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# Disulfiram Inhibition of Cyanide Formation After Acetonitrile Poisoning

Peter De Paepe,[1,2] Pieter Colin,[3] Pieter Depuydt,[4] An-Sofie Decavele,[5] Julie De Smet,[3] Koen Boussery,[3]

Christophe Stove,[6] Dominique Benoit,[4] Alain Verstraete,[5] Jan Van Bocxlaer,[3] Walter Buylaert[1]


[1] Department of Emergency Medicine, Ghent University Hospital, Ghent, Belgium

[2] Heymans Institute of Pharmacology, Ghent University, Ghent, Belgium

[3] Laboratory of Medical Biochemistry and Clinical Analysis, Faculty of Pharmaceutical Sciences, Ghent

University, Ghent, Belgium

[4] Department of Intensive Care Medicine, Ghent University Hospital, Ghent, Belgium

[5] Department of Clinical Chemistry, Microbiology and Immunology, Ghent University, Ghent, Belgium

[6] Laboratory of Toxicology, Faculty of Pharmaceutical Sciences, Ghent University, Ghent, Belgium



Correspondence:

Peter De Paepe
Ghent University Hospital, Department of Emergency Medicine
De Pintelaan 185
9000 Ghent
Belgium
Telephone 0032 9 332 5211
Fax 0032 9 332 4980
E-mail peter.depaepe@ugent.be

ABSTRACT

**Context:** Cyanide poisoning may be caused by acetonitrile, a common industrial organic solvent and

laboratory agent.

**Objective:** To describe the potential use of disulfiram in treating acetonitrile poisoning in a human

clinical case and to further study its effect in human liver microsomes in vitro.

**Case details:** A 30-year-old man initially presented with a cholinergic toxic syndrome following ingestion of aldicarb. Toxicological analysis revealed coingestion of ethanol. He subsequently developed severe metabolic acidosis caused by the cyanogenic compound acetonitrile which was erroneously interpreted as acetone in the chromatogram. After 3 treatments with hydroxocobalamin (5g i.v.) and sodium thiosulfate (12.5g i.v.) on days 2, 3 and 5, he had transient improvement but recurrent lactic acidosis. Treatment with disulfiram was associated on day 7 with resolution of metabolic acidosis and slowing of the decrease in acetonitrile concentration. He recovered from acetonitrile toxicity completely. The time course of acetonitrile, thiocyanate, and cyanide concentrations suggested that disulfiram inhibited cyanide formation.

**Results:** In vitro experiments with human liver microsomes showed the cyanide concentration was significantly lower after incubation with acetonitrile and disulfiram than acetonitrile alone (a mean 60% reduction in cyanide level).

**Discussion:** Although disulfiram was given late in the course of the poisoning it is possible that it contributed to the recovery.

Short title: Inhibition of cyanide formation after acetonitrile poisoning

Key words: toxicology, poison, antidote, hydroxocobalamin, sodium thiosulfate, acetonitrile, disulfiram

## INTRODUCTION

Cyanide poisoning may be caused by acetonitrile, a common industrial organic solvent and laboratory agent that has been used in cosmetic nail polish remover. Previous reports of acetonitrile intoxication after inhalation or oral ingestion in 23 patients have included 8 deaths.[1,2] Acetonitrile initially is slowly metabolized by cytochrome P450 2E1 (CYP2E1) to cyanohydrin, which is broken down by catalase to form cyanide and formaldehyde (Figure 1).[3] Cyanide may be transformed by rhodanese to the less toxic thiocyanate, but this reaction may be delayed by the depletion of substrates such as thiosulfate necessary for this reaction.[1] Cyanide also may be neutralized by binding to dicobalt edetate or reaction with hydroxocobalamin (Figure 1).

Acetonitrile poisoning has been treated with sodium thiosulfate alone or varied combinations of dicobalt edetate, hydroxocobalamin, sodium nitrite, amyl nitrite, and 4-dimethylaminopyridine.[1,2] Literature review showed no previous reports of treatment of acetonitrile poisoning with the combination of hydroxocobalamin and sodium thiosulfate. We treated a patient who initially presented with a cholinergic toxic syndrome following ingestion of aldicarb and who subsequently developed severe metabolic acidosis due to cyanide poisoning by acetonitrile coingestion. The protracted clinical course with recurrent lactic acidosis required treatment with the combination of hydroxocobalamin and sodium thiosulfate on days 2, 3 and 5. This was supplemented with disulfiram on day 7 since we hypothesized that disulfiram may inhibit CYP2E1-mediated toxification of acetonitrile.

