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Effects of Disulfiram on Serum Dopamine-β-hydroxylase and Blood Carbon Disulphide Concentrations in Alcoholics

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The aim of this study was to investigate the effects of single and repeated disulfiram doses on serum dopamine-β-hydroxylase activity and blood carbon disulphide concentrations in a group of abstinent alcoholics. The increase in the blood concentration of carbon disulphide was dose dependent after the oral administration of 100–400 mg of disulfiram. Free carbon disulphide peaked at 12 h while the protein-bound fraction increased at least up to 24 h. Both single (100–400 mg p.o.) and repeated (200 mg daily p.o. for ca. 1 month) administrations failed to inhibit the activity of serum dopamine-β-hydroxylase. The repeated daily administration of 200 mg of disulfiram also had no influence on copper-activated serum dopamine-β-hydroxylase, which was the same before and after 1-month treatment period. Contrary to the disulfiram group, the activity of the copper-activated enzyme in the serum of abstinent alcoholics declined significantly during the same 30 days.

INTRODUCTION

Disulfiram (ANTABUSE) has been used as an alcohol aversive drug for more than 40 years. Behavioural and neurological symptoms, as well as adverse cardiovascular effects, have been reported in abstinent alcoholics after prolonged disulfiram treatment.[1] It has been proposed that these effects are caused by the formation of carbon disulphide,[2] a well-known occupational, neurological and cardiovascular toxic agent.[3–5] In rodents, both disulfiram and carbon disulphide inhibit dopamine-β-hydroxylase (DBH) and reduce the conversion of dopamine to noradrenaline.[6,7] The mechanism of DBH inhibition involves chelation of copper, an essential cofactor of DBH, by dithiocarbamates formed either by the reduction of disulfiram or by the reaction of carbon disulphide with circulating amines.[8] It has been shown that inhibition of DBH is quickly reversed in the adrenal glands of rats exposed to carbon disulphide, but a rebound effect has been observed after repeated exposures characterized by increased content of DBH and a faster rate of catecholamine synthesis.[9,10] Increased DBH activity has also been reported in the blood of monkeys repeatedly dosed with disulfiram.[11] Based on these observations it has been suggested that repeated short-lasting inhibitions of DBH, caused either by occupational exposure to carbon disulphide or by prolonged therapy with disulfiram, trigger neurological and cardiovascular effects through the chronic stimulation of the sympathoadrenal system.

The aim of this study was to investigate the effect of single and repeated disulfiram administrations on serum DBH activity and on the metabolic formation of carbon disulphide in abstinent alcoholics.

EXPERIMENTAL

In vitro study

The effect of disulfiram on serum DBH was measured with and without copper sulphate supplementation (1 μM final concentration). Serum was separated from ca. 20 ml of blood withdrawn from the cubital vein of a healthy volunteer. Serum DBH was measured by the conversion rate of tyramine to octopamine at 37°C and pH 5.0 according to Kato *et al.*[12] A 50-μl aliquot of disulfiram dissolved in acetone was added to the test system (1 ml) to give final disulfiram concentrations between 0.1 and 0.6 μM. The addition of acetone (1:20) reduced the enzyme activity by ca. 30%.

Acute *in vivo* study

Four alcoholics (two males and two females) were hospitalized to start alcohol aversive treatment with disulfiram. They were taking a normal diet without any medication and had abstained from alcohol for 3–5 days before disulfiram therapy. A sample of blood was withdrawn from the cubital vein of each subject before taking a single oral dose of disulfiram: 100 mg (one subject), 200 mg (two subjects) or 400 mg (one subject). Three more blood samples were withdrawn from each subject at scheduled times (1, 12 and 24 h after disulfiram). The total blood for carbon disulphide determination and the serum for DBH determination were processed separately. Blood samples were placed in glass tubes containing two drops of 10% ethylendiaminetetracetate solution and stored at 4°C until

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Table 1. Characterization of the study subjects

