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Plasma protein binding of disulfiram and its metabolite diethylthiocarbamic acid methyl ester

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Abstract—The human plasma protein binding of disulfiram and its metabolite, diethylthiocarbamic acid methyl ester has been studied by an ultrafiltration technique. Both compounds were bound principally to albumin (mean 96·1 and 79·5%) over the ranges 200–800 and 345–2756 nM, respectively. The average number of binding sites was approximately 1 for both substances, whereas the average association constants were $7{\cdot}1 \times 10^4$ and $6{\cdot}1 \times 10^3$ M^{-1}, respectively. Log P (octanol/water) was 2·81 for disulfiram and 1·85 for diethylthiocarbamic acid methyl ester.

To date, disulfiram has been the only successful drug supporting treatment of alcoholic patients in clinical practice. For those patients on a standard treatment regimen of the drug either significantly fewer drinking days or a longer time to relapse after discontinuation of treatment compared with a control group was reported (Fuller & Roth 1979; Fuller et al 1986). Recently, the therapeutically active metabolite of disulfiram, diethylthiocarbamic acid methyl ester (Me-DTC), was identified as a potent inhibitor of the low K_m isozyme of aldehyde dehydrogenases (ALDH) in rat liver (Johansson et al 1989). The inhibition of this enzyme is thought to be responsible for the disulfiram-ethanol reaction when ethanol is ingested (Kitson 1978). In healthy volunteers taking disulfiram and challenged with ethanol a close relationship was found between the given dose, the plasma concentration of Me-DTC and a valid disulfiram–ethanol reaction (Johansson & Stankiewicz 1989). In alcoholics known to respond to disulfiram medication the mean plasma concentration of Me-DTC agrees with findings in healthy volunteers (Johansson & Stankiewicz 1989). However, in patients who tolerate the medication, i.e. those who in spite of the intake of disulfiram could continue drinking alcohol, the plasma concentration of Me-DTC was below the level of detection during treatment (Johansson & Stankiewicz 1989), indicating an incomplete inhibition of the low K_m isozyme in these patients. It is now possible to investigate the basic pharmacokinetics of the active principle of disulfiram. This paper reports the plasma protein binding characteristics of both the parent drug and Me-DTC.

Materials and methods

Chemicals. Tetraethyl[1-^{14}C]thiuram disulphide, 5 μCi mg^{-1} was a generous gift from A/S Dumex, Copenhagen, Denmark. [^{14}C]Me-DTC was synthesized from [^{14}C]COS in reaction with diethylamine and then methylated with methyl iodide (Johansson 1989). Labelled COS was prepared by reaction of [^{14}C]KSCN (586 μCi mg^{-1}, Amersham, UK), with a 2·5 molar excess of 50% H_2SO_4 at room temperature (Dalvi et al 1975). The radiochemical purity (98·5%) and specific activity (58 mCi $mmol^{-1}$) of the product were determined by gas chromatography and HPLC (Johansson et al 1989).

Methods. Heparinized blood from five healthy volunteers was centrifuged at 1500 *g* for 15 min, and the pooled plasma used to study plasma protein binding of [^{14}C]disulfiram and [^{14}C]Me-DTC by means of agarose electrophoresis and liquid scintillation counting of isolated protein slices of the gel (Albert 1985). Five μL (350 nmol) of [^{14}C]Me-DTC or 1 μL (15 nmol) of [^{14}C]disulfiram in ethanol was added to 50 μL of plasma. After incubation at 37°C for 2 h, 8 μL of plasma was analysed by agarose electrophoresis.

