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VARIATIONS IN DRUG FREE FRACTION DURING ALCOHOL WITHDRAWAL

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1 Free fractions of diazepam, propranolol and warfarin were determined in 15 male chronic alcoholics in alcohol withdrawal.

2 On admission the mean free fraction of diazepam was 25% above and propranolol 44% below the limits of normal range, while the mean warfarin free fraction was in high normal range. One week later mean free fraction of diazepam declined by 20% while propranolol and warfarin increased by 24% and 19% respectively ($P < 0.05$).

3 Propranolol free fraction and α_1-AGP concentrations were highly correlated (linear $r = -0.83$, $P < 0.001$). In contrast the sources of variation in diazepam and warfarin free fraction were more complex and less certain.

4 Statistically significant changes of drug free fractions in serum of chronic alcoholics were observed during alcohol withdrawal. The extent and direction of these changes differed for various classes of drugs and their potential causes appear to be quite different.

5 Clinically important changes in drug effect may be present acutely, within the dosing interval, as a result of altered drug binding. These are more likely when the clinical response is closely related to drug concentration and will occur within the dosing interval due to larger fluctuations in free drug concentration, even though the average free drug concentration will remain unchanged.

6 Total drug level changes will be observed during alcohol withdrawal even in absence of detectable changes of drug metabolism.

Introduction

Ethanol could alter plasma protein binding of drugs by modifying the number and/or affinity of binding sites for the drug on the protein. Such change could occur with acute ethanol inhibition of hepatic protein synthesis, in association with established liver disease, by change in the concentration of other plasma constituents known to bind drugs or by alteration in intermediary metabolism of endogenous substances known to modify drug binding (Sellers & Holloway, 1978). Fatty acids have been shown previously to modulate the reciprocal changes in diazepam and warfarin binding (Sellers *et al.*, 1980) whereas propranolol free fraction was inversely related to variations in α_1-acid glycoprotein (α_1-AGP) (Borga *et al.*, 1977). However, the extent of changes in free fraction over short periods of time and changes in free fraction for acidic and basic drugs during alcohol withdrawal have not been studied. We have studied the extent and origin of changes in binding of diazepam and warfarin which are bound predominantly to albumin (Sellers *et al.*, 1980) and of a basic drug, propranolol, bound extensively to the acute phase reactant protein, α_1-AGP (Borga *et al.*, 1977) during alcohol withdrawal. Changes in free fraction could be expected to have both practical clinical consequences with respect to treatment of chronic alcoholics in withdrawal as well as important but more theoretical implications for the conduct and interpretation of pharmacokinetic studies of alcohol effect on drug disposition. The most easily observed consequence of such free fraction changes will be reciprocal changes in total drug concentration (Sellers *et al.*, 1979b). The net effect is to shift the nominal therapeutic range of a drug (Greenblatt *et al.*, 1982).

Methods

Fifteen male chronic alcoholics ages 32 to 63 years in moderate to severe withdrawal, as determined by the Clinical Institute Withdrawal Assessment for Alcohol

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(CIWA-A), were admitted to the Clinical Research Unit of the Addiction Research Foundation (Shaw *et al.*, 1981) (Table 1). All had a history of consuming an average of at least 200 g of ethanol per day for at least 5 years and had been drinking continuously for at least 1 week prior to admission. Patients were initially studied no more than 24 h after ingesting their last drink. Four blood samples were obtained; one on admission and three fasting samples on the mornings of the second and third days and on the morning before discharge (5 to 7 days post-admission). Each sample was collected with an all glass non-heparinized system and tubes were covered with Teflon®. Samples were immediately refrigerated and allowed to coagulate for 30 min. Serum was separated by centrifugation at 5000 rev/min for 10 min not more than 45 min after collection. Diazepam, propranolol, and warfarin free fractions were determined in duplicate by equilibrium dialysis usually the same day, but always within 24 h of sample collection. [^{14}C]-diazepam (Amersham Corp., 97% radiochemically pure, specific activity 200 μCi/mg) was added to and mixed with non-radioactive drug to 0.5 ml aliquots of serum as a tracer to achieve a concentration of 600 ng/ml, and was dialyzed against a physiological saline phosphate buffer (pH 7.4) (Ehrnebo & Odar-Cederlof, 1975), at 37°C for 6 h to reach equilibrium (Abel *et al.*, 1979). Equilibrium of drug in this system is achieved within 3 h. [^{14}C]-R/S-warfarin (Amersham Corp., 97% radiochemically pure, specific activity 158 μCi/mg), concentration of 1.2 ng/ml and (±)-4-[^{3}H]-propranolol hydrochloride (Amersham Corp., 97% radiochemically pure, specific activity 74 μCi/mg), concentration 50 ng/ml respectively, were studied in the identical system as for diazepam. The coefficient of variation for diazepam, propranolol and warfarin free fraction determinations was less than 2%. The concentration of fatty acids in serum was determined by a modification of the method of Dole (Trout *et al.*, 1960). The coefficient of variation of duplicate fatty acid determinations was 5% and the normal range in our laboratory was 1000–1500 mEq/l, for men.

