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Alcohol Intoxication and Sialic Acid in Erythrocyte Membrane and in Serum Transferrin

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SCHELLENBERG, F., F. BEAUGÉ, C. BOURDIN, J. M. BOURRE AND J. WEILL. *Alcohol intoxication and sialic acid in erythrocyte membrane and in serum transferrin.* PHARMACOL BIOCHEM BEHAV **39**(2) 443–447, 1991.—Microheterogeneity of serum transferrin as well as erythrocyte membrane sialic acid content were examined in alcoholic patients and healthy controls. Both the sialic acid content of erythrocyte membranes and of the circulating transferrin were significantly lower in alcoholic patients than in controls. A moderate daily ethanol intake (less than 80 g) allowed to observe a proportional relationship between alcohol intake and the carbohydrate deficient forms of transferrin, and also a correlation between alcohol intake and the membrane sialic acid content. This supports the hypothesis of ubiquitary alterations of glycosylations in connection to ethanol intoxication. Additional disturbances could explain the absence of correlations between membrane sialic acid, pattern of abnormal forms of serum transferrin, and alcohol intake in heavy alcoholic patients.

Alcohol Erythrocyte membrane Serum transferrin Sialic acid

SOME years ago, abnormal forms of serum transferrin were found present in the spinal fluid as well as in the serum of alcoholic patients when analyzed by isoelectric focusing (23). These abnormal forms were characterized by their higher isoelectric point (5.7 and 5.9 versus 5.4 for the usual form). This microheterogeneity of the serum transferrin disappeared after alcohol withdrawal (17). Transferrin is a sialoglycoprotein containing 4 to 5 sialic acid residues responsible for the electrical charge of the molecule. Neuraminidase-treated serum samples exhibited the same microheterogeneity than samples from alcoholics (29), that suggested a reduced sialic acid content of the abnormal forms of transferrin molecule. This was later confirmed (25) by the determination of the sialic acid content of the transferrin forms. In order to use this microheterogeneity as a potential indicator of alcohol intoxication, different quantification methods of the abnormal fractions of transferrin were developed using either isoelectric focusing (21, 22, 33) or a radioimmunoassay (31).

Furthermore, a deficiency in sialic acid was also observed in the proteins of erythrocyte membranes of alcoholic patients (26). As other studies (13) have demonstrated that inhibition of glycosyltransferases by ethanol was involved in these two phenomena, alcohol consumption and sialic acid deficiency were possibly correlated. The purpose of the present investigation was, therefore, first to ascertain the decrease in the erythrocyte membrane sialic acid, as well as the microheterogeneity of transferrin in alcoholic patients and second to study a possible relation of these sialic acid alterations to the alcohol consumption.

POPULATION

Blood samples were collected from 27 alcoholic patients aged 23–64 years (median 36 years) who had been admitted for detoxication and rehabilitation to an alcohol treatment center, and from 17 control subjects aged 22–62 years (median 33 years) without any previous alcohol problem. Daily ethanol consumption was evaluated through a questionnaire. Control subjects were asked to complete this questionnaire at the time of the blood collection. Alcoholic patients were asked to complete it twice, first a week after they were admitted, and again two months later. All patients whose answers were divergent were excluded from the study. Those who had stopped drinking a few days before their admittance and those who had reduced their alcohol intake during the last two weeks were also excluded. According to these criteria, the daily ethanol intake was 0–60 g in the control group (mean 10 g) and 80–360 g in the alcoholic group (mean 196 g). All controls were in good health at the time of the blood collection. Alcoholic patients were supposed not to have severe liver injury (ascites or cirrhosis). For ethical reasons, it was not possible to submit alcoholic patients to liver bi-

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TABLE 1

RED CELL CHARACTERISTICS IN 17 CONTROLS AND 27 ALCOHOLIC PATIENTS

	Alcoholics Mean	p	Controls Mean
Red cell count $\times 10^{12}$/l	4.5	$p<0.05$	4.7
Hemoglobin g/l	147	ns	152
Mean corpuscular volume fl	100	$p<0.05$	91

opsies, but usual liver biochemical tests showed no serious liver damage.

METHOD

Erythrocyte Membrane Preparation

Erythrocyte membranes were prepared from blood samples anticoagulated with EDTA according to Fairbanks (5). Samples were resuspended in 0.32 M saccharose buffer pH 7.4 and stored at −25°C until used. The high concentration of saccharose prevented the membranes from aggregating during storage and permitted to obtain reproducible results even after a storage of several weeks. The protein concentration was determined with the usual Lowry method (12) using human serum albumin as standard.

