Ann. Rev. Pharmacol. Toxicol. 1981. 21:575–96

THE ACTIONS AND METABOLIC FATE OF DISULFIRAM

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INTRODUCTION

Before the 1940s, it was known that exposure to certain organic sulfur compounds including carbon disulfide, tetramethylthiuram disulfide, and tetraethylthiuram monosulfide produced unpleasant reactions upon coincident ingestion of ethanol (1). Tetraethylthiuram disulfide [disulfiram (DS)] was first produced in 1881 and was used in the rubber industry to accelerate the vulcanization of rubber (2). In the 1930s, this substance was introduced into medicine as a scabiescide (3). Disulfiram was found to be toxic to lower forms of life utilizing copper-containing respiratory enzymes. Its effectiveness appeared to be directly related to its affinity to chelate copper. Intestinal worms were especially sensitive to this compound and hence DS was introduced as a vermicide. By chance, Hald & Jacobsen (4) ingested ethyl alcohol after they had ingested DS and promptly noticed a peculiar and highly disagreeable reaction. Based upon this ethyl alcohol–DS interaction, DS was proposed for the prophylaxis of chronic alcoholism (5). A new concept was then proposed that the patient taking DS chronically would not concomitantly ingest alcohol for fear of this disagreeable reaction.

Most studies of disulfiram, especially those reported two and a half decades after DS was introduced into treatment of alcoholism, were inadequately designed and commonly lacked proper control populations. Stastical analysis of the data derived from the clinical studies as well as accurate and sensitive methods for determining DS and its metabolites in body fluids were not ideal in those early years of investigation. Varying rates of improvement in alcoholics using DS, between 19–83%, have been reported

0362-1642/81/0415-0575$01.00

(6–10). Comprehensive and explicit objective criteria for measuring improvement of alcoholism are still required to obtain a clearer picture. Nevertheless, most clinicians experienced in the treatment of alcoholism are convinced that DS is a useful drug in treatment of selected patients.

CHEMISTRY OF DISULFIRAM

Disulfiram is an off-white or light gray, odorless, almost tasteless crystalline powder that is practically insoluble in water (0.7% W/V) but has varying solubilities in organic solvents such as ethanol (3.82% W/V), and ether (7.14 W/V). The melting point is 70°C–72°C and has a density of 1.30. It has a molecular weight of 296.54 and it is a potent chelator of iron, copper, and zinc. Disulfiram and its major metabolite diethyldithiocarbamic acid (DDC) are both stable in basic medium (pH 7.0–9.0) but unstable in acid medium up to pH 7.0.

PHARMACOKINETICS OF DISULFIRAM

Administration

In the 1950s, a daily oral dose of 1.5 g of DS was administered for prophylaxis to alcoholic patients, but as a result of the toxicity commonly manifested by such a high dose, it was reduced to an initial dose of 500 mg followed by a daily maintenance dose of 250 mg. The implantation of DS under the skin in a depot reservoir was initiated in 1955 (11). This subcutaneous implantation was attractive in the sense that it provided a long-term treatment for up to six months for the often noncomplying patient. However, an inadequate understanding of the physicochemical characteristics of such an implant as well as the highly variable pharmacokinetic properties and disposition of DS have made such an approach to treatment of alcoholism of equivocal value.

Absorption

Disulfiram is rapidly but incompletely absorbed from the human gastrointestinal tract. Hald & Jacobsen (12) found 20% of administered DS unchanged in feces. DeSaint-Blanquet et al (13) reported that 70–90% of the orally administered DS was absorbed in rats. In these studies, 2 hr after dosing, the presence of DS and DDC were qualitatively demonstrated in blood, liver, kidney, and muscle.

Distribution

Because of its high lipid solubility, DS accumulated in the various fat depots. The brain consistently reveals the least detectable amounts of DS

and its metabolites. DS and its metabolites have been found in the thyroid, adrenals, pancreas, stomach, small and large intestine, muscle, liver, testes, kidney, lung, spleen, and heart. These conclusions by Faiman et al (14) and Stromme (15) are largely based upon metabolism studies using radioactive DS. Following intraperitoneal administration of ^{35}S-diethyldithiocarbamic acid (DDC), the thiol was found in highest concentration in the plasma, liver, kidney, with the lowest concentration detected in the brain.

Metabolism

REDUCTION OF DISULFIRAM The metabolism of DS (I) outlined in Figure 1 proceeds first by a reduction of the disulfide linkage to its corresponding thiol, DDC (Figure 1, II) metabolite. The kinetics of this reaction may possibly be first order with respect to the disulfide (16) and may lead to formation of mixed disulfides with protein sulfhydryl groups. The glutathione reductase system of the erythrocytes has been shown to reduce DS (17, 18). It has been estimated that up to 50 g of disulfiram can be reduced by the adult human erythrocyte within 24 hr (19). This brisk reduction reaction causes the rapid disappearance of the parent compound from the bloodstream (20–24). In in vitro studies, Cobby et al (20) found that upon addition to whole blood, disulfiram was promptly reduced to the thiol within 4 min. Disulfiram may also be pharmacologically inactivated by an interaction within protein by a mechanism similar to the drug's reaction with cystamine derivatives (25). The DDC produced is further metabolized via four different pathways. These are glucuronidation, nonenzymatic degradation, methylation, and oxidation.

