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PROTECTION WITH DISULFIRAM FROM CENTRAL AND PULMONARY OXYGEN TOXICITY*

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Abstract—Disulfiram protected mice from oxygen convulsions and lung damage. The degree of protection from convulsions was dependent upon both the dose of disulfiram administered and the time interval between disulfiram administration and oxygen exposure. Post-exposure survival of disulfiram-treated mice was very good whereas non-disulfiram-treated mice never survived the oxygen exposure period. Disulfiram protection does not appear to be related to dopamine-β-hydroxylase inhibition. It is thought disulfiram's protection may be due to its action on the cytochrome chain and its inhibition of electron transfer.

ACUTE exposure of man and animals to hyperbaric oxygenation (OHP) produces convulsions, while lung damage and death occur after prolonged exposure. The lack of adequate protection from oxygen toxicity has limited the clinical usefulness of oxygen since the hazards appear to outweigh the benefits of this type of therapy.

Many theories have been proposed to explain the mechanism(s) of oxygen poisoning, and based upon these theories, various methods of protecting against oxygen toxicity have been investigated.[1] Although numerous agents have afforded some degree of protection, complete protection from oxygen convulsions and lung damage has not been possible.

In earlier studies[2] the relationship between central oxygen toxicity and brain biogenic amines was investigated. During the course of those studies, disulfiram was found to protect against oxygen toxicity. In this communication, further studies with disulfiram are described. The data indicate that disulfiram appears to be more effective as an oxygen protectant than any other agent previously reported.

METHODS

Male albino Swiss Webster mice of the HA/ICR strain (ARS-Gibco, Madison, Wis.) weighing 22–28 g were used in these studies. Cylindrical Plexiglas hyperbaric chambers having a capacity of about 30 l. were constructed for these investigations.

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Calcium chloride (Fisher Scientific) and soda lime (W. R. Grace & Co.) were placed inside the chamber to absorb moisture and carbon dioxide respectively. During the course of all experiments pure oxygen was metered through the chamber at a rate of 1·5 l./min. The temperature within the chamber was maintained at 25° ± 1° at all times.

All drugs investigated were suspended in 1% methyl cellulose, except sodium diethyldithiocarbamate which was dissolved in saline. Mice treated with the various drugs under investigation and their corresponding controls were placed inside the hyperbaric chambers in groups of four. The methods of chamber pressurization and maintenance of oxygen concentrations have been reported previously.[3] After the desired oxygen pressure was reached, the animals were observed and the time taken for convulsions to occur was noted. Animals which did not convulse were assigned a "convulsive time" corresponding to the time the experiment was terminated. The time taken for 50 per cent of the animals to convulse (CT_{50}) and the corresponding standard error for each drug investigated was calculated on a GE-635 computer using a LD_{50} program.[4] Differences between CT_{50} values were determined by non-parametric statistical analysis (Mann–Whitney U Test).[5] Day-to-day differences in the susceptibility of mice to oxygen convulsions sometimes existed. For this reason, in all of the studies carried out, animals treated with the various drugs investigated were exposed to high oxygen pressures together with non-drug-treated controls in order to make valid comparisons.

The effectiveness of disulfiram in protecting against lung damage was studied by administering disulfiram (200 mg/kg, i.p.) to mice 4 hr before their exposure to three atmospheres of 100% oxygen for 2 hr. Control animals were given vehicle only and the same experimental procedures employed. Animals were sacrificed by cervical dislocation immediately after their removal from the hyperbaric chamber and their lungs removed, blotted dry and placed in 10% formalin. Normal staining and preparation methods were then employed for histopathological examination of the lung tissue. Lung tissue was arbitrarily graded on a scale from one to three. The greater the lung damage the higher the numerical value assigned. Differences between lung tissue from oxygen and non-oxygen-exposed animals were determined by nonparametric statistical analysis (Mann–Whitney U Test).[5] Some control animals convulsed during the 2-hr exposure to three atmospheres of 100% oxygen. These were discarded and only lung tissues from nonconvulsed animals were used for examination. None of the disulfiram-treated animals exposed to three atmospheres for 2 hr exhibited signs of oxygen toxicity.