Sialic Acid Determination

A modification of the commercially available enzymatic-colorimetric method (Boehringer Mannheim GmbH Sialic acid test ref. 784192) was developed for the sialic acid determination in membranes. The method has been fully automatized on a Progress (KONE, Helsinki) multiparameter analyzer; only 44 µl of membrane suspension are required for a duplicate determination, the total reaction time is 30 min, and the run to run precision is better than with the Warren method (16). The high concentration of saccharose in the sample does not interfere with the sialic acid determination. Results were expressed as µmol/g protein.

Serum Carbohydrate Deficient Forms of Transferrin

The carbohydrate-deficient forms of transferrin were evaluated by both the calculation of Tf index and the determination of the Carbohydrate Deficient Transferrin (CDT). Tf index was calculated as described previously (22): After iron saturation, transferrin fractions are separated by isoelectric focusing on ultrathin polyacrylamide gel. After the run, 25 µl anti-transferrin antiserum are added on the gel surface, nonbound proteins are discarded by several washes, and the transferrin-anti-transferrin complexes are stained using Coomassie blue. Tf index is the ratio Tf5.4/Tf5.7 in which Tf5.4 is the percent of the main band of tetrasialotransferrin and Tf5.7 the percent of disialotransferrin. For the determination of CDT, the method (Swedish Pat. 84 00587-5) used was that described by Stibler (31) except that radioactivity was measured directly using a γ counter. In this method, serum is iron-saturated, diluted with a piperazine-HCOOH buffer at pH 5.65 and passed through microcolumns packed with an anion exchanger. The eluate contains all proteins with a pI over 5.65, and CDT is determined in the eluate using a double antibody transferrin radioimmunoassay.

Statistical Methods

We used geometric means, Kruskal-Wallis analysis of variance, and Spearman's rank correlation coefficient.

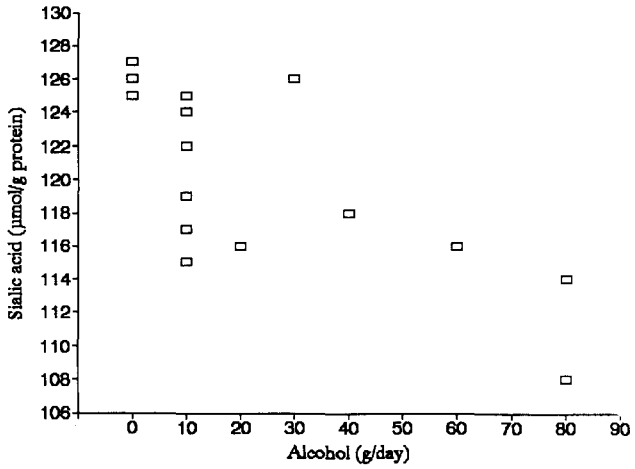

FIG. 1. Sialic acid content of the erythrocyte membrane and mean daily ethanol intake in 19 subjects consuming less than 100 g of ethanol per day.

RESULTS

Alcoholic patients had a mean daily ethanol intake of 196 g, ranging from 80 g to 360 g. Compared to the controls, they had slightly less red cells, and their mean corpuscular volume was significantly increased (Table 1). The absence of severe liver injury was assessed by their moderate liver biochemical alterations (Table 2). Standard deviations are only indicatory of the variety of the observed values. In both groups, the high σ values significate that the mean is distorted by some very high results. The increase of alkaline phosphatases and bulirubinaemia is significant, but beneath the upper limit of normal range values. We did not observe any significant decrease in the total transferrin concentrations.

Both the sialic acid concentration in membranes and the sialic acid content of circulating transferrin, evaluated by CDT and Tf index determinations, were significantly lower in alcoholic patients compared to the control group ($p<0.001$) as shown in Table 3.