GLUCURONIDATION Conjugation of DDC with glucuronic acid (Figure 2) is a major detoxification mechanism of DS in man and other animals (26). Metabolism studies of radioactive DS in the rat revealed that about 50% of a given dose is excreted in urine as the glucuronide metabolite (Figure 1, III) (18). However, it is possible that a small portion of the glucuronide conjugate may be hydrolyzed by the esterases in the intestine to yield DDC during enterohepatic circulation.

NONENZYMATIC DEGRADATION Aspila et al (27) have shown that the rate of decomposition of DDC is pH dependent. In acid medium, DDC decomposes rapidly to diethylamine (Figure 1, IV) and carbon disulfide (Figure 1, V) (15). This diethylamine may be excreted unchanged by man (28), but a possibility exists for further degradation of diethylamine to ammonia and to acetaldehyde. In the presence of NADPH and cytochrome P-450 (a mixed function oxidase), carbon disulfide is oxidatively desul-

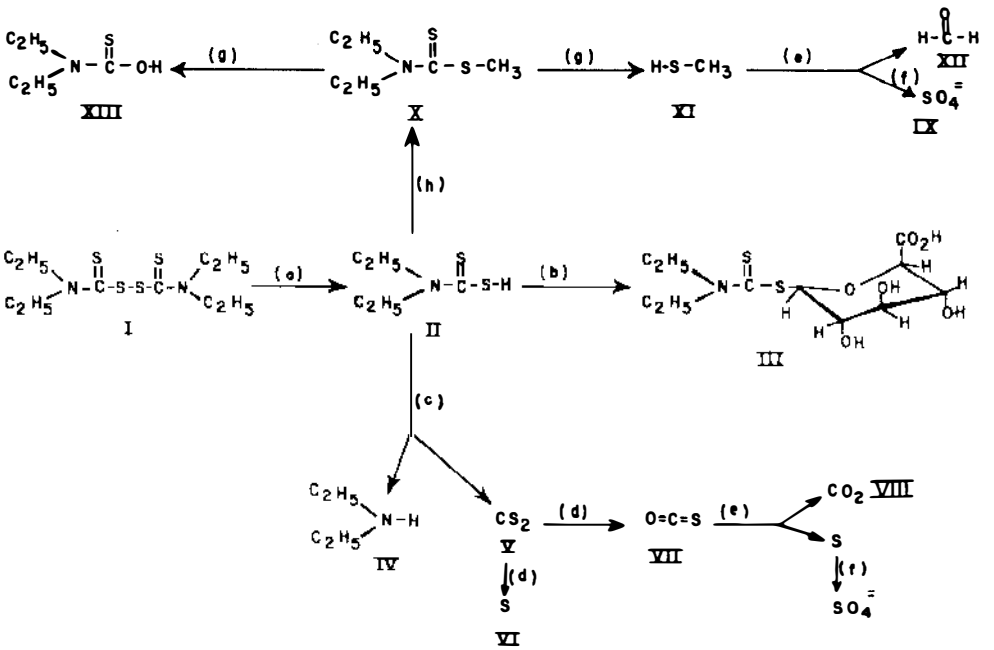

Figure 1. The metabolic fate of disulfiram. I. tetraethylthiuram disulfide (disulfiram), II. diethyldithiocarbamic acid, III. glucuronide of diethyldithiocarbamate, IV. diethylamine, V. carbonyl disulfide, VI. sulfur, VII. carbonyl sulfide, VIII. carbon dioxide, IX. sulfate, X. methyl ester of diethyldithiocarbamate, XI. methyl mercaptan, XII. formaldehyde, XIII. thiocarboxylic acid. (*a*) glutathione reductase, (*b*) conjugation, (*c*) nonenzymatic degradation, (*d*) oxidative desulfuration (C-P_{450}), (*e*) oxidation, (*f*) sulfoxidase, (*g*) esterases, (*h*) S-methylation: S-adenosyl methionine transmethylase.

furated to carbonyl sulfide (Figure 1, VII) and elemental sulfur (29). The carbonyl sulfide can be further oxidized to carbon dioxide and sulfur. The sulfur, which can be covalently bound to carbon and other compounds, can be metabolized to sulfate by the sulfoxidases (30). Carbon disulfide also reacts with certain amino acids yielding dithiocarbamates (31). In addition, dithiocarbamate can be cleaved back to carbon disulfide and amino acids or isothiocynates and H_2S. Both of these pathways may subsequently yield sulfate ions (32). From these observations and during either carbon disulfide poisoning or DS intoxication, a large number of sulfate ions are found in the urine of man and animals.

