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Inhibition of serum lactate dehydrogenase activity by disulfiram and diethyldithiocarbamate

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Treatment with oral disulfiram (DSF) is often prescribed as part of the medical management of alcohol abuse, with the objective of encouraging prolonged sobriety. Consumption of an alcoholic beverage during treatment with DSF will result in a subjectively unpleasant drug interaction characterized by malaise, flushing, palpitations and nausea [1, 2]. The mechanism of this interaction has been ascribed mainly to the non-competitive inhibition of aldehyde dehydrogenase by DSF, so that the consumption of ethanol is followed by toxic accumulation of its first metabolite, acetaldehyde [3, 4]. However, acetaldehyde toxicity does not completely account for all the clinical manifestations of the DSF–ethanol reaction, and the effects of the drug on other enzymes may also be clinically significant. DSF inhibits the activity of dopamine β-hydroxylase, which may contribute to the hypotension observed during the DSF–ethanol reaction [5]. Carper *et al.* have demonstrated recently that DSF and its major metabolite, diethyldithiocarbamate (DDC), both inhibit the activity of alcohol dehydrogenase. This unexpected new finding prompted us to investigate whether another major dehydrogenase enzyme, lactate dehydrogenase (LDH) (EC 1.1.1.27), was also inhibited by DSF.

Materials and methods

Preparation of DSF and DDC solutions. Disulfiram USP (Abbott Laboratories, North Chicago, IL) and diethyldithiocarbamate (Sigma Chemical Co., St Louis, MO) were dissolved in absolute alcohol (0.3 g DSF/100 ml and 0.17 g DDC/100 ml), stored at 2°, and used within 1 week of preparation.

Incubation procedure. Pooled human serum was prepared from blood samples submitted to the Department of Laboratory Medicine of St. Vincent's Medical Center of Richmond, and filtered through a Falcon sterile membrane filter unit No. 4620B10 (Thomas Scientific Co., Swedesboro, NJ). Duplicate 5-ml serum aliquots were incubated at 37°, and DSF or DDC solutions were added to produce 0, 50, 100, 200, 500, and 1000 μM concentrations. Samples of serum (0.5 ml) were withdrawn for assay from all incubation mixtures at zero time, and also at approximately 6, 12, 24 and 48 hr. The activity of LDH in each withdrawn sample was assayed by measuring the rate of formation of NADH in the reaction:

$$\text{lactate} + \text{NAD}^+ \xrightarrow{\text{LDH}} \text{pyruvate} + \text{NADH} + \text{H}^+$$

using a Beckman Ideal Clinical Analyzer (Beckman Instruments Inc., Brea, CA).

Results

The results of the incubations are shown in Fig. 1. Both DSF and DDC inhibited the activity of human serum LDH; the activity of LDH decreased in a log-linear fashion with time and also with the concentration of DSF and DDC. DSF appeared to be the more potent inhibitor of the enzyme, since there was no detectable activity after 24 hr of incubation with the 1000 μM solution.

Discussion

DSF and DDC both inhibited the activity of LDH in pooled human serum; the activity of the enzyme declined as the drug concentration and the duration of incubation were increased. The log-linear decline in enzyme activity with time suggests that this was a first-order process, i.e. that both DSF and DDC inactivated a constant fraction of the available enzyme per unit of time.

These findings raise a number of interesting questions which have yet to be answered. Since human serum contains at least five isoenzymes of LDH originating mainly from cardiac muscle, striated muscle, erythrocytes, liver, kidney, and brain, it is possible that not all the isoenzymes were equally susceptible to the drugs. In a clinical setting, reduced serum activity of LDH in patients treated with DSF or DDC could possibly obscure the diagnosis of acute

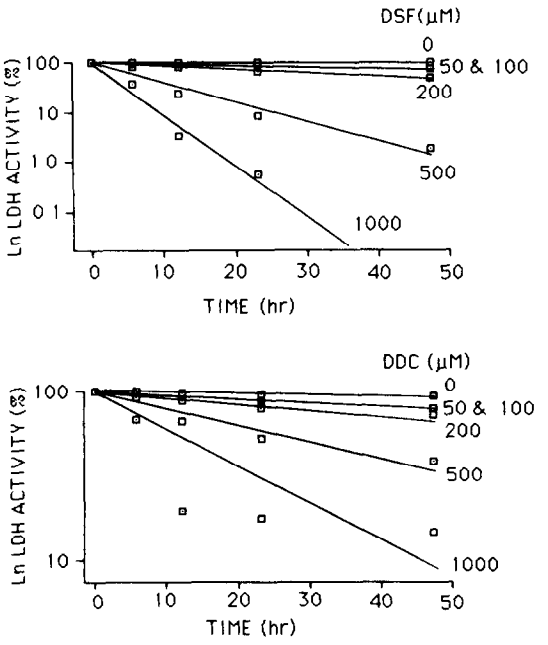

Fig. 1. Effects of disulfiram (DSF) (upper panel) and diethyldithiocarbamate (DDC) (lower panel) on lactate dehydrogenase (LDH) activity over time. Incubations were performed at 37°.

myocardial infarction, where elevation of an isoenzyme of LDH provides a useful diagnostic test [7]. Further studies of the effects of DDC and DSF will be required to determine if the purified isoenzymes differ in their sensitivities and rates of inactivation. Also, these findings do not clearly differentiate the effects of DSF and DDC on serum LDH, since DSF is rapidly converted to DDC *in vitro* in the presence of albumin [8, 9]. Further studies of the effects of these drugs on LDH need to be performed in fluids other than serum, to determine whether DSF or DDC or both agents can inhibit the enzyme in the absence of albumin.

These findings also raise questions about possible effects of DSF and DDC in humans treated with either of the drugs. LDH is a near ubiquitous enzyme which is present in most of the cells of the body in concentrations 500-fold greater than in serum [10], and the effects of inhibition of LDH activity in humans are not clearly understood. Treatment with DSF or DDC could possibly block the physiologic shift to anaerobic glycolysis that occurs during exercise and hypoxia, and blunt the development of lactic acidosis. Blockade of this pathway may also contribute to the pathogenesis of the DSF–ethanol reaction. Further studies are needed to determine the degree of inhibition of intracellular and extracellular LDH induced by DSF and DDC in humans and the physiologic significance of these effects.

The mechanism by which DSF and DDC inactivated LDH was not determined in this study. The enzyme contains no metals or disulfide bridges; however, cysteine was a possible site of action since LDH can be inactivated by the chemical modification of one cysteine per subunit [11]. The inhibitory effects of DDC upon aldehyde dehydrogenase, alcohol dehydrogenase, and lactate dehydrogenase suggest a commonality of action upon dehydrogenase enzymes which merits further investigation.

In summary, the objective of this study was to determine the effects of DSF and its major metabolite, DDC, upon the enzymic activity of lactate dehydrogenase. The activity of LDH in human serum was measured over 48 hr during incubation at 37° with solutions of DSF and DDC (50–1000 μM). A time- and concentration-dependent inhibition of enzyme activity was observed with both DSF and DDC; DSF was the more potent inhibitory agent, reducing enzyme activity to an undetectable level within 24 hr. These findings suggest that DSF and DDC therapy in humans may be accompanied by reduced tissue LDH activity, with possible effects upon clinical diagnostic testing and the normal physiologic responses to exercise and hypoxia.

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