α_1-AGP and albumin (Romach *et al.*, 1981), fatty acid concentration and cholesterol (Levine and Zak, 1964), and triglycerides (Kessler & Lederer, 1965) were determined on all samples. Paired *t*-test, analysis of variance and multiple linear regression were used for the statistical tests. The study was approved by the Human Experimentation and Ethics Committee of the University of Toronto.

Results

After admission, none of the free fractions, fatty acids, cholesterol, triglycerides, α_1-AGP or albumin changed significantly over the initial 2 days. These data were therefore combined to characterize the early or symptomatic phase of withdrawal and used for multiple linear regression calculations. Data collected subsequent to this time period when the patients were clinically improved were referred to below as those observed in the late withdrawal phase. At the

Table 1 Clinical characteristics of the subjects at the time of admission

Subject	*Age (years)*	*Weight (kg)*	*Abuse (years)*	*Consumption (g/day)*	*Assessment CIWA-A**
1	32	72.4	10	360	21
2	35	73.4	10	—	20
3	42	60.4	10	224	19
4	52	56.2	4	234	34
5	38	85.7	13	600	23
6	50	75.0	22	—	19
7	56	51.0	30	—	23
8	49	57.5	28	—	20
9	46	71.8	25	210	19
10	52	66.0	35	—	35
11	40	53.5	24	514	24
12	54	70.6	4	234	21
13	50	47.0	25	374	22
14	38	—	3	234	27
15	63	101.8	35	400	25
Mean	46.5	67.3	18.6	338	23.5**
± s.d.	8.7	10.9	11.3	13.6	5.1

*Clinical Institute Withdrawal Assessment for Alcohol

**Corresponds to a clinical assessment by clinicians of moderately severe withdrawal.

time of admission, mean diazepam free fraction was 25% above the normal range reported from our laboratory (Abel *et al.*, 1979), propranolol free fraction was 44% lower than lower limit of normal (Borga *et al.*, 1979), while mean warfarin free fraction was in high normal range (Sellers *et al.*, 1980) (Figures 1 and 2). One week later, when patients were no longer symptomatic, mean diazepam free fraction declined by 20% while mean propranolol and warfarin free fraction increased by 24% and 19% respectively (Table 2). All changes were statistically significant ($P < 0.05$); concurrently α_1-AGP and fatty acids decreased significantly ($P < 0.05$); but albumin, cholesterol, and triglycerides did not change.

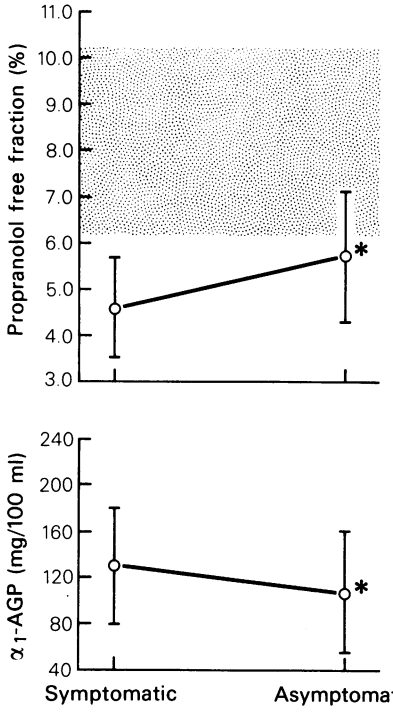

Figure 1 Changes in propranolol free fraction and α_1-AGP concentration in 15 male chronic alcoholics during symptomatic and asymptomatic phase of alcohol withdrawal. The differences of means between symptomatic and asymptomatic phase (*) are statistically significant ($P < 0.05$). The shaded area indicates the 95% confidence band of normal values for males in our laboratory (propranolol free fraction 8.24 ± 0.99% and α_1-AGP concentration 80 ± 30 mg/100 ml; mean ± s.d.).