METHYLATION In radiochemical experiments (33) DS was administered intraperitoneally and 2 hr later methyl ester of DDC (MeDDC) was calculated to represent 0.05% of the given dose. In another experiment, DDC

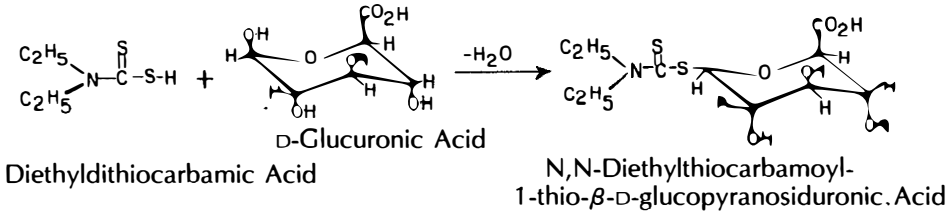

Figure 2 Glucuronidation of diethyldithiocarbamic acid.

was administered to dogs intravenously and S-methylation accounted for approximately 27% of metabolism of the administered dose (34). A highly active S-adenosyl methionine transmethylase catalyzes the methylation of DDC. This enzyme, found both in kidney and liver, is associated with the 100,000 X g microsomal fraction. Biotransformations from mercaptans to methylthioesters have also been reported for some aliphatic thiol compounds (35, 36). Similarly, S-methylation is involved in the metabolism of thiopurines, thiopyrimidines (37–39), and thiopentals (40). The methyl ester of the DDC derivative (Figure 1, X) may be attacked by esterases, generating a methyl mercaptan which may in turn be oxidized to sulfate and formaldehyde. To support this conclusion, it is known that the production of sulfate from other thioester compounds proceeded rapidly. For example, 6-methylmercaptopurine generates sulfates more rapidly than 6-mercaptopurine when administered to rats (41). This may be due to a possible cleavage of methyl mercaptan from the S-methyl compound and the latter's oxidation to produce inorganic sulfate (33, 42). S-methyl thioglycollic acid is also readily oxidized to sulfate while the corresponding S-phenylthioglycollic acid yields no significant amount of sulfate.

This similar oxidative pathway may act as an important sulfate-forming pathway in the metabolism of disulfiram. Approximately 62–74% of sulfate formed from the metabolism of DS involves methylmercaptan oxidation (34). The methyl ester of DDC is chemically very stable in blood (33). While slowly cleared from the body, it does not go through the covalent disulfide interchange reaction by which DS inhibits aldehyde dehydrogenase (43). A thioalcohol (Figure 1, XIII) formed from this methylester can be glucuronidated and excreted in urine.

OXIDATION Stromme (44) demonstrated the reoxidation of DDC back to DS. However, this reoxidation involved only about 4% of DDC formed. It is known that in the presence of atmospheric oxygen, DDC can be reoxidized to DS. Such a reoxidation can be effected by the oxidases in the body.

DISULFIRAM AND THE ENZYMES OF THE BODY

The Dehydrogenases

Shortly after the introduction of DS in the management of alcoholism, the effect of the drug on important enzyme systems was determined. In in vitro experiments, DS produced a long-lasting decrease in liver aldehyde dehydrogenase activity (45). This suggested that recovery of activity might depend upon synthesis of new enzymes.

Certain workers claimed that DDC is the active inhibiting compound in vivo (46), while others found DDC to be ineffective at inhibiting aldehyde dehydrogenase (45). Stromme (44) found that ^{35}S DDC and ^{35}S DS actively bind liver and plasma proteins. The interaction of either DS or DDC with protein–SH groups can yield mixed disulfides. Alternately, it is also possible that the sulfhydryl groups of aldehyde dehydrogenase (47, 48) might participate in this reaction in vivo. Additionally, one of the mixed disulfides could interact with and inhibit aldehyde dehydrogenase. Inhibition of aldehyde dehydrogenase (ALDH) can be prevented but not reversed by sulfhydryl-containing compounds (46) such as dithiothretol.

Fructose-1,6-diphosphate dehydrogenase and succinic dehydrogenase are also inhibited by DS (49, 50). Therefore, glycolysis, the tricarboxylic acid cycle, and pentose phosphate shunt are affected by DS. Glyceraldehyde-3-phosphate dehydrogenase is also inhibited by DS (51).