	Group A	Group B
No. of subjects	15	20
Male/female[a]	12/3	14/6
Age (years)[b]	46.2 ± 11.2	51.4 ± 10.3
Alcohol intake (g day^{-1})[b]	161.0 ± 44.0	152.0 ± 33.0
Smokers/non-smokers[a]	8/7	12/8

[a] Not significantly different, Pearson chi-squared test.
[b] Not significantly different, Student's *t*-test.

assayed. Carbon disulphide was determined as free and bound (acid-labile) carbon disulphide. Acid hydrolisis was performed by heating two parts of 1% hydrochloric acid with one part of blood at 100°C for 1 h. Carbon disulphide was measured according to Brugnone *et al.*[13] The concentration of bound carbon disulphide was calculated from the difference between total (free + acid-labile) and free carbon disulphide concentrations. Serum was separated soon after blood sampling and stored at −80°C until assayed. The DBH activity with and without the addition of copper (1 μM) was measured in all samples on the same day according to Kato *et al.*[12]

Patients were aware that blood sampling was aimed at obtaining experimental results that were of no clear therapeutic or diagnostic value for themselves. Informed consent was obtained from the patients before starting the experimental procedure.

Subchronic *in vivo* study

Thirty-five hospitalized alcoholic patients (26 males and 9 females) selected for a detoxification programme were divided into two groups according to their willingness to undergo disulfiram therapy. The two groups (group A: 15 subjects not treated with disulfiram; group B: 20 subjects selected for disulfiram treatment) did not differ in age or sex distribution, smoking habit or alcohol intake (Table 1). Informed consent was obtained from patients before joining the study.

A blood sample was withdrawn from the cubital vein of all patients 6.1 ± 2.4 (mean ± SD) days after the beginning of the abstinence period (5.8 and 6.3 days for group A and B, respectively; not significantly different). Disulfiram therapy (group B) with daily oral doses of 200 mg of disulfiram (equal to 2.9 ± 0.36 mg disulfiram kg^{-1} body wt., mean ± SD) started on the next day. Serum was taken and stored at −80°C until assayed. A second blood sample was withdrawn 31.2 ± 8.2 days after the first sampling (33.8 and 29.3 days for group A and B, respectively; not significantly different) and serum was taken and stored as reported above. The second sample from patients treated with disulfiram was taken between 2 and 24 h after the last dose. Between the first and second samplings the subjects were not hospitalized, but all participated in group psychotherapy and abstained from alcohol up to the end of the study. Circumstantial evidence for the continuity of disulfiram therapy was obtained at the 2-weekly psychotherapy sessions by patients and the relatives of patients, who were asked to be present at the daily tablet ingestion. Serum DBH activity was measured in both samples from each subject on the same day with and without the addition of 1 μM copper.[12]

Table 2. Effect of disulfiram and copper addition to human serum dopamine-β-hydroxylase (DBH)

Disulfiram (μM)	Copper (1 μM)	DBH (μmol min^{-1} l^{-1} serum)
0	−	20.8
0.1	−	18.7
0.2	−	16.3
0.4	−	11.3
0.6	−	6.4
0	+	35.0
0.2	+	34.1
0.4	+	36.9
0.6	+	34.0

RESULTS

The addition of copper to the test system increased DBH activity from 20.8 to 35 μmol min^{-1} l^{-1} of serum. This is a known effect of copper on serum DBH and it is due to the neutralisation of endogenous sulphydryl inhibitors present in serum.[14] Table 2 shows the effect of copper and disulfiram supplementation on DBH activity. Disulfiram inhibited serum DBH activity with an I_{50} of ca. 0.4 μM. The addition of copper completely neutralized the effect of disulfiram up to the highest tested concentration of 0.6 μM.