Binding parameters were studied at room temperature (23°C) (Me-DTC) and at 4°C (disulfiram) in ultrafiltration units (Centrifree Micro partition System, Amicon Corporation, Lexington, MA, USA). The protein binding of [^{14}C]disulfiram in plasma was investigated at pH 4·5, because of its instability under physiological conditions (Johansson 1988), and at pH 4·5 and 7·4 added to an isotonic 10 mM phosphate buffer solution containing human albumin (45 g L^{-1}). Portions (2 mL) of the blood pool were acidified with an equal volume of disulfiram stabilizing agent (Johansson 1988) consisting 0·15 M of sodium chloride, 0·2 M acetic acid and, 10 mM diethylene triamine pentaacetic acid (DTPA). The plasma phase was separated by centrifugation at 4°C. Portions (2 μL) of labelled disulfiram were added to 500 μL fractions of stabilized plasma or albumin solution and to pure isotonic albumin solution to give 200, 400 and 800 nM. The plasma protein binding of [^{14}C]Me-DTC was studied after its addition at 345, 689, 1378 and 2756 nM to 1·0 mL of plasma. The ultrafiltrate, containing the free drug fraction, was obtained by centrifugation at 2000 *g* for 30 min. All measurements were made in duplicate. The radioactivity in 100 μL sample or ultrafiltrate was measured by liquid scintillation counting (Johansson 1989).

Percentage fractions of bound drug were calculated as $(C_T - C_F)/C_T \times 100$, where C_T is total concentration of the drug in the sample and C_F the concentration of free drug in the ultrafiltrate. The number of binding sites per molecule of protein and the mean association constant, K_a, were determined from a direct linear plot (Eisenthal & Cornish-Bowden 1974).

The stability of unlabelled disulfiram in pure albumin solution 45 g L^{-1} at pH 7·4 added at a concentration range of 100–800 nM was determined by HPLC (Johansson & Stankiewicz 1989). The partition coefficient of both substances between octanol/water (log P, where P is equal to $C_{octanol}/C_{water}$) was determined according to Hansch et al (1963).

Results and discussion

The plasma protein binding data are illustrated in Table 1. Albumin is the only binding protein of both [^{14}C]disulfiram and [^{14}C]Me-DTC, as shown by the single band of radiolabelled albumin that separated from other plasma proteins on an agarose electrophoretic gel. In the blood of patients on disulfiram treatment or in fresh blood to which disulfiram had been added, the drug was rapidly reduced to the monomer diethyldithiocarbamic acid and further converted to its bis(diethyldithiocarbamato) copper complex (Johansson & Stankiewicz 1985; Johansson 1990). Before quantitative determination of disulfiram concentrations in plasma could be obtained, it was essential to add stabilizing agents to the blood collection tubes promptly at the time of collection or at preparation when disulfiram was added to fresh blood or plasma in-vitro. Disulfiram in human blood or plasma is most stable at pH 4·5 in the

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Table 1. Protein binding of disulfiram (DSF) and diethylthiocarbamic acid methyl ester (Me-DTC) in human plasma and albumin solution (45 g L^{-1}).

Sample	% bound (±s.d.)	K_a M^{-1}	N
Plasma, pH 4·5, + DSF (n = 3)	88·4 (±1·8)	$2·6 \times 10^4$	1
Albumin soln (n = 3) pH 4·5, + DSF	88·1 (±1·5)	$2·2 \times 10^4$	1
Albumin soln (n = 3) pH 7·4, + DSF*	96·1 (±0·2)	$7·1 \times 10^4$	1
Plasma, pH 7·4, + Me-DTC (n = 4)	79·5 (±1·4)	$6·1 \times 10^3$	1

n = number of independent samples analysed in duplicate.
The association constant, K_a and the number of binding sites per molecule of protein, N, were evaluated graphically from a direct linear plot (Eisenthal & Cornish-Bowden 1974).
* Recovery of disulfiram (100–800 nM) in human albumin solution (45 g L^{-1} in 10 mM phosphate buffer, pH 7·4) was 100% as determined by HPLC (Johansson & Stankiewicz 1989).

presence of sodium chloride and with DTPA added as a metal chelating agent (Johansson 1988). However, the recovery of disulfiram in the concentration range of 100–800 nM added to pure albumin solution (45 g L^{-1}) at pH 7·4 was close to 100% without need of stabilizing agents. The average binding of [^{14}C]disulfiram to albumin at pH 4·5 in plasma or pure albumin solution was 88·4 (±1·8) and 88·1 (±1·5)%, respectively, whereas at pH 7·4 in albumin solution binding was 96·1 (±0·2)%. Thus, protein binding of labelled disulfiram in stabilized plasma resembles the binding in pure albumin solution at pH 4·5, whereas the binding in albumin solution at pH 7·4 increases to about 96%. These results not only suggest albumin to be the major binding protein, a finding which agrees with the electrophoretic data, but also that the percentage binding of disulfiram in fresh untreated human plasma resembles that in albumin solution at pH 7·4. The decrease in pH of both plasma and albumin solution may affect the structure of the albumin molecule, leading to detachment of the bound drug, and a change in association constant (Table 1). The more hydrophilic Me-DTC metabolite is bound to a lesser extent with a mean value of 79·5 ± 1·4%.

