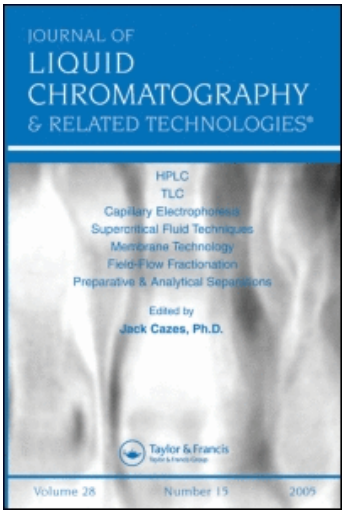

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Analysis of Plasma Diethyldithiocarbamate-A Metabolite of Disulfiram

H. G. Giles[ab]; J. Au[c]; E. M. Sellers[ab]

[a] Addiction Research Foundation, Toronto, Ontario, Canada [b] Departments of Pharmacology and Medicine, University of Toronto, [c] Department of Chemical, Biological and Materials Technology, Ryerson Polytechnical Institute,

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ANALYSIS OF PLASMA DIETHYLDITHIOCARBAMATE A METABOLITE OF DISULFIRAM

H.G. Giles,[1] J. Au[2] and E.M. Sellers[1]
[1]Addiction Research Foundation, 33 Russell Street, Toronto, Ontario, Canada and Departments of Pharmacology and Medicine, University of Toronto.
[2]Department of Chemical, Biological and Materials Technology, Ryerson Polytechnical Institute.

ABSTRACT

A reversed-phase high-performance liquid chromatographic analysis was developed for diethyldithiocarbamate in plasma. Following treatment of plasma (1 ml) with methyl iodide (250 µl), biphenyl (internal standard, 1.8 µg) was added in chloroform (6 ml). After shaking (30 min.), the chloroform was separated and evaporated under nitrogen (to 50 µl). Acetonitrile (250 µl) was added and the solution was again evaporated under nitrogen (to 100 µl). Aliquots (25 µl) were chromatographed using acetonitrile: acetate buffer (65:35, pH 4) at 2.5 ml/min on a 5 micron C-8 column with detection at 276 nm. Recovery of methyldiethyldithiocarbamate (MeDDC) was 92.5 ± 3.2%. Retention times and theoretical plates for MeDDC and biphenyl were 3.0 and 4.6 min., and 4660 and 6336 respectively. The analysis was linear over the range 25 to 400 ng/ml with a coefficient of variation of 3.2%. Analysis of samples after intravenous disulfiram (10 mg) administration to rats yielded a total body clearance of 343 ml/min. This supports the view that metabolism is principally by extra-hepatic routes.

INTRODUCTION

Disulfiram (tetraethylthiuramdisulfide) has been used in the treatment of alcoholism for 30 years (1). The contribution of the disposition of disulfiram and its major metabolites in efficacy and toxicity studies in animals and man has not been investigated adequately because of difficulties

in drug analysis. Few specific chromatographic techniques have been developed. A flame-ionization gas chromatography method has been used (2) but it is not very sensitive and a normal phase high performance liquid chromatographic (HPLC) technique has been developed (3).

It seems to be accepted that *in vivo* disulfiram is subjected to immediate reduction to the parent thiol, and the diethyldithiocarbamate (DDC) so formed is rapidly metabolized (3-5). One metabolite is the methyl derivative. This paper describes the analysis of DDC as its methyl ester (MeDDC) in human plasma and the application of the method in determining the kinetic profile of DDC after intravenous administration of disulfiram in rats.

EXPERIMENTAL

Sample preparation

MeDDC was prepared by the method of Cobby *et al* (2). It is somewhat volatile under nitrogen so accommodation must be made in both adding the compound to plasma and in handling the extract.

Aliquots (100 µl) of the standard solutions of MeDDC in methanol were added to plasma (1 ml) and these solutions were then treated in the same manner as the unknown samples.

The plasma samples were placed in screw-capped tubes (15 ml) and methyl iodide (250 µl) was added. The tubes were then capped, agitated on a shaker for 30 min., and centrifuged. Chloroform (5 ml) was removed and concentrated under a stream of nitrogen to 50 µl. Acetonitrile (250 µl) was then added and mixed and this solution was again evaporated under nitrogen to a final volume of 100 µl. This procedure is necessary because of the

volatility of MeDDC. Aliquots (25 µl) of this extract were analyzed on the chromatograph.

