased considerably after discontinuation of these drugs (Fig. 3). The direct experimental data are also available that neuroleptics and tranquillizers increase the levels of EE in blood of human subjects (8). The rise of the EE levels in the patients could indicate that the treatment changed the status of the central nervous system, but the elevated EE concentration itself might contribute to the improvement in the patients’s state. During the treatment, the concentrations of EE were 10–25 mg/l in some of our patients, while it was shown that the low-dose intravenous infusion of ethanol, which provided blood alcohol levels of 20 to 80 mg/l, prevented the appearance of withdrawal and delirium tremens symptoms in abstinent patients with alcoholism (15).

It is worth noting that the successful use of acupuncture (13) or psychotherapy (22) at the period of withdrawal increased the EE concentrations in alcoholic patients.

In agreement with our results are the data that in alcoholic patients actualization of the pathologic craving for alcohol and other types of emotional excitement are attended by reduced EE levels, whereas disactualization of the pathologic craving for alcohol and relaxation increase the EE concentrations. It is suggested that modulation of catecholamine levels ensures a connection between EE levels and the status of the central nervous system (22). Another mechanism of such a relationship can be manifested in a correlation between the concentrations of EE and β-endorphin in blood of healthy individuals and alcoholic patients (10).

We observed a rise in the blood EE levels in alcoholic patients treated with disulfiram. The ability of this drug to increase blood EE has also been shown in experiments on animals (42) and in human alcoholics after implantation of Esperal (22). First, this disulfiram effect may be related to its inhibitory action on the activity of tissue aldehyde dehydrogenase which can increase the level of the EE precursor, endogenous acetaldehyde. An elevation of the endogenous acetaldehyde concentration up to measurable values was found in blood of disulfiram-treated rats (11). Second, disulfiram is also an inhibitor of liver alcohol dehydrogenase (43) and can slow down EE catabolism, like it was demonstrated for pyrazole (21).

Thus, EE is a normal intermediate of metabolism in animals and humans. Its level changes as a function of various physiological conditions and after administration of drugs used for treatment of alcoholism. Since a correlation has been found between the levels of EE and the severity of the AWS or emotional state in alcoholic patients, further, more detailed investigations are necessary to study this parameter in humans with alcohol-related problems.

REFERENCES