PARADIGM TO TEST A DRUG-INDUCED AVERSION TO ETHANOL

E. GARVER1, A. D. ROSS2, G.-C. TU3, Q.-N. CAO3, F. ZHOU3 and Y. ISRAEL1,3*

1Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, Philadelphia, PA 19107, 2Department of Medicine, National Jewish Medical and Research Center, Denver, CO 80206, USA and 3Center for Gene Pharmacotherapy, Millennium Institute, University of Chile, Santiago, Chile

(Received 17 January 2000; in revised form 19 April 2000; accepted 5 May 2000)

Abstract — The screening of new agents for aversive therapy of alcoholism requires a simple animal model. Animals trained to ingest ethanol solutions and subsequently administered a drug known to produce an aversion to ethanol in humans, do not readily make the association between the malaise induced by the aversive drug–ethanol reaction and the consumption of the same ethanol-containing solution that has been consumed previously without ill effects. An experimental paradigm is reported in which the malaise of the drug–ethanol reaction is quickly recognized by rats as derived from ethanol. Disulfiram was used as the model drug. Lewis rats were deprived of water for 18 h after which 6% (v/v) ethanol was offered as the only fluid. During the first hour of ethanol access, both controls (vehicle) and disulfiram (100 mg/kg)-treated animals consumed intoxicating amounts of ethanol (0.7–0.9 g ethanol/kg). Plasma acetaldehyde levels developed were 3–5 μM and 40–50 μM in the two groups respectively. After this time, disulfiram-treated animals virtually ceased consuming alcohol (90% inhibition), indicating that the disulfiram–ethanol reaction is associated with alcohol ingestion. Control animals continued consuming the alcohol solution for the additional 4–5 h tested. This model should be of value in the testing of new agents that reduce aldehyde dehydrogenase levels for prolonged periods for their potential as an aversive treatment in alcoholism.

INTRODUCTION

Disulfiram is an ‘anti-alcohol’ drug that non-specifically inhibits aldehyde dehydrogenase (ALDH2-1) by reacting with sulphhydryl groups in the enzyme (Weiner, 1979; Vallari and Pietruszko, 1982). Supervised disulfiram administration is effective in reducing alcohol consumption in alcoholics (Brewer, 1993). However, the effectiveness of non-supervised disulfiram has been criticized (Swift, 1999). In a well controlled study of non-supervised administration of disulfiram to 202 subjects, a therapeutic dose of the drug significantly reduced by 45% the number of drinking days (Fuller et al., 1986). However, the time elapsed to consume their first drink was not reduced. The latter results are not unexpected; an aversion to ethanol would only occur after the patient challenges the effect of the drug.

Disulfiram has serious disadvantages, including a number of side-effects, such as severe hypotension, paraesthesias, and motor neuropathies (Gallant, 1987; Peachey and Annis, 1989; Dupuy et al., 1995), which markedly reduce compliance with self-administration. Brewer (1984) and Christensen et al. (1991) showed that when disulfiram is administered in tolerable doses, only 50% of patients develop the disulfiram–ethanol reaction.

In 70–90% of humans, a single gene mutation protects against abuse of alcohol or alcoholism (Harada et al., 1982; Thomasson et al., 1991; Higuchi, 1994; Tu and Israel, 1995). Such a gene encodes high-affinity ALDH2 (Yoshida et al., 1985), a mitochondrial enzyme that metabolizes acetaldehyde, the first product of ethanol oxidation (Svanaes and Weiner, 1985; Cao et al., 1988; Klyosov et al., 1996). Means to specifically inhibit the transcription or translation of this gene on a long-term basis could be used as potential agents for the treatment of alcoholism.

The development of new aversive means to reduce alcohol consumption requires an experimental paradigm to test the drug-induced aversion to ethanol. We describe here studies designed to test the effect of an aversive medication.

MATERIALS AND METHODS

Animals and treatment

Male Lewis rats (Harlan, Indianapolis, IN, USA) weighing 200–300 g, were used. Prior to the ethanol consumption studies, all rats were acclimatized for at least 3 days in either a plastic shoebox cage or a specially designed plexiglass cage with stainless-steel wire bottom. All animals were maintained on a 12 h light:12 h dark cycle and had free access to laboratory rodent diet 5001 (PMI Feeds, Inc., St Louis, MO, USA) and tap water. Finely powdered disulfiram was suspended in 1% carboxymethylcellulose in saline to yield a final concentration of 15 mg/ml and was administered orally.