Propranolol free fraction and α_1-AGP concentration were highly correlated (linear $r = -0.83$; $P < 0.001$). In fact, the relationship was slightly curvilinear and addition of a quadratic term in the multiple linear

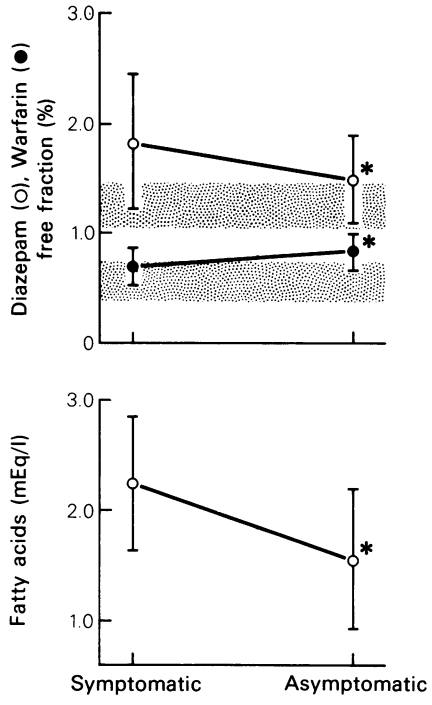

Figure 2 Changes in diazepam and warfarin free fractions and fasting fatty acids in 15 male chronic alcoholics during symptomatic and asymptomatic phase of alcohol withdrawal. The differences of means between symptomatic and asymptomatic phase (*) are statistically significant ($P < 0.05$). The shaded area indicates the 95% confidence band of normal values for males in our laboratory (diazepam free fraction 1.25 ± 0.11%, warfarin 0.561 ± 0.084% and fasting fatty acids 1.73 ± 0.67 mEq/l, mean ± s.d.).

regression improved the correlation to multiple $r = 0.94$ ($P < 0.001$), accounting for 89% of the variance in propranolol free fraction. Addition of albumin to the regression equation only accounted for an additional 1% of propranolol free fraction variance (Table 3). The sources of variation in diazepam and warfarin free fraction are far more complicated and less certain than with propranolol. Multiple linear regression indicated that duration of drinking immediately prior to admission and γ-glutamyl transpeptidase levels (GGT) on admission had an association with diazepam free fraction observed on the day of admission (Table 4). With the exception of subject's age, the remaining variables did not contribute significantly to the variance when duration of drinking was controlled. In contrast, the effect of the duration of drinking on warfarin free fraction at the time of admission was only of borderline significance ($0.05 < P < 0.1$) (Table 5). Interestingly, warfarin free fraction showed a strong negative

Table 2 Observations in 15 male chronic alcoholics during early (days 1 and 2) and late (days 3 to 7) alcohol withdrawal

Variable		n	*Early*	n	*Late*	*P <*	*Normal range (Male)*
DFF	(%)	25	1.84 ± 0.59	27	1.48 ± 0.39	0.02	1.05 – 1.47
WFF	(%)	24	0.70 ± 0.16	26	0.82 ± 0.18	0.02	0.4 – 0.7
PFF	(%)	25	4.62 ± 1.1	27	5.66 ± 1.4	0.02	5 – 12
FFA	(mEq/l)	21	2228 ± 585	23	1558 ± 632	0.001	1000 – 1500
AGP	(mg/100 ml)	25	134.3 ± 47.8	27	116.1 ± 44.2	0.05	< 90
ALB	(g/100 ml)	25	4.2 ± 0.6	27	4.2 ± 0.5	n.s.	3.3 – 5.5

Mean ± s.d. of diazepam, warfarin, propranolol free fraction (DFF, WFF and PFF, respectively), fatty acid concentration (FFA), α_1-acid glycoprotein concentration (AGP) and albumin concentration (ALB).

correlation with α_1-AGP (linear correlation coefficient $r = 0.40$, $P < 0.005$). There was a weak, but significant inverse relationship between diazepam and warfarin free fraction (linear correlation coefficient $r = -0.28$, $P = 0.05$).

Discussion

We have observed significant changes of drug free fractions in serum of chronic alcoholics during alcohol withdrawal. Free fraction of diazepam decreased whereas propranolol and warfarin free fraction increased. Not only is the extent and direction of these changes different for various classes of drugs, but also the potential causes of these changes appear to be quite different. Propranolol, a basic drug ($pK_a = 9.45$) binds to albumin and α_1-AGP and changes in the concentration of the latter protein are primarily responsible for the changes in free fraction that occur in health as well as in disease states (Borga *et al.*, 1977). In alcohol withdrawal, as in other disease states, α_1-AGP variations accounts for virtually all the propranolol free fraction variations. Whether withdrawal itself, or concurrent diseases in chronic alcoholic patients cause the increase α_1-AGP is not known. However, after 1 week of alcohol withdrawal α_1-AGP levels are still 15–20% above normal although significantly lower than in the symptomatic phase of withdrawal ($P < 0.05$). The curvilinear relationship between propranolol free fraction and α_1-AGP is predicted by the law of mass action.

Warfarin is known to bind primarily to albumin, however, only small changes in albumin concentration were observed during alcohol withdrawal and these accounted for only a small portion of warfarin free fraction variance. Furthermore, albumin on α_1-AGP together accounted for only 30% of observed warfarin free fraction variance, implying that other factors are important determinants of variations in warfarin binding. The inverse relationship between diazepam and warfarin free fraction has been reported recently (Sellers *et al.*, 1980) and seems to occur because diazepam and warfarin bind to albumin at two different sites with reciprocal modulations of binding affinity (Sudlow, 1979).