Table 3 shows a dose-related increase of both free

Table 3. Blood carbon disulphide (CS_2) concentration and serum dopamine-β-hydroxylase (DBH) activity of abstinent alcoholics after a single oral disulfiram dose

Subject and disulfiram dose	Time of blood sampling after disulfiram (h)	Blood CS_2 (nM)		Serum DBH (μmol min^{-1}l^{-1})	
		Free	Bound	No Cu^{2+}	Cu^{2+}
Subject 1	0	1	10	14	29
100 mg p.o.	1	1	9	13	27
	12	10	64	13	27
	24	7	119	10	26
Subject 2	0	3	15	24	53
200 mg p.o.	1	4	26	20	48
	12	162	446	28	57
	24	157	986	25	53
Subject 3	0	4	24	17	52
200 mg p.o.	1	3	177	19	59
	12	50	242	24	54
	24	5	508	20	55
Subject 4	0	5	13	16	34
400 mg p.o.	1	11	77	16	34
	12	617	2397	15	32
	24	260	2562	16	32

Table 4. Serum dopamine-β-hydroxylase (DBH) activity without copper supplementation in abstinent alcoholic patients with and without a 1-month disulfiram therapy

		Serum DBH activity (mean ± SD)	
No. of subjects	Disulfiram (200 mg day^{-1})	Before the first disulfiram dose (μmol min^{-1} l^{-1})	After the last disulfiram dose (% change)
15	–	13.5 ± 9.6	−3.0 ± 23.6
20	+	18.0 ± 14.0	−6.4 ± 21.5

and bound carbon disulphide concentrations in the blood of four abstinent alcoholics after a single dose of disulfiram. Free carbon disulphide peaked at 12 h after disulfiram administration and the bound fraction was still increasing after 24 h. Serum DBH activity measured both with and without copper supplementation remained unaffected even by 400 mg of disulfiram.

Table 4 shows that repeated disulfiram ingestion (200 mg daily p.o.) also failed to cause significant serum DBH inhibition in a group of abstinent alcoholics after ca. 1 month of treatment. Table 5 shows that the mean value of copper-activated serum DBH activity did not differ between the two groups of alcoholic patients at the beginning of the study, i.e. a few days after abstinence started. The only difference between the two groups was in the change of DBH activity during abstinence. While DBH activity was not significantly changed in patients treated with disulfiram, copper-activated DBH declined by a significant 11% in the 15 control alcoholics who started abstinence simultaneously with the disulfiram group.

DISCUSSION

Dopamine-β-hydroxylase is localized to catecholamine-containing vescicles in the sympathetic nerves, brain and adrenal medulla but it is released from these sites into plasma simultaneously with noradrenaline.[15] Thus, the serum concentration of DBH depends on the DBH content of the noradrenaline synthesizing sites and the activity of the sympathetic nervous system. As the activity of this enzyme depends on the presence of endogenous and exogenous inhibitors, which act through the chelation of copper in DBH, *in vitro* estimated enzyme activity is always lower without copper supplementation than with copper supplementation. Tables 3–5 show that the DBH activities of serum samples obtained from disulfiram-treated or control abstinent alcoholics were twofold higher with copper supplementation (which relates to the enzyme content) than without copper supplementation. Although, after a single dose of disulfiram the blood concentration of bound carbon disulphide, which includes disulfiram, diethyldithiocarbamate and the dithiocarbamate-type compounds of carbon disulphide, sharply increased, the ratio of copper activated and unactivated enzyme activities remained unchanged.

As the I_{50} for disulfiram in serum (where it is rapidly metabolized) is 0.4 μM (see Table 2), some inhibition was expected at least at the two highest concentrations: 0.99 μM in subject 2 and 2.6 μM in subject 4, corresponding to 0.5 μM and 1.3 μM disulfiram, respectively. The lack of inhibition suggests that either the distribution of bound carbon disulphide in blood follows the distribution of free carbon disulphide, which is predominantly (90%) in the red blood cells,[16] or the dilution of the assay system counteracted the disulfiram effect. Lake *et al.* were also unable to detect any inhibitory effect on serum DBH after prolonged disulfiram treatment (250 and 500 mg/day^{-1}).[17]

The only difference between the disulfiram-treated and control groups suggests an effect on DBH synthesis in DBH stores. After 1 month of treatment with 200 mg disulfiram day^{-1} the serum DBH activities of copper-supplemented samples were approximately the same as before treatment, while DBH activities significantly declined in the serum of control abstinent alcoholics. It is a point of interest that this difference between the two groups can be shown only when individual changes in activity are compared. The reason for this is that in humans, DBH shows a genetically determined great interindividual variation[18] that could disguise a statistically significant effect when mean activities are compared.