Animals were administered disulfiram (100 mg/kg) or vehicle (between 09:00 and 10:00) for 4 days. On the evening of day 3, water was removed for 18 h, whereas food was provided ad libitum. On the morning of day 4, animals were dosed as described above, and 2 h later the animals were allowed access to a 6% (v/v) ethanol solution as the only drinking fluid. Consumption of the ethanol solution was measured at 1, 2, 3, 4, and 5 h. In some experiments, plasma acetaldehyde was determined at 0, 1, 3, 5, and 24 h after the animals were given access to 6% ethanol. For the latter studies, blood samples were removed from femoral catheters while the animals were freely consuming the ethanol solution.

Plasma acetaldehyde levels

Blood was collected in heparin, placed immediately on ice and centrifuged at 10 000 g for 5 min to harvest plasma for subsequent acetaldehyde determinations. Acetaldehyde was determined by high-pressure liquid chromatography (HPLC) using a modification of the method described by Lucas et al. (1986). Plasma (250 μl) was mixed with 4 ml of ice-cold

*Author to whom correspondence should be addressed at: Department of Pathology, Anatomy and Cell Biology, Thomas Jefferson University, 1020 Locust Street, Suite 275, Philadelphia, PA 19107, USA.

© 2000 Medical Council on Alcoholism
perchloric acid (0.6 M in 0.15 M sodium chloride) and centrifuged at 10,000 g for 10 min at 4°C. Acetaldehyde present in the supernatant was derivatized by the addition of 2,4-dinitrophenylhydrazine. The absorbance of the eluted derivatives was monitored at 365 nm. A five-point standard curve with an acetaldehyde concentration range of 0–20 μM was prepared for HPLC analysis. All samples were quantified from a standard curve prepared on the same day the samples were extracted into iso-octane. The linear correlation of each of the standard curves was ≥0.991.

**Determination of mitochondrial ALDH2 activity**

Mitochondria from the livers collected from control and disulfiram-treated animals were isolated as described by Tank et al. (1981). Mitochondria were resuspended in 0.1 M sodium phosphate buffer, pH 7.4 and stored at −70°C until protein content and ALDH activities were determined. Prior to enzyme activity determinations, the thawed mitochondrial samples were incubated with Triton X-100 (2% v/v) at 37°C for 15 min to ensure complete lysis and release of ALDH.

The low $K_m$ ALDH activity in the isolated mitochondria was assayed as previously described by Tank et al. (1981) with minor modifications. The assay was performed at 37°C in 1.0 ml of reaction mixture, and was initiated by the addition of acetaldehyde to a final concentration of 10 μM. ALDH2 activity was determined by recording the change in absorption at 340 nm, due to NADH formation, with a Beckman DU®640 spectrophotometer. Protein concentration in the resuspended mitochondria was measured by the Micro BCA Protein Assay Reagent Kit (Pierce, Rockford, IL, USA) as described by the manufacturer, with bovine serum albumin as the standard.

**RESULTS**

The consumption of 6% ethanol was determined at intervals over a 5-h period in 18-h water-deprived rats pretreated with disulfiram (100 mg/kg) or vehicle (Fig. 1). Cumulative intake is shown at hourly intervals throughout the first 5 h. During the first hour of 6% ethanol presentation, ethanol consumption was similar in the disulfiram and vehicle control groups, amounting to $0.87 ± 0.12$ g ethanol/kg (disulfiram) and $0.69 ± 0.12$ g ethanol/kg (controls) (means ± SEM). However, after the first hour, control animals continued to drink the ethanol solution, whereas the disulfiram-treated animals virtually ceased consuming it. During the subsequent 4-h interval (hours 1 to 5), disulfiram-treated animals consumed 88.1% less ethanol than controls ($P < 0.002$).

In a separate group of disulfiram-treated animals, alcohol consumption and plasma acetaldehyde concentrations were measured simultaneously in animals with implanted femoral catheters (Fig. 2). Data show that disulfiram animals consumed the 6% ethanol solution during the first hour of access ($0.8$ g/kg), as previously observed, then stopped consuming ethanol for 4–5 h when minor ethanol consumption was resumed. Alcohol consumption in the first hour led to marked elevations in plasma acetaldehyde averaging $59.6 ± 11.1$ μM. Acetaldehyde levels were reduced to $24.4 ± 10.8$ μM by the third hour and to $4.0 ± 1.1$ μM at 5 h.