Mobile phase

Acetonitrile (Burdick and Jackson Laboratories, Inc.) was filtered through a 0.5 micron filter (Millipore Corporation). The buffer was prepared by adding 0.72 g of sodium acetate to 1 L of 0.05 M acetic acid. The pH was 4.0. The buffer was filtered through a 0.45 micron filter. The mobile phase consisted of 65:35, v:v, acetonitrile:buffer. It was degassed by bubbling in a stream of helium. The flow rate was 2.5 ml/min and the pressure was 2500 p.s.i.

Apparatus

The apparatus consisted of a pump (Waters Associates 6000A), automatic injector (Waters Intelligent Sample Processor), column (Brownlee, C-8, 5 micron, 25 cm), variable wavelength detector (Perkin-Elmer LC75) set at 276 nm, and an integrator (Hewlett-Packard 3380A).

Animal experiment

Seven male, Sprague-Dawley rats (320-350 g) were cannulated in the jugular vein and allowed 10 days to recover from surgery. Disulfiram (10 mg) was administered in 400 µl of a vehicle consisting of 80% ethanol and 20% isotonic saline. Blood samples were obtained after decapitation at 0, 1 and 5 min., and 1, 2, 3, and 7 hours. Each sample was divided into two for the analysis of DDC (methylation with methyl iodide) and the metabolite MeDDC (no methylation).

Calculations

The standard curve was generated from peak height of MeDDC/peak height biphenyl.

Analytical recovery was calculated from the concentration of MeDDC obtained from plasma divided by the concentration obtained from methanol.

Rat plasma DDC concentrations were determined after subtracting (on a molar basis) MeDDC quantities produced by metabolism. The pharmacokinetic profile (Fig. 2) shows a bi-exponential decline. The apparent elimination half-life disposition rate constant, clearance, and apparent volume of distribution were determined by standard methods (6).

RESULTS AND DISCUSSION

Figure 1 shows a chromatogram of MeDDC and biphenyl extracted from spiked plasma. There was no interference from endogenous compounds under the conditions stated. Although there was no notable influence of pH on the elution of either MeDDC or biphenyl, variations in pH do affect the elution of endogenous compounds. Under neutral conditions there is interference with MeDDC. The recovery of MeDDC was 92.5 $\pm$ 3.2% of the theoretical maximum. The capacity factors for MeDDC and biphenyl were 1.61 and 3.06, respectively, while the efficiencies were 4660 and 6336 theoretical plates, respectively. Table 1 summarizes the reproducibility of the method. The average coefficient of variation over the range studied was 3.2%. The limit of detection is 3 ng/ml. This assay has the specificity, sensitivity, and reproducibility necessary and its use is illustrated in the rat experiment.

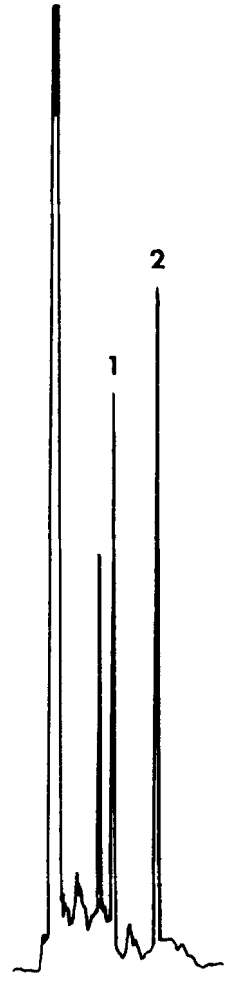

Fig. 1 Chromatogram of plasma extract containing MeDDC (200 ng/ml). Retention times of MeDDC (peak 1) and biphenyl (peak 2) are 3.0 and 4.6 min., respectively.