The Oxidases

Kjeldgaard (52) found that DS at a concentration of 1.2×10^{-7} mole/ml in rabbit liver homogenate caused marked inhibition of aldehyde oxidase, a flavoprotein. He also demonstrated that the reduced disulfiram (i.e. DDC) was not as effective an inhibitor of aldehyde oxidase as was DS. Further, following inhibition by DS, the enzyme could not be reactivated with glutathione. He concluded that the S–S linkage of DS may be important for the inhibition of aldehyde oxidase. Another flavoprotein enzyme, xanthine oxidase, not only oxidizes certain purines but also utilizes acetaldehyde as a substrate, converting it to acetic acid by direct utilization of oxygen. Disulfiram inhibits xanthine oxidase activity in rat liver, lung, and spleen (53) but not in milk. Studies of this enzyme (54, 55) suggest that it is composed of two different prosthetic groups; one is an oxidase system responsible for reoxidation of the reduced enzyme by atmospheric oxygen, and the other group has a dehydrogenase action capable of transferring hydrogen from xanthine to methylene blue.

In a study involving 32 male alcoholics, 7 developed psychosis. Interestingly, the platelet monoamine oxidase and plasma amine oxidase activity was significantly lower and the red cell catechol-O-methyl transferase was

higher in the patients who developed psychosis than those that did not develop psychosis (56). It is possible that this decrease in amine-oxidizing activity may result in a persistence of catecholamine action and hence the psychotic reaction. The disulfide grouping of DS is important for the irreversible inhibition (57) of DS on D-amino acid oxidase.

Dopamine-β-Hydroxylase

Another important amine degradative enzyme inhibited by disulfiram is dopamine-β-hydroxylase (DBH), an enzyme that catalyses the conversion of dopamine to norepinephrine (58). Experiments in vitro show that DS, at concentration of 10^{-5} m, inhibits by 100% the enzymatic conversion of dopamine to norepinephrine. This inhibition is sufficient to become the rate-limiting step in catecholamine synthesis for patients taking this drug. Under normal physiologic conditions, tyrosine hydroxylase is the rate-limiting enzyme (59). It appears that DDC, a metabolite of disulfiram, also inhibits DBH. The loss of activity may be caused by the chelation of copper.

Other Enzymes Inhibited by Disulfiram

The aldolases are inhibited by DS and Stromme (60) has shown that DS (but not DDC) is a potent inhibitor of hexokinase.

General Mechanism of Enzyme Inhibition

The reduction of the disulfide bond of DS by glutathione SH-containing compounds leads to the formation of mixed disulfides which in turn may inhibit selected enzymes. It is possible that disulfiram inhibits more or less all –SH enzymes and cofactors with –SH groups, e.g. CoA and thioctic acid.

DS and DDC are potent chelators of copper and other metals. Chelation of the metal portion of an enzyme by DS or DDC might lead to the inactivation of that enzyme. Metal-containing enzymes inhibited by either DS or DDC include the zinc-containing enzyme such as aldehyde dehydrogenase and glyceraldehyde phosphate dehydrogenase (17, 61) as well as the copper-dependent enzymes, adlehyde oxidase and dopamine-β-hydroxylase (62).

PHARMACODYNAMICS OF DISULFIRAM

The Disulfiram-Ethanol Reaction

Administration of DS to a subject 12 hr prior to consuming about 15 ml of alcohol produces a series of unpleasant effects within 15 min. These reactions begin with a deep cutaneous flushing, mainly of the face and upper trunk. This cutaneous flushing is caused by vasodilation and is accompanied by palpitation, dyspnea, hyperventilation, tachycardia, headache, and, oc-

casionally, chest pain (simulating cardiac ischemia). If the vasodilation is significant, hypotension may occur. The hypotension may produce pallor, weakness, vertigo, nausea, and vomiting. Confusion, drowsiness, and sleep usually follow with eventual complete recovery within 2–4 hr (63–65) providing, of course, that the amount of alcohol ingested is modest. Frequently, there are transient changes in the electrocardiographs such as flattening of the T waves, depression of the S-T segment, and Q-T prolongation in a pattern suggestive of right ventricular "strain" (66, 67). These reactions are collectively called the disulfiram-ethanol reaction (DER).

As might be expected, absorption of very small amounts of alcohol by a patient on DS treatment may not precipitate the DER. For example, a small amount of wine ingested during religious communion, the absorption of the dilute alcohol medium in a bronchial nebulizer spray, or ear drops containing ethyl alcohol did not result in DER in some patients (68).

ACETALDEHYDE AND DER In the beginning, the reactions that developed following the administration of alcohol and DS were attributed to increase in acetaldehyde formation in the body. Disulfiram was proposed to inhibit the dehydrogenases, aldolases, and oxidases that utilize acetaldehyde as substrate.