**ALDH activity and acetaldehyde levels**

Three hours after the administration of 100 mg/kg disulfiram (given daily for 4 days), liver mitochondrial ALDH activity was found to be inhibited by 87% ($P < 0.0001$) when...
compared to that in control animals given the vehicle. Under the same conditions, the administration of ethanol (1 g/kg orally) increased plasma acetaldehyde from 5.0 ± 0.2 μM (before ethanol) to 41.9 ± 4.9 μM, 1 h after administration of ethanol ($P < 0.001$). In line with studies by Eriksson (1985), endogenous acetaldehyde levels prior to any exposure to ethanol, were increased in disulfiram-treated animals (5.03 ± 0.21 μM) vs those in control animals (1.28 ± 0.43 μM) ($P < 0.001$).

**DISCUSSION**

Alcoholics given disulfiram are normally informed of the dysphoria that may result from the disulfiram–ethanol reaction, and thus a guided association can be made between ethanol consumption and the malaise that ensues. This is not feasible with animals. Earlier work, which led to the present study, suggested that animals which have learned to consume ethanol do not quickly associate that the same ethanol solution which has been consumed over a prolonged learning period without ill effects has (after drug administration) become the cause of the malaise. In one study (Schlesinger et al., 1966) in which mice were offered the choice of water or 10% ethanol for 14 days, the subsequent administration of disulfiram (100 mg/kg/day) for 3 days led to only small reductions (20%) in ethanol consumption. In another study (Amit et al., 1976), rats were trained for 50 days to drink solutions containing increasing concentrations of ethanol up to 10% in the two-bottle choice condition. Subsequent administration of disulfiram or calcium carbimide every second day for 10 days led to only minor reductions (10%) in ethanol consumption, despite the fact that treatment with disulfiram (50 mg/kg/day) and calcium carbimide (25 mg/kg/day) markedly elevated blood acetaldehyde levels following the i.p. administration of a 1.5 g/kg dose of ethanol.

The use of the limited access paradigm to assess the effect of aversive medication has resulted in conflicting data which also limits its use. In this paradigm, rats were trained for about 60 days to consume alcohol solutions which were available to the animal for 10 min/day. Cyanamide, an inhibitor of ALDH, increased, rather than decreased ethanol consumption in this model (Aragon et al., 1993).

The method used in the present study was to pair the putative dysphoric effects of the disulfiram–ethanol reaction with the taste of ethanol, a variant of the conditioned taste aversion paradigm in which a novel taste is paired with the administration of a noxious substance or a noxious condition (the ethanol–disulfiram reaction). It is well established (Nolan, et al., 1997; Scalera et al., 1997; Barber et al., 1998; Yasoshima and Yamamoto, 1998) that a robust conditioned taste aversion quickly develops after the first pairing of the new taste with a noxious agent. In our studies, the effect was fully demonstrable in as little as 5 h following ethanol presentation, which allows fluid deprivation of the animals for slightly less than 24 h. Such a paradigm requires that: (1) ethanol be consumed for the first time by animals that have been pretreated with disulfiram; (2) enough ethanol be consumed by the animal in a short time to generate high blood acetaldehyde levels which lead to the malaise of the disulfiram–ethanol reaction; (3) ideally, an inbred strain be used to reduce variability and increase inter-laboratory reproducibility.

Data obtained after the disulfiram dosing regimen described here indicate that a strong aversion to ethanol is observed when plasma acetaldehyde levels are in the range of 40–60 μM resulting from a reduction in mitochondrial ALDH activity of 87%. This reduction in activity is in line with the reduction of ALDH activity observed in human ALDH2-2/ALDH2-1 heterozygotes (Enomoto et al., 1991; Xiao et al., 1995) who are markedly protected against alcohol abuse and alcoholism (Harada et al., 1982; Thomasson et al., 1991; Higuchi, 1994; Tu and Israel, 1995). In ALDH2-deficient individuals, the oral consumption of 0.5 g/kg ethanol yielded plasma acetaldehyde concentrations of 35.4 μM as compared to 2.1 μM in controls (Harada et al., 1981).

Overall, studies presented here show that a simple animal model can be used to determine the aversion against ethanol elicited by drugs that reduce ALDH activity and which result in increases in acetaldehyde levels comparable to those seen in humans who consume alcohol and who carry an inactive ALDH allele.

**Acknowledgements** — This work was supported by the National Institute on Alcohol Abuse and Alcoholism (AA 96-003), Fondacot (1981049) and Catedra Presidente de la Republica.

**REFERENCES**