CASE DETAILS

A 30-year-old male technician was found comatose in his laboratory. A mobile intensive care unit team diagnosed a cholinergic toxic syndrome. Blood glucose level was normal and oxygen saturation on room air was 89%. The patient was intubated and atropine (3.5 mg) was given intravenously. Broken bottles with chemicals were found at the scene, and a witness saw him ingesting aldicarb 30 minutes earlier.

After arrival at the emergency department, treatment included gastric lavage, activated charcoal (via an orogastric tube), atropine (2 mg/h i.v.), and pralidoxime (30 mg/kg once, then 8 mg/kg/h i.v.). Past medical history included previous episodes of self-intoxication with methanol and heavy tobacco use.

Laboratory test results at admission were as follows: red blood cells $5.30 \times 10^6/\mu L$ (normal range: $4.35–5.87 \times 10^6/\mu L$), white blood cells $10.76 \times 10^3/\mu L$ (normal range: $4–10 \times 10^3/\mu L$), thrombocytes $291 \times 10^3/\mu L$ (normal range: $136–356 \times 10^3/\mu L$), serum creatinine 81 $\mu$mol/L (normal range: 64–103 $\mu$mol/L), urea 0.16 g/L (normal range: 0.13–0.43 g/L), glucose 8.2 mmol/L (normal range: 4.0-5.9 mmol/L), sodium 142 mmol/L (normal range: 135-144 mmol/L), potassium 3.2 mmol/L (normal range: 3.6-4.8 mmol/L), chloride 103 mmol/L (normal range: 98-106 mmol/L), calcium 8.9 mg/dL (normal range: 8.5-10.5 mg/dL), aspartame aminotransferase (ASAT) 17 UI/L (normal range: 0–37 U/L), alanine aminotransferase (ALAT) 15 U/L (normal range: 7–40 UI/L), gamma-glutamyltransferase 17 UI/L (normal range: 12-64 UI/L), lipase 66 UI/L (normal range: 0-60). Hemostasis parameters were as follows: prothrombin time 80 % (normal range: 70-120 %), index normalized ratio (INR) 1.17 (normal range: 0.9-1.1), fibrinogen 325 mg/dL (normal range: 200–400 mg/dL). Initial blood gas showed pH of 7.40 (normal range: 7.35-7.45), $pCO_2$ of 34.1 mmHg (normal range: 35-45 mmHg), $pO_2$ of 244 mmHg (normal range: 83-108 mmHg), $HCO_3$ of 20 mmol/L (normal range: 22-26 mmol/L), base excess of -3.6 mmol/L (normal range: -2-+3 mmol/L) and lactate 2.3 mmol/L (normal range: 0.5-2.2 mmol/L).

Ethanol level was 2.3 g/L; osmolal gap was high (31 mOsm/kg after correction for ethanol); and blood cholinesterase activity was low (3920 U/L; normal range, 11188 to 16698 U/L). Fomepizole treatment was not initiated because the criteria for toxic alcohol treatment were not met and the ethanol blood level was well above the therapeutic range for treatment of toxic alcohols.

Toxicology blood screening was negative for tricyclic antidepressants, salicylates, paracetamol, benzodiazepines, barbiturates, phenothiazines, opioids. Gas chromatography-mass spectrometry showed the presence of venlafaxine. The presence of methanol and ethylene glycol in blood was excluded with gas chromatography with flame ionization and gas chromatography-mass spectrometry respectively. Urine toxicology testing was negative for paraquat and para nitrophenol. Gas chromatography with flame ionization suggested that the elevated osmolal gap was caused by acetone.