Hald & Jacobsen (69) documented an eightfold increase in acetaldehyde concentration in the blood when 40 ml of alcohol was consumed after 1.5 g of DS was taken the previous day. Using gas chromatographic methods, Truitt et al (70) showed that the blood concentration of acetaldehyde in a nonalcoholic subject who had consumed a moderate amount of alcohol was less than 1.0 mg/ml. However, the level of acetaldehyde produced by a standard dose of alcohol in an alcoholic subject appears to be highly variable. Majchrowicz et al (71) found no difference in the rate of alcohol or acetaldehyde metabolism in rats after a two month period of forced consumption of 20% alcohol or acetaldehyde. This finding is in agreement with other results published (72–75). On the other hand, there is some evidence that chronic administration of alcohol may increase formation of acetaldehyde by inducing the synthesis of alcohol dehydrogenase or NADP-dependent hepatic microsomal enzymes (76). The highest level of acetaldehyde found in the central nervous system appears in the cerebellum, which controls movement, position, and equilibrium.

Mitochondria have the greatest sensitivity to acetaldehyde in vitro (77, 78). Alcohol dehydrogenase found in the soluble fraction of brain homogenates (79) may be responsible for the higher level of acetaldehyde found in brain following alcohol consumption. Twice as much acetaldehyde has been reported (77, 80) in the brain following alcohol treatment (81). The amounts of acetaldehyde in rabbit blood increases with increasing dose of DS with

the alcohol dose as a constant (69). Again, when the animals were adequately dosed with DS, the amount of acetaldehyde increased with the increase in alcohol (82). Following perfusion of liver from normal and DS-treated animals with blood to which ethyl alcohol had been added, there was a marked increase of acetaldehyde in the liver treated with DS as compared to the untreated liver. The tentative inference from this study is that the liver may be the major organ involved in the acetaldehyde production which in turn is important to the DER phenomena (1). While other tissues may be involved in this acetaldehyde production, they play only a minor role when compared to the amount of acetaldehyde produced in the liver.

Recently, it has been made clear that acetaldehyde alone does not duplicate all of the reactions noted during DER. In dogs anesthetized with chloralose, intravenous administration of acetaldehyde in doses of 8–9 mg/kg body weight, caused an increase in arterial blood pressure, because of peripheral vasoconstriction (83, 84). However, this peripheral vasoconstriction does not explain the cutaneous flushing, vasodilation, and consequent hypotension that attend the DER. Interestingly, however, acetaldehyde inhibits dopamine-β-hydroxylase and thus inhibits the conversion of dopamine to norepinephrine. The resultant depletion of norepinephrine stores in the heart and blood vessels then allows acetaldehyde to act directly on these tissues to yield hypotension and vasodilation (85). This might explain this seeming paradox. At concentrations of 0.2 to 0.7 mg/100 ml acetaldehyde infused intravenously into the body of normal humans produced marked increase in heart rate and ventilation similar to the hyperventilation observed during DER (86–88).

DISULFIRAM AND BIOGENIC AMINES Through a condensation reaction, acetaldehyde can react with dopamine in vitro to form salsolinol, a tetrahydroisoquinoline (89, 90). The tetrahydroisoquinolines are suspected to be involved in the addiction component of alcoholism.

It is possible that DS treatment causes the production of salsolinol via two diverse mechanisms. First, DS greatly increases the accumulation of acetaldehyde by blocking aldehyde dehydrogenase. Second, DS inhibits dopamine-β-hydroxylase and thus allows dopamine to accumulate. As a result, the in vivo condensation of these two substances to form salsolinol is enhanced (see Figure 3). A possible role of salsolinol in DER has not been established.

DISULFIRAM-INDUCED HEPATOTOXICITY Some of the adverse effects and toxicity of DS may be camouflaged or overlooked as ethanol-induced reactions. Disulfiram has been shown to cause hepatotoxicity in

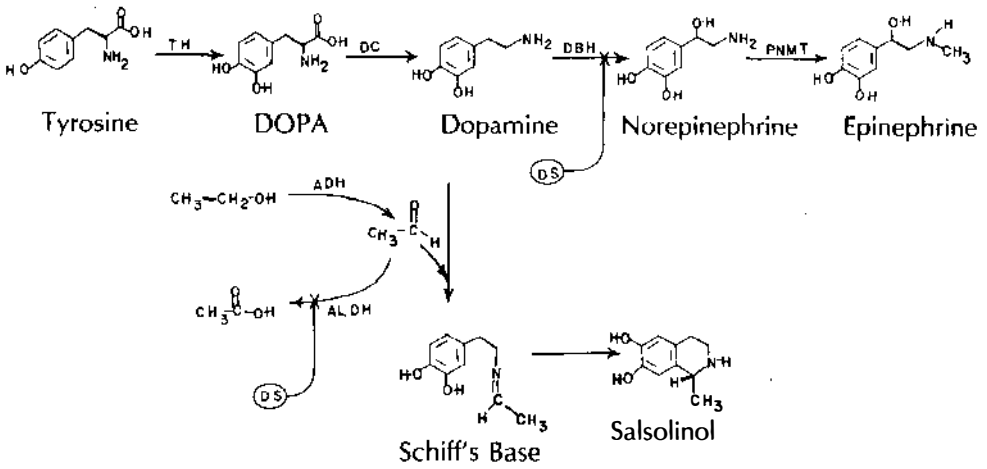

Figure 3 Condensation of dopamine and acetaldehyde. X = inhibition; ADH = alcohol dehydrogenase; ACDH = aldehyde dehydrogenase; DβH = dopamine β hydroxylase; DC = DOPA decarboxylase; DS = disulfiram; PNMT = phenyl-*N*-methyl transferase; TH = tyrosine hydroxylase.