The percentage binding was found to be linear over the concentration ranges tested with mean association constants at pH 7·4 of $7·1 \times 10^4$ M^{-1} (disulfiram) and $6·1 \times 10^3$ M^{-1} (Me-DTC). The mean number of binding sites per molecule of protein was approximately 1 for both substances, indicating a single type of binding site.

The hydrophobicity, expressed as log P (octanol/water) was found to be 2·81 for disulfiram and 1·85 for Me-DTC. The affinity of albumin for Me-DTC is less than for disulfiram, probably because of the metabolite's more hydrophilic structure. In view of the adverse effects observed in some alcoholic patients on disulfiram treatment (Rainey 1977), these findings may be of considerable importance especially for those patients with liver disease associated with impaired protein synthesis and a decrease in the blood albumin concentration (Albert 1985).

References

Albert, A. (1985) Selective Toxicity. The Physico-chemical Basis of Therapy. 7th edn, Chapman and Hall, London & New York, pp 65–117

Dalvi, R. R., Hunter, A. L., Heal, R. A. (1975) Toxicological implications of the mixed-function oxidase catalyzed metabolism of carbon disulfide. Chem. Biol. Interac. 10: 347–361

Eisenthal, R., Cornish-Bowden, A. (1974) The direct plot. A new graphical procedure for estimating enzyme kinetic parameters. Biochem. J. 139: 715–720

Fuller, R. K., Roth, H. P. (1979) Disulfiram for treatment of alcoholism. Ann. Intern. Med. 90: 901–904

Fuller, R. K., Branchey, L., Brightwell, D. R., Derman, R. M., Emrick, C. D., Iber, F. L., James, K. E., Lacoursiere, R. B., Lee, K. K., Lowenstam, I., Maany, I., Neiderhiser, D., Nocks, J. J. Shaw, S. (1986) Disulfiram treatment of alcoholism. A Veterans Administration study. J. Am. Med. Acs. 256: 1449–1455

Hansch, C., Muir, R. M., Fujita, T., Maloney, P. P., Greiger, F., Streich, M. (1963) Biological activity of plant growth regulators. J. Am. Chem. Soc. 85: 2817–2829

Johansson, B. (1988) Stabilization and quantitative determination of disulfiram in human plasma samples. Clin. Chim. Acta 177: 55–64

Johansson, B. (1989) Diethylthiocarbamic acid methyl ester: a suicide inhibitor of liver aldehyde dehydrogenases? Pharmacol. Toxicol. 64: 471–474

Johansson, B. (1990) Distribution of disulfiram and its metabolites over erythrocyte cell membranes and inactivation of erythrocyte aldehyde dehydrogenase activity. Pharmacol. Toxicol. 66: 104–108

Johansson, B., Stankiewicz, Z. (1985) Bis-(diethyldithiocarbamato) copper complex: a new metabolite of disulfiram? Biochem. Pharmacol. 34: 2989–2991

Johansson, B., Stankiewicz, Z. (1989) Inhibition of erythrocyte aldehyde dehydrogenase activity and elimination kinetics of diethyldithiocarbamic acid methyl ester and its monothio analogue after administration of single and repeated doses of disulfiram to man. Eur. J. Pharmacol. 37: 133 138

Johansson, B., Petersen, E., Arnold, E. (1989) Diethylthiocarbamic acid methyl ester. A potent inhibitor of aldehyde dehydrogenase found in rats treated with disulfiram or diethyldithiocarbamic acid methyl ester. Ibid. 38: 1053–1059

Kitson, T. M. (1978) Studies on the interaction between disulfiram and sheep liver cytoplasmic aldehyde dehydrogenase. Biochem. J. 175: 83–90

Rainey, J. M. (1977) Disulfiram toxicity and carbon disulfide poisoning. Am. J. Psych. 134: 371–378.