ISOFORMS OF TRANSFERRIN IN PSORIASIS PATIENTS ABUSING ALCOHOL

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Abstract — The different isoforms of transferrin have been quantified by isoelectric focusing in the sera of psoriasis patients with and without a history of abusing alcohol. In both male and female psoriasis subjects abusing alcohol, there were significant increases in the 2-sialylated forms by comparison to the control subjects. Psoriasis patients who had no evidence of alcohol abuse had similar profile for the isoforms of transferrin to that of the controls. Other groups of patients with alcohol-induced tissue damage, i.e. liver, brain or muscle, used as positive controls, similarly showed significant increases in the 2-sialylated forms, by comparison to controls. These results substantiate the current use of carbohydrate-deficient transferrin as a sensitive marker of alcohol abuse, particularly in subjects not drinking in excess of 60 g of ethanol/day but showing alcohol-related psoriasis.

INTRODUCTION

In Western society, alcohol abuse has a prevalence of between 10 and 15% in the population. Within this small section, there is a striking variability in an individual's response to the effects of excessive alcohol intake. A variety of symptoms will be exhibited by these subjects ranging from life-threatening alcohol induced liver damage or brain damage, to cardiovascular disturbances, myopathy or merely skin disorders. Alcohol abuse is now recognized to precipitate or exacerbate certain cutaneous diseases, in particular psoriasis (Higgins and du Vivier, 1992 and 1994), and these skin problems appear to occur as an early manifestation, often before evidence of classical hepatic toxicity is apparent. A recent survey of 100 psoriasis patients attending the Dermatology Out-patients' Department of a London Teaching Hospital showed that 39 of these patients were abusing alcohol to a limited extent, marginally exceeding the recommended alcohol intake of 21 units/week for men and 14 units/week for women.

Only three subjects had any features of dependency (Higgins *et al.*, 1992). On cessation of alcohol intake the psoriasis improved.

There has been much interest to establish a biochemical test which will identify subjects abusing alcohol. Over the past 20 years, the use of the carbohydrate-deficient transferrin assay, which quantifies the degree of sialylation of transferrin, particularly the di- (2-Si), mono- (1-Si) and asialylotransferrin (collectively termed carbohydrate-deficient transferrin, CDT), has shown both specificity and sensitivity, in identifying individuals severely abusing alcohol. However, in the few studies of this parameter in the sera of individuals who abuse alcohol to a limited degree, the suitability of this biochemical marker to identify the abusers of alcohol has been questionable.

We have therefore assessed in these present studies the degree of sialylation of transferrin in male and female subjects with psoriasis, who have an intake of alcohol marginally above the recommended guidelines, and compared these results with another group of psoriatic patients with no evidence of abusing alcohol. Patients with alcohol-induced pathologies (myopathy, brain

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damage and liver damage) induced by excessive alcohol intake, have been used as positive controls.

MATERIALS AND METHODS

Selection of patients

Psoriasis patients. Patients (n = 52) were recruited from the Dermatology Out-patients' Department at King's College Hospital, London, UK as part of a prospective study examining the relationship between alcohol consumption and skin disease. Drinking habits were assessed by means of a frequency/quantity questionnaire (Bernhadt *et al.*, 1982). Of these 52 patients, four females and 18 males were identified as heavy drinkers (the mean intake of alcohol was 36 ± 17 units/week, or 284 ± 136 g alcohol/week). Each of these subjects was abusing alcohol at the time of the study.

Patients with alcohol-induced liver cirrhosis or myopathy. Patients with alcohol-induced cirrhosis (n = 6) or alcoholic myopathy (n = 6), which had been confirmed by liver and muscle biopsy, respectively, were consuming alcohol up to the time of the blood sampling (mean intake of alcohol/week 295 ± 30 units or 2360 ± 240 g alcohol/week), and were selected as positive controls for alcohol abuse.

Patients with alcohol-induced brain damage. Patients with alcohol-induced brain damage (n = 6), which had been confirmed by both biochemical (altered parameters for the erythrocyte transketolase enzyme) and cognitive tests, were also tested. Such subjects were not necessarily drinking at the time of blood sampling.

Control subjects. Healthy individuals (n = 10) were recruited for the study, with an age range comparable to the patient groups. These controls consumed only small quantities of alcohol per week (<4 units).