patients (91–94). Although Goyer & Major (95) found no dose-related hepatotoxicity in 35 alcoholics receiving the drug over a three week period, nine of the patients did show non-dose–related subclinical hepatotoxicity. These investigators attributed these findings to "idiosyncratic" response of the patients. Other investigators demonstrated that chronic DS and ethanol treatment in animals significantly enhanced hepatic lipid peroxidation. Further, incubation of liver homogenates with acetaldehyde damaged membrane lipids of the hepatocyte (96).

CARBON DISULFIDE AND DS NEUROPATHIES Disulfiram has been implicated in the development of sensorimotor peripheral neuropathy (97–100). In all cases, this neuropathy improved upon discontinuation of DS treatment and reappeared on resumption of DS therapy (101). The denervation potentials were detected in all cases in distal muscles and the conduction velocities ranged from normal to slightly reduced. These electrophysiological findings suggest that DS induces degeneration of the axon similar to that observed in experimental isoniazid neuropathy (102, 103) and during the earliest stages of Wallerian degeneration following transection or crushing injury (104). With no observed myelin breakdown in the absence of axonal degeneration, the stages of the degeneration resemble axonal lesion (101). Disulfiram has been shown to cause optic atrophy and encephalopathy (105). These neuropathies occur in the absence of the DER suggesting that more than inhibition of acetaldehyde dehydrogenase or DBH may be involved in this neuropathy.

Carbon disulfide, a metabolite of disulfiram, has been shown to cause axonal neuropathies in animal (106, 107) and in man (108, 109). Carbon disulfide is deposited in all tissues in the body (110) with the brain having the slowest uptake and longest retention time of this compound. Generally, 76% to 95% of all carbon disulfide that enters the body is deposited and metabolized by body tissues. Five to thirty percent is exhaled and less than 1% is excreted in the urine. Lewey (111, 112) has suggested interesting possible explanations of the mechanism underlying carbon disulfide–induced generalized neuropathy. They proposed that DS may induce a pyridoxine deficiency similar to that described with carbon disulfide (99, 113). The carbon disulfide reacts with pyridoxamine, causing pyridoxine deficiency (114). In general, carbon disulfide interferes with primary amino acid groups. In intact animal models carbon disulfide leads to decreased excretion of pyridoxic acid and enhanced excretion of xanthurenthic acid (both of which are indicators of pyridoxine deficiency) and decreased plasma level of pyridoxal phosphate (115, 116). The thiamine deficiency state that results from chronic liver injury that is commonly present in alcoholic patients receiving DS treatment, makes them especially vulnerable to the neuropathy associated with DS. Further, such alterations may be of major importance in perpetuating liver injury since interference with dependent biochemical reactions may contribute to liver injury and interfere with its repair (108).

DISULFIRAM-INDUCED PSYCHOSIS Since its introduction into clinical medicine, DS has provoked psychological and neurologic abnormalities (117). Major symptoms include a peculiar disorientation in which certain patients are conscious of their environment but uninterested in it, gradual amnesia, anxiety, insomnia, and violent restlessness. Often, these symptoms necessitate reduction of DS dose by the consulting physician (118). These psychoses might relate to the personality difficulties of the patient that originally led to alcoholism and then became manifest after pharmacologic prohibition of alcohol. These psychological difficulties may be handled by regressing to a psychotic reaction.

DISULFIRAM-INDUCED ACETONEMIA DeMaster & Nagasawa (119) have shown that chronic DS administration increases concentration of serum acetone in man and the rat. For example administration of an oral dose of disulfiram (0.5 g/kg) to rats for three consecutive days increased the fasting blood acetone 25-fold. Similarly, in humans treated with 250 mg of DS daily for a minimum of one month, the increase in acetone in expired air was 15-fold greater than in matched controls. Acute administration of DS 0.5 g/kg to rats orally raised the serum acetone level fivefold. This acetonemia may not be related to ALDH inhibition by DS because other

ALDH inhibitors such as cyanamide and pargyline did not produce increase in blood acetone. The exact mechanism leading to the increased production of acetone in man by DS remains unclear.

INHIBITION OF OXIDATIVE PHOSPHORYLATION Disulfiram enhances the toxicity of normobaric oxygen to rats in a manner similar to paraquat (120, 121). An interaction of DS with the electron transport system may increase the production of superoxide ions (122) which in turn may cause edematous lung lesions. DS inhibits NAD^+-dependent mitochondrial oxygen consumption (123, 124) and oxidative phosphorylation and ion transport in rat liver mitochondria.