Table 1 Reproducibility using spiked human plasma[a]

AMOUNT ADDED (ng/ml)	AMOUNT DETERMINED (ng/ml)	PERCENT DETERMINED	C.V.(%)
0	0	---	---
25	24	96	4.0
50	48	97	4.0
100	102	102	2.5
200	202	101	1.4
300	306	102	3.9
400	394	99	3.6

[a]Eight samples were determined in each case

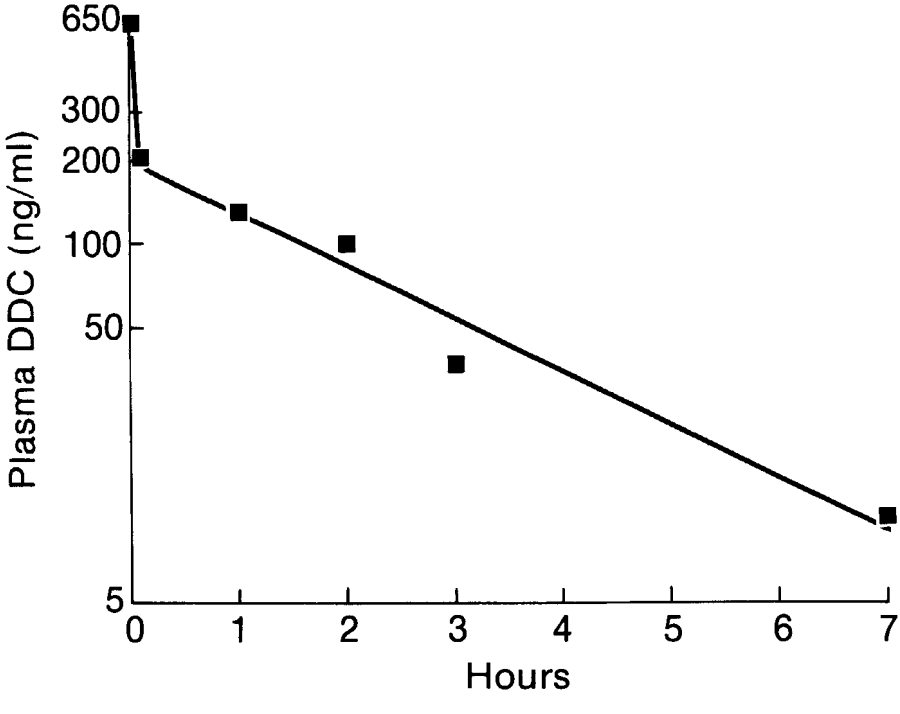

Fig. 2 Kinetic profile of DDC after disulfiram (10 mg, intravenously) administration to rats.

The dose of disulfiram (10 mg or 33 mg/kg) was larger than that normally given to humans (3.5-7 mg/kg). This dose is the same dose that was given to rats in another study (4) and far less than the dose (200 mg/kg) given to mice in other work (3). A complicating feature, however, is that because of solubility problems (3,4), the drug was given in an ethanolic vehicle. Because ethanol is known to inhibit drug metabolism (7) the clearance value determined must be seen as a minimum value.

Metabolically produced MeDDC is a relatively minor and constant constituent (30±6 ng/ml) but its subtraction from the total MeDDC concentration (35 ng/ml) at 7 hours was significant. The DDC concentration versus time profile is shown in Fig. 2. The terminal half-life, apparent volume of distribution, and clearance were found to be 1.59 hr., 47.1 L, and 343 ml/min, respectively. Since liver plasma flow in the rat is only 8 ml/min and the total body clearance is 343 ml/min, our results support the view that clearance is principally be extra-hepatic routes of elimination.

REFERENCES

1. Hald, J. and Jacobsen, E., Lancet, 255, 1001 (1948).

2. Cobby, J., Mayersohn, M., and Selliah, S., J. Pharmacol. Exp. Ther., 202, 724 (1977).

3. Jensen, J.C., and Faiman, M.D., J. Chromatogr., 181, 407 (1980).

4. Stromme, J.H., Biochem. Pharmacol., 14, 393 (1965).

5. Cobby, J., Mayersohn, M., and Selliah, S., J. Pharmacokinet. Biopharm., 6, 369 (1978).

6. Gibaldi, M., and Perrier, D., Pharmacokinetics, Marcel Dekker, Inc., New York, 1975.

7. Sellers, E.M., and Holloway, M.R., Clin. Pharmacokinet., 3, 440 (1978).