TABLE 2

BIOCHEMICAL CHARACTERISTICS OF THE CONTROLS (n = 17) AND OF THE ALCOHOLIC PATIENTS (n = 27)

	Controls			Alcoholics	
	Mean	σ	p	Mean	σ
Total bilirubin	10	6	ns	15	5
Direct bilirubin	1	1	ns	3	3
Aspartate aminotransferase	22	6	$p<0.01$	49	66
Alanine aminotransferase	25	14	ns	34	40
Alkaline phosphatases	60	16	$p<0.01$	90	37
Gamma glutamyltransferase	26	41	$p<0.001$	282	258
Transferrin	2.78	0.28	ns	2.65	0.34

Normal ranges: Total bilirubin: 5–18 µmol/l. Direct bilirubin: 0–5 µmol/l. Aspartate aminotransferase: 5–30 UI/l. Alanine aminotransferase: 6–40 UI/l. Alkaline phosphatases: 40–105 UI/l. Gamma glutamyltransferase: 5–40 UI/l. Transferrin: 2.1–3.8 g/l.

TABLE 3

ALCOHOL CONSUMPTION, MEMBRANE SIALIC ACID CONTENT, Tf INDEX AND CDT IN 17 CONTROLS AND 27 ALCOHOLIC PATIENTS

	Controls				Alcoholics		
	Mean	σ	Range	*p*	Mean	σ	Range
Alcohol g/day	10		0–60		196		80–360
Sialic acid μmol/g prot.	121.8	4.8	115–127	<0.01	111.7	5.1	99–122
Tf index × 1000	47	12	22–65	<0.001	140	173	50–296
CDT mg/l	54.4	12.4	31–72	<0.001	128.8	44.8	57–275

A significant relationship ($Rs = -.617$, $p = 0.0001$) was found between membrane sialic acid content and alcohol consumption in the whole population, control subjects and alcoholics together (Table 4). When considering separately the control and alcoholic patients groups, no significant correlation was observed in the alcoholic patients. In the control group, a slight correlation ($p < 0.19$) was found, which may be considered significant because of the lack of precision in evaluating alcohol consumption. This difficulty is generally responsible for low correlations between alcohol consumption and other parameters. We considered separately a group containing all subjects with a mean daily ethanol intake equal to or below 80 g. In this excluding very high consumers subgroup, a high correlation ($Rs = -.675$, $p = 0.0115$) was found between membrane sialic acid and alcohol consumption (Fig. 1).

When considering the relationship between membrane sialic acid and carbohydrate-deficient transferrin, a high correlation was found in the whole population, but none in the control or alcoholic patients group. Nevertheless, sialic acid was significantly correlated to CDT ($Rs = -.714$, $p = 0.0076$) in the group consuming less than 80 g/day of ethanol.

DISCUSSION

Other studies showed that the transferrin alteration in drinkers did not solely consist in sialic acid deficiency (25), but also in other terminal carbohydrates such as galactose and N-acetylglucosamine (27). The decrease in transferrin galactose and N-acetylglucosamine suggested that the sialic acid defect was included in a wide range of alterations of the glycosylations. Some authors (17) suggested that sialic acid deficiency was probably caused by an impaired uptake of sialic acid-deficient transferrin due to membrane dysfunction. We had previously shown that the normalization of the transferrin pattern occurred in alcoholics during hospitalization with the same rate than serum transferrin turnover, and without any significant variation of the total serum transferrin (21). It seems more likely that the transferrin carbohydrate deficiency is caused by an impaired synthesis, as suggested by the normal levels of sialidase and β-galactosidase activities in the serum of alcoholics (30).

TABLE 4

SPEARMAN'S RANK CORRELATIONS BETWEEN ALCOHOL INTAKE AND STUDIED PARAMETERS

	Controls		Alcoholics		All	
	Rs	*p*	Rs	*p*	Rs	*p*
Alcohol/Sial	−.38	0.19	.01	0.97	−.62	0.0001
Alcohol/Tf ind.	.46	0.06	−.07	0.71	.68	0.0001
Alcohol/CDT	.62	0.01	.04	0.82	.73	0.0001

In the present study, a significant correlation was found between the increase of the sialic acid-deficient transferrin, as assessed by CDT or Tf index, and the ethanol intake in the control group. This group is homogeneous, consisting of abstainers or moderate drinkers whose alcohol consumption did essentially take place at meal times. No correlation was found in alcoholics between alcohol intake and the increase of the carbohydrate-deficient forms of transferrin. As mentioned above, there is no proportion in the dose-effect relations of ethanol when the mean daily intake exceeds 80 g. Several factors may be involved in this result. First, as we showed previously (20), the drinking habits could play a role in the synthesis of the carbohydrate-deficient forms of transferrin. An adaptative response to high concentrations of ethanol in the cell environment could be another factor. We never did measure Tf index over 0.45 or CDT over 340 mg/l, even for daily ethanol intakes near 700 g (22). This would suggest the existence of a threshold in the synthesis of the carbohydrate-deficient glycoproteins.