This decline in serum DBH activities in the control group and no change in the disulfiram group seem to be paradoxical without considering the effects of alcohol withdrawal and disulfiram on noradrenaline-synthesizing and -releasing sites. Ethanol withdrawal syndrome, characterized by tremor, agitation, hallucination, dilated pupils, seizures, tachycardia and increased blood pressure, frequently develops in alcoholics 6–60 h after the discontinuation of long-term ethanol consumption.[19] Most alcoholics do not develop every symptom and the usual clinical picture is mild. Similar symptoms are seen in hyperadrenergic states, such as anxiety and thyrotoxicosis, and direct observations, including increased plasma noradrenaline levels,[20] support the view that the sympathetic nervous system is activated upon withdrawal of ethanol. Treatment with β-adrenergic blockers such as propanolol and timolol has been shown to control some of the

Table 5. Copper-activated serum dopamine-β-hydroxylase (DBH) activity in abstinent alcoholic patients with or without a 1-month disulfiram therapy

		Serum DBH activity[a] (mean ± SD)	
No. of subjects	Disulfiram (200 mg day^{-1})	Before the first disulfiram dose (μmol min^{-1} l^{-1})	After the last disulfiram dose (% change)
15	–	30.3 ± 21.7	−11.1 ± 17.6[b,c]
20	+	37.0 ± 28.3	+2.6 ± 20.6

[a] Serum DBH activity measured with 1 μM copper addition.
[b] Significantly different from first sampling results. Student's *t*-test for correlated samples: $P < 0.05$, two-tailed.
[c] Significantly different from change in disulfiram-treated patients. Student's *t*-test for independent samples: $P < 0.05$, two-tailed.

signs of alcohol withdrawal,[21,22] otherwise clinical and biochemical changes tend to disappear spontaneously during the first week of abstinence.[20] Direct evidence of serum DBH increase after ethanol withdrawal as compared with pre-abstinence values is lacking, but in a group of alcoholics serum DBH activity declined between day 1 and 4 of abstinence,[23] suggesting that this parameter behaves like noradrenaline levels. The decline in the level of copper-activated DBH from the early days of abstinence to 1 month later can be the sign of normalization and the abatement of the withdrawal syndrome. The absence of the same decline in disulfiram-treated patients is unlikely to be the sign of a prolonged withdrawal syndrome. It is most likely the consequence of the effect of disulfiram on DBH in noradrenaline-synthesizing sites. Firstly, it has been reported that in rats the *in vivo* inhibition of adrenal DBH activity by diethyldithiocarbamate[24] or carbon disulphide[10] was associated with increased adrenal DBH content. Secondly, in these rats the shift in adrenaline catecholamine concentrations simulates stress-like effects such as hypothermia, immobilization or hypoglycaemia, which consequently induce less additional response in carbon disulphide-exposed rats than in control rats.[25] Thus, it seems reasonable to assume that the catecholamine system of patients treated with disulfiram is constantly in a stress-like situation. The result is that from the elevated DBH depots more DBH is released and this increased release is sufficient to keep the serum DBH content at the high initial level.

In summary, serum DBH is not a sensitive test for the biological monitoring of disulfiram ingestion or occupational exposure to dithiocarbamates or carbon disulphide. However, disulfiram is not without effect on DBH. While in control alcoholics during the first month of the abstinence period the serum DBH content declined, this decline was prevented by the daily ingestion of disulfiram. As serum DBH derives from noradrenaline-synthesizing sites, this observation demonstrates that at these sites DBH and, through DBH, the whole catecholamine system is affected by disulfiram.

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