DISULFIRAM IN CANCER THERAPY Disulfiram inhibits metabolism of the carcinogen azomethane. It is interesting that the oxymetabolite of azomethane produces neoplasia (125) and DS offers radioprotection from the oxymetabolite neoplasia. The mechanism of carcinogenesis postulated for azomethane is the inhibition of its oxidation to azoxymethane during the metabolism of 1,2-dimethylhydrazine. In the radioprotection, it is possible that the formation of mixed disulfides during the metabolism of DS provides groups that bind to intra- and extracellular proteins and other body constituents and by a yet unknown mechanism decrease the ionization of molecules by the radiation (25). However, Stromme (44) found that these protein-bound metabolites play only a minor role in radioprotective action.

OTHER SIDE EFFECTS OF DISULFIRAM Disulfiram has led to convulsions in alcoholics receiving therapy (126). While the mechanism of this disulfiram-induced seizure in alcoholics remains unknown, it must be understood that the chronic alcoholic is particularly prone to seizure disorders for a variety of reasons. Discontinuation of DS therapy has been suggested in these cases.

An unusual sign of increased difficulty with colostomy due to DS has been reported. This difficulty was relieved on discontinuation of DS (127). Disulfiram should be avoided in pregnancy because of suspected teratogenicity (128).

DISULFIRAM INTERACTION WITH OTHER DRUGS

The duration and intensity of drug action often are modified by the activities of drug-metabolizing enzymes primarily located in the hepatocytes. These enzymes catalyze the metabolism of drugs by many pathways such as hydroxylation, dealkylation, deamination, sulfoxidation, and glucuronide formation. One drug can inhibit the metabolism of a second drug by inhibit-

ing the enzyme necessary for the detoxification of the second drug. As a result, the plasma and tissue half-life is prolonged and both the therapeutics and the toxic effects of the drug are potentiated. On the contrary, the administration of one drug may reduce the pharmacologic activity of a second drug by stimulating the hepatic drug-metabolizing enzymes and thus increasing the inactivation of the second drug. Fewer drugs are known to inhibit drug-metabolizing enzymes than are known to induce the activity of the same enzymes. Drugs known to inhibit drug-metabolizing enzymes include nortriptyline (129), methandrostenolone (130), oxyphenbutazone, allopurinol, methylphenidate (131), phenyramidol (132), and DS (133, 134). Drugs that stimulate drug metabolism include the barbiturates, antihistamines, hypoglycemics, and uricosuric agents.

Disulfiram increases the half-life of antipyrine (134) by inhibition of the hepatic microsomal mixed function oxidases. Rothstein (133) reported the enhanced effect of warfarin following DS administration. O'Reilly (135) studied the pharmacologic mechanism for the interaction of DS and warfarin and suggested that DS inhibited the enzymes responsible for the hydroxylation of warfarin. This impairs the metabolic clearance of warfarin and prolongs the serum half-life for the unchanged warfarin, which in turn inhibits the synthesis of vitamin K–dependent clotting factors resulting in an exaggerated hypoprothrombinemic effect.

Disulfiram inhibits the metabolism of phenytoin (136, 137). Svendsen et al (138) confirmed the later study on phenytoin but showed that DS did not inhibit tolbutamide metabolism. The metabolisms of phenytoin, antipyrine, and coumarins all involve hydroxylation by the hepatic microsomal enzyme system of man and animals. However, Svendsen et al (138) found that although tolbutamide is also oxidized by hepatic microsomal enzymes, its metabolism was not inhibited by DS. He suggested that DS may be a more specific inhibitor in the microsome's enzyme system. Other drugs whose biotransformations are inhibited by DS include chlordiazepoxide, hexobarbital, and thiopental (7). The toxicity of certain centrally acting drugs has been increased by DS in rats. These drugs include morphine, meperidine, amphetamine, and barbital (139). Disulfiram increases the carcinogenicity due to ethylene bromide (140). While the combined use of DS and metronidazole are not synergistic in the management of alcoholics, their combined use may increase the incidence of confusional psychosis (141).

Lang et al (142) demonstrated that DS and DDC diminished the inductive effect of phenobarbital on cytochrome P-450 content and *p*-nitroanisol O-demethylation, while both compounds showed additive effects in inducing NADPH-dependent cytochrome reductase.

Contrary to other investigations, it was found that glucuronidation and hydroxylation enzymes are not in close functional relationship as might be

suggested by their similar response to induction or inhibition by foreign compounds (143–145). It is possible that glucuronidation could be stimulated while there is coincident inhibition of the hydroxylation enzymes.

OTHER DRUGS WITH DISULFIRAM-LIKE ACTIONS

Tetramethylthiuram disulfide, tetramethylmonosulfide, and tetraethylthiuram monosulfide all affect the metabolism of ethanol in a fashion similar to disulfiram. Interestingly, in rabbits, the thiuram monosulfides have greater power to induce acetaldehydemia than does the corresponding disulfides (145).