The cholinergic crisis subsided and the patient was extubated 10 hours after admission. However, he developed agitation and severe lactic acidosis 12 hours later necessitating reintubation (Figure 2). After typical causes of lactic acidosis were ruled out, poisoning with a cyanogenic compound was considered, and the patient was treated with hydroxocobalamin (5 g i.v.) and sodium thiosulfate (12.5 g i.v.) (Figure 2). This caused prompt clinical improvement and decrease in plasma lactate level, and he was extubated. The analytical toxicologist reinspected the chromatogram and suspected that acetonitrile had been mistaken for acetone. Injection of a standard of acetonitrile in the gas chromatography with flame ionization revealed that the retention time of the standard corresponded completely with that of the peak in the patient sample that was first erroneously reported as acetone. The concentrations of blood cyanide and plasma thiocyanate were determined by a colorimetric method[4,5] and the plasma acetonitrile was determined by headspace gas chromatography (Figure 2).

The patient had relapsing lactic acidosis, and treatment with hydroxocobalamin (5 g i.v.) and sodium thiosulfate (12.5 g i.v.) was repeated 2 more times (Figure 2). On the 7th day, thiocyanate

levels remained high and lactate level increased again. After disulfiram was given (400 mg oral), the metabolic acidosis resolved and the decrease in acetonitrile concentration slowed (Figure 2). The patient recovered completely, received psychiatric care, and informed the health care providers that he had ingested acetonitrile with aldicarb in a suicide attempt and informed us that he was familiar with their toxic effects. He gave deferred informed consent for disulfiram treatment and blood sampling.

This case suggested that disulfiram was a useful antidote for acetonitrile poisoning. Therefore, the effect of disulfiram was evaluated in human liver microsomes in vitro. Human liver microsomes (BD UltraPool, BD Biosciences, Billerica, MA) were incubated with acetonitrile (600 mM) and the effect of disulfiram (1.35 µM) was studied using a modification of a previously described protocol.[6] Cyanide was measured as previously described.[7] The concentration of disulfiram used corresponded with free plasma concentration in humans. On 3 different days, experiments were performed with 18 samples (9 samples with and 9 samples without disulfiram). The mean cyanide concentration was significantly lower after incubation with acetonitrile and disulfiram (mean ± SD; 4 ± 3 µM) than acetonitrile alone (10 ± 3 µM; one-sided $t$ test, $P \le .001$); disulfiram caused a mean 60% reduction in cyanide level (Figure 3).

DISCUSSION

The patient presented with a cholinergic toxic syndrome and developed severe metabolic acidosis that was caused by acetonitrile and that responded temporarily to repeated administration of hydroxocobalamin and sodium thiosulfate. After relapse of metabolic acidosis, treatment with disulfiram caused a slowing of cyanide production and resolution of the toxic syndrome.

A high index of suspicion is necessary in treating patients who have acute poisoning. In the present patient, the elevated osmolal gap was initially attributed to acetone but later shown to be caused by acetonitrile. Misdiagnosis because of close retention times between different substances on chromatography has been reported previously.[1,8] Acetonitrile intoxication might have been

suspected earlier if accurate information had been available about substances present at the scene of the poisoning. In addition, a high index of suspicion is necessary with a clinical course that is atypical for the suspected toxic substance, and multiple substances may be used in self intoxication. In this case, the suspicion about the presence of a second substance occurred from routinely available tests used to evaluate and monitor acute poisoning, including the osmolal gap, blood gases, and lactate level.

The clinical course was remarkable for the delayed onset of symptoms. After acetonitrile exposure, patients typically remain well for several hours before developing signs of cyanide poisoning. This delay may be explained by the metabolism of acetonitrile, which is broken down by CYP2E1 and catalase to form cyanide (Figure 1).[6,9] In addition, ethanol may prevent toxicity of nitriles by inhibiting the formation of cyanide.[10] This may be attributed to competition between ethanol and acetonitrile for CYP2E1 and competition between ethanol and cyanohydrin for peroxidation by catalase (Figure 1).[6] The prolonged delay in the development of symptoms in the present patient (24 hours) compared with other cases (3 to 12 hours)[1,2] may have occurred because of ethanol.