Methods

Carbohydrate-deficient transferrin. Sera were prepared from the blood specimens collected from the control and different patient groups, and were stored at $-20°C$ until analysis. Isoelectric focusing was by the previously described methodology (de Jong and van Eijk, 1988). Essentially serum (50 µl) was incubated with 2 µl of 0.5 M $NaHCO_3$ and 2 µl of 10 mM Fe(III) citrate for a few minutes at room temperature prior to its application to the Phast-System with Immobiline Dry Plate pH 4–7 (Pharmacia LKB, Uppsala, Sweden) with a pH gradient between the electrodes of 5.0–6.0. For the visualization of the separated transferrin bands 200 µl anti-Tf IgG (Dako IgG a/s, Copenhagen, Denmark; titre 1080) were spread over the surface of the gel immediately at the completion of the run and left to incubate at room temperature for 20 min. All other serum proteins were removed from the gel by washing the gel in 0.15 M saline for 48 h at 4°C with several changes of the saline. The gel was incubated with 200 g/l trichloroacetic acid and stained with Coomassie Brilliant Blue R-250, 1 g/l, at 50°C for 10 min. The gel was destained at 20°C. The purified sialic-acid-depending transferrin bands were quantified with an Ultroscan XL densitometer (He/Nelaser, 633 nm, Pharmacia, Sweden), connected to a personal computer with GelScan XL software (version 2.1, Pharmacia, Sweden).

Statistical analysis

Statistical analysis was by ANOVA with protected Tukey *t*-test.

RESULTS

Table 1 shows the results for the different isoforms of transferrin in sera of patients with psoriasis. There were small and insignificant changes in the percentages of isoforms of the highly sialylated form of transferrin present, while there were significant increases in the percentage of the 2-Si form present in the sera of psoriasis patients abusing alcohol, both male and female, by comparison to control subjects. In addition, the 3-Si form was also increased in the female psoriatic patients abusing alcohol. The profiles for the isoforms of transferrins in the patients with psoriasis not abusing alcohol were comparable to the controls.

Each of the patient groups in whom excessive amounts of alcohol had induced tissue damage (liver, brain or muscle) showed significantly increased percentages of the 2-sialylated forms of transferrin (Table 2). The increase in the 2-sialylated form in each of these groups appeared to be at the expense of the 6-Si (apart from liver-

Table 1. Isoforms of transferrin (%) in sera of psoriasis patients and controls

Subjects	Transferrin isoforms					
	7-Si	6-Si	5-Si	4-Si	3-Si	2-Si
Psoriasis patients						
Male (n = 20)	0.66 ± 0.39	3.88 ± 1.1	20.9 ± 2.9	63.9 ± 4.6	6.96 ± 2.29	2.7 ± 1.34
Female (n = 18)	0.52 ± 0.23	4.90 ± 1.14	23.6 ± 3.34	60.8 ± 4.0	7 13 ± 2.29	2.98 ± 1.06
Abusing alcohol						
Male (n = 10)	0.60 ± 0.36	4.19 ± 0.87	20.4 ± 2.15	63.9 ± 5.5	7.49 ± 3.1	3.42 ± 2.69*
Female (n = 4)	n/d	4.48 ± 1.76	23.8 ± 3.79	56.9 ± 6.76	10.23 ± 1.80*	4.68 ± 0.67**
Control subjects (n = 10)	n/d	4.92 ± 1.70	25.6 ± 1.70	60.6 ± 2.6	6.56 ± 1.83	2.23 ± 0.47

Results are mean ± SD. Statistical significance by ANOVA with protected Tukey t-test: *$P < 0.05$, **$P < 0.01$ by comparison to control subjects. n/d Denotes not detectable.

damaged patients) and 5-Si isoforms of transferrin, which were both decreased.

DISCUSSION

In this present study, both male and female psoriasis patients who marginally abused alcohol had significantly elevated levels of the 2-Si form of CDT, whereas the 3-Si form was also elevated in female subjects. Patients with psoriasis who did not abuse alcohol showed similar profiles for the isotransferrins to that of the controls. This study has also confirmed the large elevation of di-sialylated transferrin in dependent alcoholic patients with alcoholic liver disease and brain damage.

Earlier procedures used non-quantitative methods of isoelectric focusing, as reported in this present communication, while more recently, quantitative methods have been developed to separate transferrin components above pI 5.65 by micro-anion-exchange chromatography (Stibler et al., 1986) followed by radioimmunoassay (RIA) to evaluate CDT. This latter method has been further modified by adoption of a different elution buffer that achieves a more stable anion-exchange chromatography of isotransferrins. Studies which have compared isoelectric focusing and anion exchange chromatography (e.g. Schellenberg et al., 1989; Xin et al., 1992; Lof et al., 1993; Anton and Bean, 1994) have found a somewhat higher sensitivity and specificity with isoelectric focusing immunofixation than with the anion-exchange chromatography–RIA method. In addition, the analysis of CDT by anion-exchange chromatography will also suffer from the fact that total transferrin concentration is somewhat variable among individuals regardless of whether or not they are alcoholic, while the presence of anti-coagulants in the sample may interfere with the chromatographic procedure.

