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PLASMA CONCENTRATIONS OF DISULFIRAM AFTER INJECTION OF SUSPENDED MICROPELLETS INTO ALCOHOLIC SUBJECTS

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ABSTRACT

The disposition of disulfiram after administration in a new formulation was studied in rats and alcoholic patients. The suspension consisted of microcrystals suspended in a mixture of propylene glycol and water and injected subcutaneously into rats and humans; the course of the plasma concentration of diethyldithiocarbamate with time was followed by gas–liquid chromatography. Systemic delivery of disulfiram was observed to occur for a month. The data demonstrate that this form has the properties of a sustained-release formulation when implanted subcutaneously. There was no evidence of local or systemic adverse reactions.

KEY WORDS Disulfiram Sustained-release formulation Implants Alcoholism treatment Plasma concentrations Disposition

INTRODUCTION

Disulfiram, tetraethylthiuram disulfide (DSF), has been utilized clinically since 1948 in the treatment of alcoholism.[1,2] This compound has practically no pharmacologic effect of its own but it is an important inhibitor of the metabolism of ethanol. DSF inhibits aldehyde dehydrogenase[3] enzyme that normally oxidizes acetaldehyde to acetic acid in the pathway ethanol → acetaldehyde → acetic acid;[4] this occurs in the liver during normal alcohol catabolism. By competing with nicotinamide adenine dinucleotide for aldehyde dehydrogenase,

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DSF produces an apparently irreversible inhibition of enzyme activity. When alcohol is ingested after administration of DSF, the acetaldehyde concentration in blood may increase to 5–10 times the concentration found during metabolism of the same amount of alcohol alone.[5] Accumulation of acetaldehyde in blood is commonly believed to be responsible for the unpleasant symptoms of the DSF-alcohol reaction; this is known as the 'acetaldehyde syndrome' characterized by flushing, dypsnea, nausea, vomiting, chest pain, palpitation, tachycardia, and hypotension.

Alcoholic patients taking DSF as tablets can themselves stop the treatment and thereby revert to the alcoholic state. Failures of treatment with oral therapy have stimulated the use of new forms of administration. The surgical implantation of DSF pellets has been, in this sense, important in long-term therapy because it precludes discontinuation of the treatment by the patient.[6,7]

Previously[8] we reported the disposition kinetics of DSF administered in tablets and as pellet implants, to normal and alcoholic patients.

In the present work we report the disposition of DSF, in rats and alcoholic patients; the drug was administered in a new implantation form, i.e. micropellets suspended in an aqueous vehicle.

MATERIALS AND METHODS

Materials

DSF (USP grade, Ayerst Laboratories) powder with a particle size of 90 μm obtained by sieving was conditioned in sealed glass vials and then sterilized by gamma radiation. DSF was assayed after sterilization by the method of Cobby *et al.*;[9] no degradation or contamination of the drug was detected.

Prior to injecting rats or patients, the microcrystals were suspended in a mixture of propylene glycol: water (5:1) sterilized in 10 ml sealed ampuls by autoclaving at 121° for 30 min.

The internal standard, bibenzyl (1,2-diphenylethane, Aldrich Chemical Company, Inc, Milwaukee, Wisconsin, USA, catalog number B3, 370–6) was used in the chromatographic method.

Animals

Ten female Wistar rats weighing 250–300 g, maintained on a normal laboratory diet, were used. Each animal was injected subcutaneously in the abdominal region with a suspension of DSF in a dose of 5 mg and kept in individual cages during the entire experiment.

Blood samples were obtained several times in the same animals by cardiac puncture before each DSF injection and 3, 7, 14, 21, 37, and 44 days after. Blood was received in an anticoagulant mixture (ammonium oxalate and potassium oxalate) and centrifuged at 2,000 rev min^{-1} for 10 min. Plasma was refrigerated at 2–5° until analysis.

Patients

This investigation was conducted under medical supervision in the Alcoholism Unit of the Hospital del Salvador (Santiago, Chile) and the protocol was approved by the Ethics Committee of this Unit. Five alcoholic male volunteers, age range 22–41 years (mean 38 years) and weight 52–71 kg (mean 68 kg), who were deemed healthy on the basis of routine clinical examination and biochemical and haematological profiles and who were not receiving any medication, were included in the study. All subjects gave written informed consent to participate in the study after having read a volunteer information sheet followed by an oral explanation.

Each subject received by injection in the adipose zone of the low abdomen, 1 g DSF suspended in 6 ml of the liquid vehicle by means of a 10 ml disposable syringe with a 18 G × 1½ needle. Prior to the injection, the abdominal zone was anaesthetized with 1% lidocaine. Blood samples were withdrawn by venipuncture at times 0, 1, 2, 3, 4, 7, 10, 17, 21, 25, and 30 days after injection into an oxalate anticoagulant mixture and centrifuged; separated plasma was frozen until analysis for diethyldithiocarbamate (DDC) concentration.