The present case illustrates the importance of understanding the mechanism of toxicity, especially because some poisonings from toxins such as acetonitrile are rare with limited previous reports. The combination of hydroxocobalamin and sodium thiosulfate is based on the neutralization of cyanide by binding to hydroxocobalamin to form cyanocobalamin and the conversion of cyanide and thiosulfate to thiocyanate, catalyzed by rhodanese (Figure 1). The combination of these 2 antidotes is beneficial because they have negligible adverse effects and they are the antidotes of choice for cyanide poisoning.[11,12] The same combination has been used successfully in a patient after severe poisoning with propionitrile, another cyanogenic compound.[13] The duration of antidote treatment should be sufficiently long because of the slow elimination of acetonitrile.[2] The need for repeated administration of antidote for 5 days after acetonitrile poisoning has been previously reported, but time course may vary (Figure 2).[1,2]

Disulfiram has been proposed as an inhibitor of CYP2E1-mediated toxification of volatile solvents.[14] Before administration of disulfiram, bicarbonate levels decreased, which suggested ongoing formation of cyanide and/or of formate which unfortunately was not analyzed (Figure 2). Disulfiram is a strong inhibitor of CYP2E1 in humans and may be useful in treating halothane toxicity mediated by CYP2E1 and toxicity from industrial solvents (Figure 1).[14-16] The disulfiram-mediated decrease in acetonitrile metabolism was less harmful than the formation of cyanide because nonmetabolized acetonitrile itself has little toxicity.[17] Disulfiram is contraindicated in patients who are simultaneously poisoned with ethanol and acetonitrile, but fomepizole may be considered for treatment of disulfiram-ethanol reactions.[18] In the present patient, disulfiram was used more than 6 days after exposure to acetonitrile and ethanol, and the elevated ethanol level presumably had resolved.

Although disulfiram was given late in the course of the poisoning and following treatment with gastric decontamination, hydroxocobalamin and thiosulfate treatment and supportive critical care, it is possible that disulfiram contributed to the revovery. This was suggested by the improvement of bicarbonate and lactate levels and the time course of acetonitrile, thiocyanate, and cyanide concentrations. After administration of disulfiram, the decrease in acetonitrile concentration became slowed for approximately 50 hours (Figure 2), suggesting that disulfiram inhibited acetonitrile metabolism by inhibition of CYP2E1 (Figure 1).[14,16] The decreased acetonitrile elimination was accompanied by a log-linear decline in thiocyanate concentration, which was faster than the decline in the concentration of the parent compound acetonitrile (Figure 2). This is evidence that disulfiram may inhibit acetonitrile metabolism as under normal conditions (i.e. without inhibition of metabolite formation), the half-life of a metabolite may only equal or even exceed the half-life of the parent compound. Subsequently, thiocyanate elimination became slower, possibly because of a decrease in the inhibitory effect of disulfiram. The fact that the acetonitrile concentrations still decreased after the inhibition may be explained by other elimination pathways.

In the present patient, the high thiocyanate level on admission may be attributed to heavy tobacco use, and the initial decrease may have been caused by smoking cessation after acetonitrile ingestion (Figure 2).[19] The cyanide level had a fluctuating pattern, possibly because of the temporary effect of hydroxocobalamin and sodium thiosulfate. After disulfiram administration, cyanide levels decreased unequivocally (Figure 2).

The decreased cyanide formation observed in the in vitro experiment using human liver microsomes supported the therapeutic potential of disulfiram in acetonitrile intoxication. The acetonitrile concentration in the in vitro experiment was in excess of acetonitrile levels observed in humans. High concentrations of acetonitrile were used because of the high detection limit of cyanide. Therefore, the findings may have underestimated the in vivo effect of disulfiram on acetonitrile metabolism. Furthermore, the potential role of disulfiram metabolites in CYP2E1 inhibition was not evaluated. However, the in vitro findings are consistent with in vivo human observations after administration of disulfiram, including a reduction in CYP2E1 activity by 90% and a reduction in CYP2E1-mediated halothane metabolism by 85%.[14,16]

The maximum cyanide level in the present patient (30 μg/mL) exceeded the highest value previously reported in acetonitrile poisoning (17 μg/mL).[1] Although cyanide blood levels > 3 μg/mL are considered potentially lethal, the patient survived.[20] Previous studies in mice suggested that there is no relation between blood cyanide level and lethality, and brain (not blood) cyanide levels may be the best indicator of lethality.[3,21] Nevertheless, there may be large overlap of cyanide blood levels between patients who survive or die after acetonitrile exposure.[1,2]

