

Cefamandole – A Competitive Inhibitor of Aldehyde Dehydrogenase

Cefamandole (CMD), a betalactam antibiotic with a 1-methyl-tetrazole-5-thiol group, evokes in man a disulfiram-like alcohol incompatibility with flush, tachycardia, hypotension and nausea (1, 2). An accumulation of acetaldehyde (ethanol metabolite) is thought to be responsible for these symptoms (disulfiram-like reaction). This explanation is derived from an increase in the acetaldehyde concentration found in the blood of alcoholized experimental rats after pretreatment with CMD (3–5). The increase of the acetaldehyde concentration in blood is caused by an inhibition of aldehyde dehydrogenase (ALDH) by CMD, as found *in vitro* with low- K_m ALDH preparations from rat liver (5). The inhibition of the ALDH activity is of a competitive character, as shown by the results of our enzyme-kinetic investigations (Figure 1).

Our experiments were carried out with isolated ALDH from the livers of female Sprague-Dawley rats (230 g; breeding station and holding conditions described elsewhere [6]) using a conventional method (7) modified as follows: The hepatic microsomes and mitochondria containing fraction (preparation described previously [8]) were resuspended in a 3% EDTA solution (for inactivation of NADH-consuming systems) with glutathion (0.6%; for protection against ALDH oxidation) and ultracentrifuged ($105,000 \times g$; 30 min; $4^\circ C$). The sediment was resuspended in an Na-phosphate buffer pH 7.4 (0.2 M) containing 1% Triton (to solubilize the ALDH from the lipid membranes of the cell organelles), and ultracentrifuged ($105,000 \times g$; 30 min; $4^\circ C$) again after one hour ($0^\circ C$). The amount of protein in the ALDH containing supernatant was determined (standard: albumin, bovine, fraction V; Sigma, St. Louis/U.S.A.) with Folin reagent

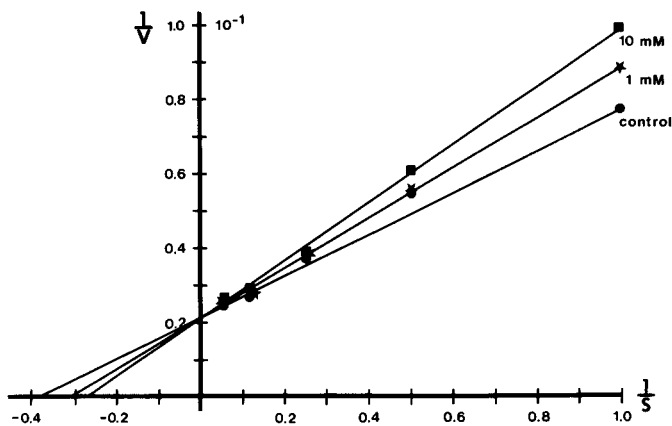


Figure 1: Lineweaver-Burk diagram (10) of the enzyme-kinetic values under the influence of CMD on isolated ALDH from rat liver. Each point on the curves was determined from eight observations (using parts of eight rat livers). $V_{max} = 49$ nmol/mg protein/min; $K_m = 2.7$ mM (control), 3.3 mM (1 mM CMD), 3.8 mM (10 mM CMD).

(9). The *in vitro* reaction mixture contained successively: 500 μ l K_2HPO_4 buffer pH 9.2 (0.5 M); 100 μ l NAD solution (0.02 M); 100 μ l ALDH (3 mg protein/ml); 290, 280, 260, 220 and 140 μ l doubly distilled water (blank: 300 μ l, 100 μ l less in CMD containing reaction mixtures); 100 μ l CMD solution (variable concentration in doubly distilled water); 10, 20, 40, 80 and 160 μ l acetaldehyde solution (0.1 M) (blank: 0). The NADH produced was determined spectrophotometrically (346 nm) and the ALDH activity (nmol acetaldehyde/mg protein/min) measured. CMD was purchased from Eli Lilly, Gießen, F.R.G., the other reagents from Merck, Darmstadt, F.R.G.

The small augmentation (Figure 1) of K_m (Michaelis constant) with constant V_{max} (maximum transformation velocity) following both realistic inhibitor concentrations of 1 and 10 mM CMD, respectively, is indicative of a small and competitive inhibition of ALDH. Although we are aware of the problems involved in transferring results from animal studies to human beings, this characteristic explains the incompatibility of CMD with alcohol (1, 2) that may appear during CMD therapy and is known to be of short duration and limited extent.

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