Patients were submitted to a psychotherapy and clinical control during the 2 months after injection.

Analytical Methods

The principal metabolite of DSF, the diethyldithiocarbamate (DDC) in plasma was determined by gas chromatographic procedure of Cobby *et al.*[9] with minor changes: 1.2 ml of plasma sample was methylated with methyl iodide (50 μl with the addition of bibenzyl as internal standard (25 μl of a solution 3.51×10^{-5} M in carbon tetrachloride). The resulting solution was extracted with carbon tetrachloride (2 ml). After concentration under nitrogen, an aliquot (2 μl) was injected into an isothermal gas chromatograph (Varian model 3700) equipped with a glass column containing 3 percent OV-101 on Chromosorb W-HP 80/100 mesh. The measured drug peak was monitored with a flame ionization detector. All plasma concentrations are reported in terms of DDC anion. This assay is linear and reproducible, with a lower limit of sensitivity of 3 ng ml^{-1}.

Analysis of the results

To evaluate elimination rate (k_e), the slope of the terminal portion of the log-linear plot of concentration of DDC vs time was determined by linear regression analysis.

The total area under the plasma concentration curve (AUC) was calculated for each subject for the entire 30 days after injection, using the linear trapezoidal method and from this time to infinity by dividing the last experimental plasma concentration by the slope of the log-linear terminal phase.

The observed maximum plasma concentration (C_{max}) and the time at which it occurred (t_{max}) were tabulated for each subject as well as the Mean Residence Time (MRT) obtained by dividing the area under the first moment of the plasma concentration curve versus time by the total AUC.[10–12]

Statistical Analysis

The confidence intervals for pharmacokinetic parameters were carried out at a significance level of $p = 0.05$ in order to ascertain intersubject variations in those parameters.[13]

RESULTS AND DISCUSSION

The rapid and quantitative reduction of DSF to its active metabolite (DDC) in blood and plasma has been reported by Cobby *et al.*[9] The mean DDC plasma concentration profile in rats after subcutaneous injection is shown in Figure 1. DDC concentration in plasma increases during the first week and then decreases slowly. The lipid solubility of DSF leads us to think that in the first week the drug is dissolved in the fatty tissues and then it is released slowly

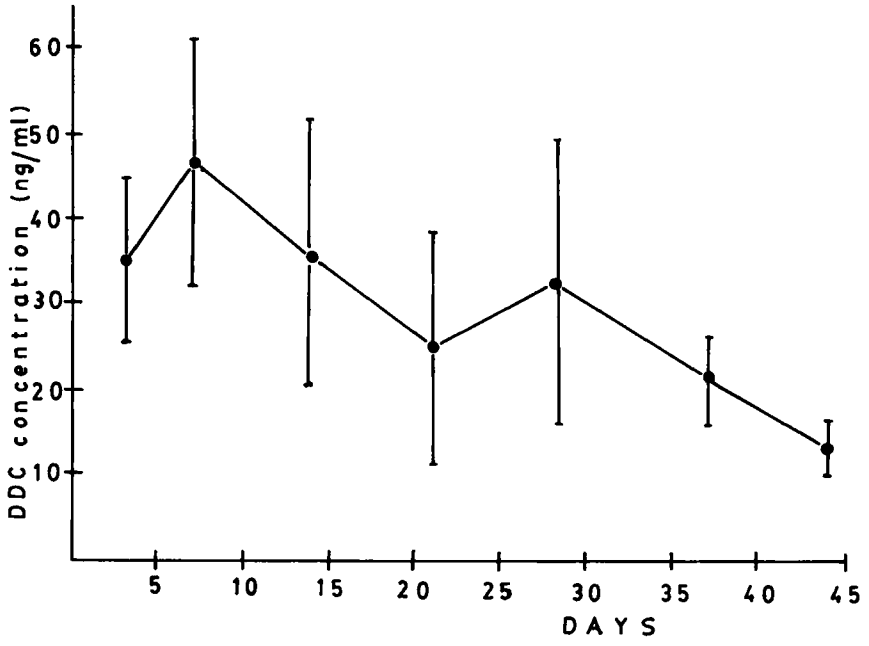

Figure 1. Mean diethyldithiocarbamate plasma concentrations after injection of 5 mg of Disulfiram subcutaneously to rats. Vertical bars indicate one standarad deviation