The present case demonstrates the importance of considering the mechanism of toxicity during treatment of suspected poisoning. When the clinical course is atypical, the involvement of substances other than the primary toxin should be considered. In clinical toxicology, well-designed clinical studies usually are unavailable and case reports frequently are the only source of clinical information. In vitro studies may provide additional insight into the treatment of uncommon

intoxications. The present clinical and in vitro observations suggest that disulfiram may be a useful antidote in treating delayed cyanide poisoning after acetonitrile intoxication, possibly by inhibiting CYP2E1-mediated metabolism of acetonitrile.

DECLARATION OF INTEREST

The authors report no declarations of interest.

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**Figure 1.** Metabolism of acetonitrile and cyanide.
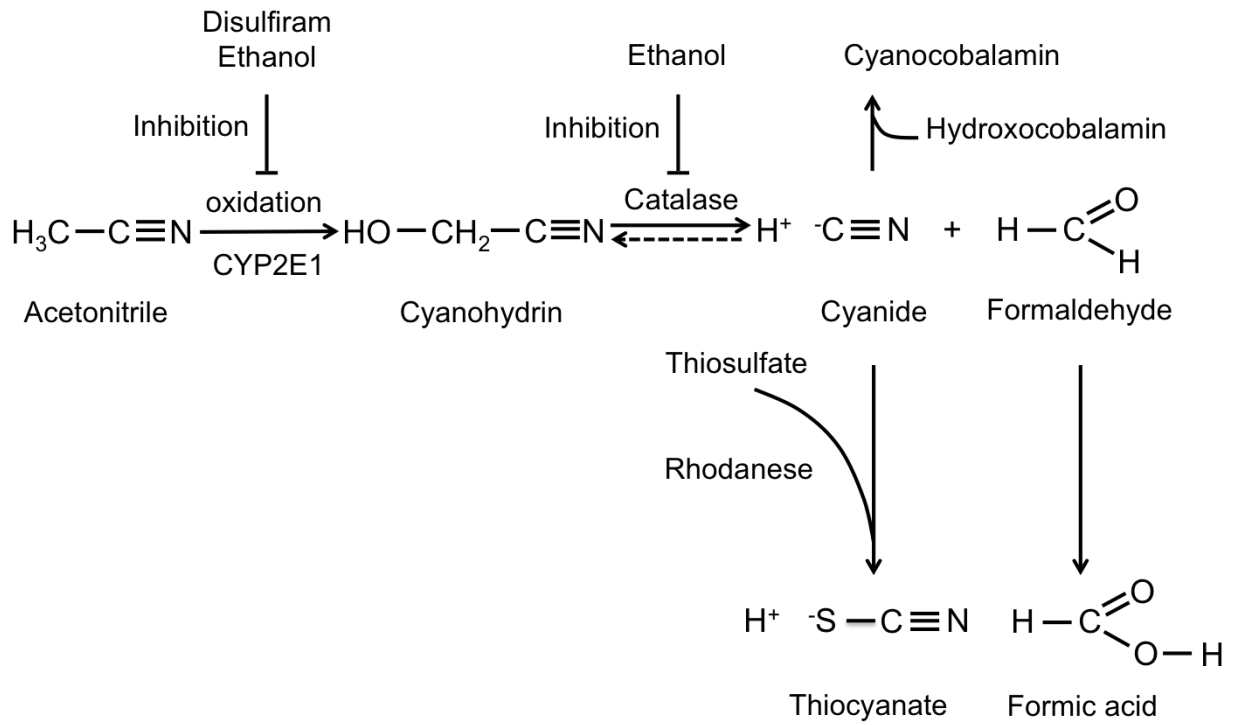
**Figure 2.** A 30-year-old man who had cyanide poisoning after ingestion of acetonitrile. Panel A shows the time course of concentrations of lactate and bicarbonate, and panel B shows the time course of acetonitrile, cyanide, and thiocyanate. The patient was treated with 3 doses of hydroxocobalamin (5 g i.v.) and thiosulfate (12.5 g i.v.) (open arrows) and 1 dose of disulfiram (400 mg oral) (filled arrow).
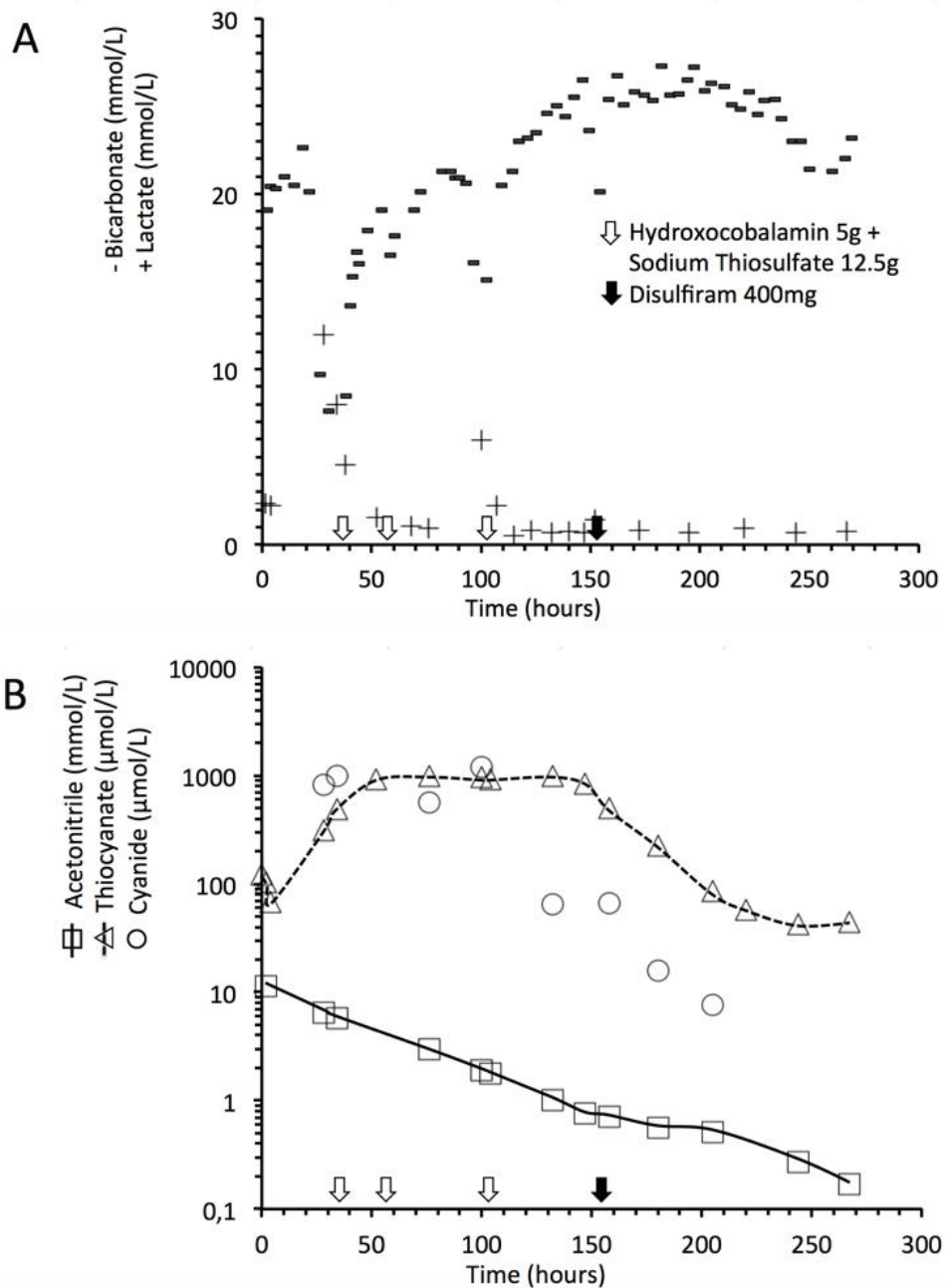
**Figure 3.** Results of the incubation experiments carried out in triplicate on three different days (n=9). Concentrations of acetonitrile and disulfiram added to the final incubate were 600 mM and 1.35 µM respectively.