There have been numerous studies investigating the suitability of CDT as a pertinent marker of alcohol abuse. Sensitivity and specificity >70–80% for alcohol misusers have been identified, particularly in patients with liver cirrhosis

Table 2. Isoforms of transferrin (%) in sera of alcohol misusers with myopathy, liver or brain damage, and healthy controls

Subjects	Transferrin isoforms					
	7-Si	6-Si	5-Si	4-Si	3-Si	2-Si
Alcohol misusers						
Liver damage (n = 6)	n/d	5.16 ± 1.39	20.9 ± 3.9	59.1 ± 5.3	7.72 ± 2.3	6.58 ± 4.86**
Myopathy (n = 6)	n/d	3.05 ± 1.09	17.7 ± 6.2	64.2 ± 5.7	7.3 ± 1.8	4.5 ± 2.3*
Brain damage (n = 6)	n/d	3.2 ± 0.32	20.18 ± 2.36	62.9 ± 3.13	8.33 ± 1.53	5.4 ± 3.0*
Control subjects (n = 10)	n/d	4.92 ± 1.36	25.6 ± 1.70	60.6 ± 2.6	6.56 ± 1.83	2.23 ± 0.47

Results are mean ± SD. Statistical significance by ANOVA with protected Tukey t-test: *$P < 0.05$, **$P < 0.01$ by comparison to control subjects. n/d Denotes not detectable.

(Stauber *et al.*, 1995) or recently consuming large amounts of alcohol (Lesch *et al.*, 1996*a*). The most critical unresolved question is what exact parameters are needed to alter the CDT levels, e.g. how much alcohol needs to be consumed, what is the duration of the drinking history needed and does there have to be associated tissue damage? In the few studies where CDT values have been assayed in healthier and younger alcohol misusers with shorter drinking histories, CDT has been shown to have a sensitivity of <30% (Takase *et al.*, 1985). Furthermore, in one study, only 20% of normal subjects consuming 60 g/day for 3 weeks showed CDT levels that exceeded the upper normal level (Salmela *et al.*, 1994), while no elevation of CDT was evident in control subjects consuming 80 g alcohol/day for 3 weeks (Lesch *et al.*, 1996*b*). Such results have led to the belief that it is necessary for there to be some degree of physical damage before CDT is elevated (Takase *et al.*, 1985; Bisson and Milford-Ward, 1994). In addition, in one study, female alcohol abusers were shown to have a lower specificity and sensitivity for CDT than male abusers (Anton and Moak, 1994), although this was not confirmed in this present study. The few female psoriatic patients abusing alcohol showed highly elevated amounts of 2-Si CDT. A study of 439 treated hypertensives showed that there was a relationship between CDT and high alcohol intake, the sensitivity of the test being 87%, but there was a considerable number of false positives (Fagerberg *et al.*, 1994*a*). Serum CDT concentrations were also associated with insulin sensitivity (Fagerberg *et al.*, 1994*b*) indicating that factors related to insulin may be operative.

The iron-binding protein transferrin plays an important role in iron homeostasis, binding to transferrin receptors on cell surfaces prior to internalization and release of iron into the cell. It remains unclear as to whether excessive alcohol consumption actually alters iron homeostasis. It is clear that alcohol will increase gut permeability, and possibly increases absorption of iron into the enterocyte (Thomson *et al.*, 1995) and therefore iron uptake. There was a twofold increase in the mean concentration of serum ferritin in the psoriasis patients with increased alcohol intake by comparison to the non-drinking psoriasis group (165.9 ± 146 vs 87.8 ± 6.75 ng/l respectively), although only five of the former group had values

for ferritin increased above the normal range (unpublished results). Other diseases where there are perturbations of iron metabolism have been investigated, to ascertain whether changes also occur in the transferrin isoforms. Patients with haemochromatosis (increased serum ferritin levels) showed marginally increased CDT values, particularly during phlebotomy (Jensen *et al.*, 1994), although the values at this time were not necessarily elevated above the reference range. Iron deficiency caused either as a normal physiological response to pregnancy (decreased serum ferritins) or as a result of chronic inflammation (increased serum ferritin), as observed in rheumatoid arthritis patients, showed a tendency for an increase in the highly sialylated transferrins, rather than the disialotransferrin which maintained a constant value (de Jong, 1993).

These studies have indicated that CDT is a sensitive marker of alcohol intake in subjects not necessarily showing gross physical damage. CDT may therefore prove to be an effective marker of early alcohol misuse, possibly before the onset of irreversible organ damage. Although other markers of alcohol abuse show high specificity and sensitivity either alone or in combination, such as apo A-II, plasma γ-GT, plasma uric acid and MCV (Burke *et al.*, 1992), the simplicity of a single test to assess alcohol abuse remains attractive. Further studies are required to identify what role these different isoforms of transferrin may play within biochemical systems.

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