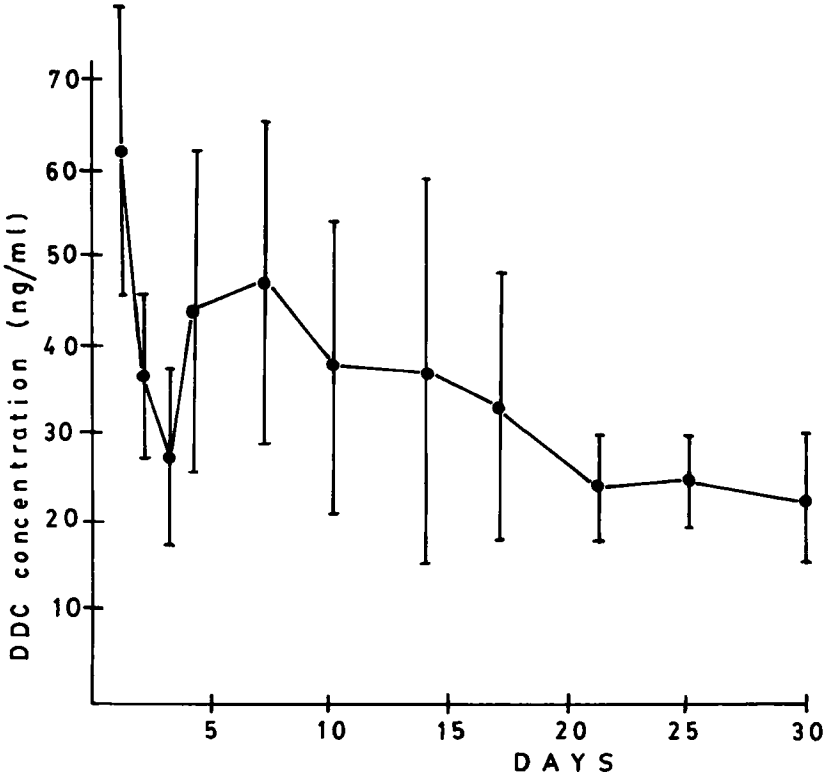

Figure 2. Mean diethyldithiocarbamate plasma concentrations after injection of 1·0 g of Disufiram subcutaneously to alcoholic patients. Vertical bars indicate one standard deviation

maintaining a measurable level of DDC for the 44 days of the experiment. This result agrees with the observation of Faiman *et al.*[14]

Figure 2 shows the mean plasma concentrations of DDC as a function of time after subcutaneous injection of 1.0 g DSF suspended in 6 ml of propylene glycol–water to 5 alcoholic patients. A high concentration of DDC is observed 24 h after implantation of micropellets due, probably, to a little dissolution of DSF in the suspension liquid; this would allow faster absorption in the initial stages. Then, a decrease of DDC concentration is observed followed by another increase giving a maximum level 7 days after the injection. This period may be due to the diffusion of DSF to the fatty tissues in accord with the physicochemical characteristics of the drug.

From day 7, the elimination of DDC proceeds by apparently first-order kinetics with a mean rate constant of 0.053 day^{-1} and an apparent $t_{½}$ of 15.4 days. the rate constant is apparently due to the constant liberation of DSF from the site of injection or from the lipidic compartment.

Table 1. Mean pharmacokinetic parameters following injection of 1 g of disulfiram suspended in propylene glycol–water to 5 alcoholic subjects

	$\bar{X}$	Range	S.D.	C.V.	Confidence interval ($p = 0{\cdot}05$)
k_e (days^{-1})	0·053	0·032–0·079	0·002	35·47	0·024–0·082
$t_{1/2}$ (days)	15·41	11·0–21·8	15·7	5·1	9·4–22.1
AUC (μg day m^{-1})	1337	1208–1504	144	10·81	1158–1516
C_{max} (μg ml^{-1})	75·04	61·41–92·50	12·85	17·22	59·08–90·99
t_{max} (days)	4·6	2·0–10·0	3·9	85·0	0·25–9·45
MRT (days)	16·93	14·36–21·01	4·75	28·05	12·75–21·11

S.D.: standard deviation.
C.V.: coefficient of variation (%).

The plasma concentrations obtained with this suspension are twice as high as those obtained with pellets reported in our previous paper,[8] but the duration of plasma levels is considerably less, about 1 month compared with 5 months for pellets.

Table 1 lists the mean pharmacokinetic parameters obtained in the 5 alcoholic subjects injected with 1.0 g of DSF in suspension. The confidence intervals revealed no individual differences in patients, probably because their physical characteristics were similar, especially in weight or fat distribution.

The continuing clinical evaluation of the subjects revealed no evidence of relapse 2 months after implantation of micropellets. No systemic reactions, hypersensitivity or adverse effects to DSF were observed. In the injection zone a slight pain was noted that disappeared after 3–5 days.

The clinical evaluation allows us to conclude that this dosage form, as well as pellets, may be of great utility in the treatment of alcoholism if it is accompanied by a psychiatric or/and psychological reinforcement.

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