In brain membranes from young rats, an in vivo experimental ethanol exposure leads to a sialic acid defect (9,32) that can be correlated with ethanol tolerance development (18). In mice synaptic membranes, chronic ethanol treatment elicits also an increase in the surface-exposed galactose (6) that may be connected to a sialic acid defect. As well, brain gangliosides are affected by an acute administration of ethanol (11, 14, 15), while a chronic intoxication has no significant effects (34); this suggests an adaptive response of the membrane to the intoxication (10). The importance of sialic acid in mediating ethanol effects is confirmed by the antagonization of ethanol intoxication by gangliosides and sialic acid (3,8).

In human erythrocyte membranes, a decrease in the sialic acid content has been repeatedly observed (1, 2, 24, 26) in connection with alcohol intoxication. The ganglioside pattern was also found to be affected by chronic alcohol ingestion (7). The membrane glycosyl transferases did not appear to be directly involved in this alteration (28), suggesting that this membrane alteration reflected a more general phenomenon. This can be related to the membrane modifications during erythrocyte aging (4). The role of ethanol could be similar to an increase of the erythrocyte normal aging.

The sialic acid content we have measured in our control group is significantly higher than in the other published studies. This can be explained either by some differences in the membrane composition or the sialic acid determination. The decrease in the sialic acid content is also less marked in our sample (9% versus 11 to 14%) than in these previous studies. First, the mean daily ethanol intake appears somewhat lower in the present study (196 g/day versus 200–500 g/day). It should, nevertheless, be pointed out that no direct relation has been established between the alcohol amount and its biological consequences for daily ethanol intake over 80 g (35). Second, the drinking habits could be involved in this difference. Indeed, both the diet and the drinking habits are different in Northern European countries, where the previous studies had been carried out, and in France. Concerning the drinking habits, the kind of beverages and their distribution in the daytime could play a role in this lower sialic acid decrease. We have recently shown (20) that peripheral markers of alcohol abuse (Tf index, γGT) had various sensitivities in the detection of alcohol abuse when the alcoholic patients were regular or paroxystic drinkers, even when their mean daily ethanol intake was similar.

A highly significant correlation was found (Table 4) between the alcohol consumption and the decrease of the erythrocyte membrane sialic acid in the whole population. This correlation is not due to a separate group effect because the sialic acid variations are continuous and moderate as shown in Fig. 1. Considering separately controls and alcoholics, the results appear somewhat different. A slight correlation was found between alcohol intake and the sialic acid content of the erythrocyte membrane only in the control group. As a moderate ethanol intake appears to be of importance in the alteration of the glycosylations, a direct role of ethanol intoxication, more than an expression of a toxic effect on liver, seems to be at the origin of this phenomenon. This suggestion is supported by the absence of liver alterations in the control group and the moderate liver disturbancies in the alcoholics.

The increase in the carbohydrate-deficient forms of transferrin does not appear to go with the decrease in the membrane sialic acid content. This could partly result from analytical reasons, for the variation of each parameter is weak when compared to the accuracy of each determination (33,31). The influence of ethanol might also differ according to the region of the membrane where it has its action. Furthermore, the turnover rate of membrane proteins is probably different from that of serum transferrin (19).

Nevertheless, the correlations between alcohol intake and both the sialic acid content of erythrocyte membrane and the carbohydrate-deficient forms of serum transferrin tend to support the hypothesis of a common origin of the glycosylation disturbancies in the alcohol intoxication.

CONCLUSIONS

We have confirmed in this study the existence in human alcoholic patients of a decrease in the sialic acid content both in the erythrocyte membrane and in the circulating transferrin, as assessed by the measurement of its carbohydrate-deficient forms. A moderate daily ethanol intake (less than 80 g) allows to observe a proportional relation between alcohol intake and the carbohydrate-deficient forms of transferrin, and also a correlation between alcohol intake and the membrane sialic acid content. This result supports the hypothesis of a general problem of glycosylations in some aspects of the physiopathology of alcoholism. Further studies are needed to understand the absence of correlation between membrane sialic acid, isoforms of serum transferrin, and alcohol intake in heavy alcoholic patients.

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