Citrated calcium cyanamide was introduced as a less powerful alternative to DS (147, 148). This compound inhibits rabbit liver aldehyde dehydrogenase in vivo. However, the severity of the ethanol withdrawal reaction in mice (149) is less than that demonstrated with DS. Cyanamide also potentiates the ethanol-induced shift in the metabolism of norepinephrine. The same unpleasant symptoms of DER are experienced on drinking alcohol after eating the common mushroom, *Coprinus atramentarius*. The active ingredient that elicits this reaction is under investigation (150, 151).

Some hypoglycemic sulfonylurea compounds such as carbutamide, chlorpropamide, and tolbutamide exhibit a disulfiram-like action (70, 138, 152, 153). Podgainy & Bressler found (154) that these sulfonylureas noncompetitively inhibit a aldehyde dehydrogenase and stated that this observation accounts for the DER-like syndrome either by an accumulation of acetaldehyde, or by an alteration in the metabolism of serotonin. However, Asaad & Clarke (152) found no specific interaction in vitro between the sulfonylureas and aldehyde dehydrogenase. Pyrogallol also increases acetaldehyde concentrations during ethanol metabolism (155) by inhibiting the aldehyde oxidation pathways.

Other compounds with DS-like action include hydrogen sulfide, tetraethyl lead, animal charcoal, and aminophenazone with phenylbutazone (156). Metronidazole has been reported to reduce the craving for alcohol (157).

METHODS FOR THE DETERMINATION OF DISULFIRAM AND ITS METABOLITES

A severe handicap to the study of the metabolism of DS has been lack of very sensitive assay methods for determining the drugs and its metabolites. Analytical methods that have been used include (*a*) colorimetric assays (21, 23, 158–162), (*b*) polarographic methods (31, 163–166), (*c*) proton magnetic resonance (167), (*d*) radiometric assay (22, 44, 168, 169), and (*e*) chromatographic assays.

Colorimetric Methods

The basis for colorimetric assays is the ability of a compound to form a complex that has a color. Disulfiram forms a chemical compound with copper ion, which is yellow in color and stable in carbon tetrachloride. Cupric diethyldithiocarbamate is determined photometrically at 430 nm (21, 23, 162):

$$CuI + (C_2H_5)_2\ NCSSSCSN\ (C_2H_5)_2 \rightarrow$$

$$(C_2H_5)_2\ NCSSCuSCSN\ (C_2H_5)_2 + 1/2\ I_2.$$

Diethyldithiocarbamic acid also reacts with copper to yield a yellow color which absorbs at 430 M (21, 23, 162):

$$2(C_2H_5)_2\ NCSS^- + Cu^{2+} \rightarrow (C_2H_5)_2\ NCSSCuSCSN\ (C_2H_5)_2$$

Disulfiram, cyanide, and ethanol react to give a color maximum between 520 and 580 nm (14) with an absorption that can be spectrophotometrically determined.

The pitfalls of these methods include lack of sensitivity and specificity, and often proteins interfere with the spectrophotometric determinations.

Other Methods

Polarographic and proton magnetic resonance methods are not suitable for biological fluids and tissues. The radiometric assay is of limited use, a consequence of high protein-binding characteristics of disulfiram.

Chromatographic Assays

The assays developed so far involve the methylation of DDC, which is then chromatographed either by HPLC or gas chromatography (20, 168, 170). In the HPLC assay, DDC is methylated using dimethyl sulfate and chromatographed on Porasil ® column. In gas chromatography, methyl iodide is used in the methylation process. We have confirmed the gas chromatographic assay and also made some modifications. We have successfully ethylated DDC and methylated dipropyl-dithiocarbamic acid for use as internal standards. We believe that the methyl ester of dipropyldithiocarbamic acid has more of the extraction characteristics of MeDDC and would serve as a better internal standard than biphenyl as previously reported internal standard. We are also trying to identify the glucuronide conjugate directly by acylation and silylation and analyze the products with GC/MS.

CONCLUSION

Treatment of a social disease like alcoholism with disulfiram adjunctive to other series of counseling programs. Success has been achieved with most chronic alcoholics who volunteer for this type of therapy, are well motivated, compulsive, and socially stable. However, this group comprises the minority of alcoholics. The majority of alcoholics must receive careful counseling in addition to the drug therapy to obtain success. The introduction of chromatographic assays in studies with disulfiram, the use of adequate controls, and the comparison of disulfiram alcoholism treatment protocol will help achieve a better understanding of this drug in terms of its pharmacodynamics, pharmacokinetic properties, and relative efficacy.

ACKNOWLEDGMENTS

The authors wish to thank Mrs. Aline Marleau for secretarial assistance in the preparation of this manuscript.

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