Diazepam free fraction depends primarily on albumin concentration and phase of withdrawal. In another study, we have reported that increased fatty acid concentrations are associated with increased

Table 3 Multiple linear regression (MLR) of propranolol free fraction (PFF) *vs* concentration of α_1-acid glycoprotein (AGP), second power of AGP and albumin concentration (ALB)

MLR	*Independent variable*	r^2 (%)	F	*Simple* r	*beta*	F
I	AGP	69	111.4	−0.83	−0.83	111.4*
II	AGP	89	212.6	−0.83	−2.84	186.2*
	AGP**2			−0.71	2.06	97.9*
III	AGP	90.2	147	−0.83	−3.0	157.5*
	AGP**2			−0.71	2.3	87.0*
	ALB			−0.23	0.09	2.5 (NS)

*$P < 0.001$

Table 4 Multiple linear regression (MLR) of diazepam free fraction (DFF) *vs* duration of the last binge (DB), serum concentration of γ-glutamyl transpeptidase (GGT) at the time of admission and age of subject (AGE)

MLR	*Independent variable*	r^2 (%)	F	*Simple* r	*beta*	F
I	DB	36	8.0*	0.6	0.60	8.0*
II	DB	64	11.5*	0.6	0.61	13.5**
	GGT			0.51	0.52	9.8**
III	DB	75	11.7	0.60	0.49	10.3*
	GGT			0.51	0.51	12.6*
	Age			0.51	0.34	5.01*

*$P < 0.05$, **$P < 0.01$

diazepam free fraction (Sellers *et al.*, 1980). In our patients this association is weak, probably masked by heterogeneity of alcohol population with respect to liver function, nutritional status and metabolic abnormalities, which are difficult to quantitate with current methods. GGT in this context perhaps may be viewed as a measure of metabolic derrangement and disturbed liver function secondary to ingestion of alcohol (Rollanson *et al.*, 1972). Fatty acid concentrations had no detectable effect on diazepam binding in the setting of alcohol withdrawal.

Both diazepam and warfarin are highly bound and their calculated total body clearance is binding sensitive (Blaschke, 1977). Hence a change in plasma protein binding results in a changed steady state concentration of *total* drug, however, steady state *free* drug concentration will remain relatively unaffected (Sellers *et al.*, 1979a). Because the free drug level is the determinant of pharmacological activity, total steady state levels may not correlate with pharmacological effect. Hence, at steady state the binding changes that we have observed may appear of no clinical significance. However, of greater importance for some drugs would be the changes in free concentration as a function of time *within* the dose interval (Levy, 1976), e.g. non-steady state kinetics in situations in which the total body clearance of a drug is long relative to the plasma clearance during distribution, e.g. diazepam, an increase in free drug concentration in the plasma compartment will be associated with a decrease in *total* drug concentration as free drug enters tissue, transiently higher initial free concentrations than with 'normal' binding, but a more rapid distribution of drug. The consequence of this homeostatic adjustment will be greater fluctuations in free drug concentration within a dose interval, even though the average free concentration will be the same.

The clinical importance of a change in the free concentration-time curve within a dose interval will be determined by the nature of the response. If the response is closely related to concentration, then one can imagine increased therapeutic or toxic effects early in a dose interval and the reverse late in the dose interval. Benzodiazepine toxicity in alcoholic patients during withdrawal may be explained in this fashion. Conversely, for a response that is damped, *average* free concentration would be expected to be

Table 5 Multiple linear regression of warfarin free fraction (WFF) *vs* serum albumin concentration (ALB), duration of the last binge (DB) and subject age

MLR	*Independent variable*	r^2 (%)	F	*Simple* r	*beta*	F
I	ALB	15	2.63	−0.40	−0.40	2.63
II	ALB	29	2.64	−0.40	−0.62	5.1*
	DB			−0.09	−0.43	2.4
III	ALB	36	2.29	−0.40	−0.73	6.5*
	DB			−0.09	−0.38	1.9
	Age			−0.11	−0.30	1.4

*$P < 0.05$

the better predictor of clinical effects, e.g. warfarin where acute changes in free concentration within dosing intervals are of little clinical significance.

Propranolol is a highly extracted drug with total body clearance comparable to hepatic clearance. In this situation, decreased binding will cause higher free concentration and lower total drug concentration resulting in less drug being available for the essentially flow restricted elimination and consequently decreased total body clearance (Sellers *et al.*, 1979b).

Since total drug concentration is inversely dependent on free fraction, the most frequently observed practical implication of this work will be, that total drug levels measured for therapeutic or kinetic purposes during alcohol withdrawal will vary even in the absence of detectable changes in drug biotransformation.

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