RESULTS

Disulfiram significantly protected mice from oxygen convulsions and lung damage. Variation in the dose of disulfiram administered (Table 1) and the time interval between disulfiram administration and oxygen exposure (Table 2) markedly influenced the onset of oxygen convulsions. Optimal protection was obtained when the time interval between drug administration and oxygen exposure was approximately 4 hr. Diethyldithiocarbamate (DDTC), the corresponding thiol of disulfiram, also protected against oxygen convulsions (Table 3); however, DDTC was not found to be as effective as disulfiram. In addition, optimal protection with DDTC occurred much earlier than that found with disulfiram.

TABLE 1. EFFECT OF DISULFIRAM ON OXYGEN CONVULSIONS*

Dose		Four atmospheres Pretreatment time (hr) $CT_{50} \pm$ S.E.				Six atmospheres Pretreatment time (hr) $CT_{50} \pm$ S.E.			
(mg/kg)	(m-moles/kg)	Control (1)	Treated (1)	Control (4)	Treated (4)	Control (1)	Treated (1)	Control (4)	Treated (4)
120	0·41	217 ± 21 (15)	219 ± 8 (15)	107 ± 7 (13)	213 ± 20 (12)	23 ± 2 (15)	27 ± 2 (14)	20 ± 1 (14)	21 ± 1 (13)
				$P < 0·002$					
200	0·68	126 ± 10 (14)	360 ± 0† (15)	110 ± 9 (13)	304 ± 28 (11)	13 ± 1 (14)	30 ± 3 (16)	12 ± 1 (14)	47 ± 5 (16)
		$P < 0·002$		$0·010 < P < 0·020$		$0·002 < P < 0·010$		$P < 0·002$	
400	1·35	175 ± 15 (10)	360 ± 0† (15)	92 ± 7 (4)‡	360 ± 0† (12)	8 ± 1 (13)	86 ± 3 (16)	8 ± 1 (13)	84 ± 6 (15)
		$0·002 < P < 0·010$		$0·002 < P < 0·010$		$P < 0·002$		$P < 0·002$	

* Disulfiram was suspended in 1% methyl cellulose and administered to mice i.p. either 1 or 4 hr before exposure to oxygen at the doses indicated. Number of animals in Parentheses.

† Experiments were terminated after 360 min at four atmospheres and 90 min at six atmospheres.

‡ Twelve animals did not survive the exposure period.

TABLE 2. EFFECT OF DISULFIRAM PRETREATMENT TIME ON OXYGEN CONVULSIONS*

Pretreatment time (hr)	$CT_{50} \pm$ S.E. (min)		Per cent change	Significance
	Control	Treated		
1	13 ± 1 (14)	30 ± 3 (16)	+ 131	$0.002 < P < 0.010$
2	12 ± 1 (16)	30 ± 3 (16)	+ 150	$P < 0.002$
4	12 ± 1 (14)	47 ± 5 (16)	+ 292	$P < 0.002$
8	11 ± 1 (14)	13 ± 1 (15)	+ 18	N.S.
16	9 ± 1 (15)	14 ± 1 (13)	+ 56	N.S.
24	19 ± 1 (15)	12 ± 1 (15)	− 37	N.S.

* Disulfiram, 200 mg/kg (0·68 m-moles/kg) suspended in 1% methyl cellulose and administered to mice i.p. at the times indicated prior to exposure to six atmospheres of 100% oxygen. Number of animals in parentheses. N.S. = not significant.

TABLE 3. EFFECT OF DIETHYLDITHIOCARBAMATE PRETREATMENT TIME ON OXYGEN CONVULSIONS*

Pretreatment time (hr)	$CT_{50} \pm$ S.E. (min)		Per cent change	Significance
	Control	Treated		
0·5	7 ± 0·8 (8)	10 ± 1 (11)	+ 43	N.S.
1	9 ± 0·6 (16)	18 ± 2 (14)	+ 100	$P < 0.002$
1·5	10 ± 1 (15)	13 ± 1 (15)	+ 30	N.S.
2	10 ± 1 (15)	15 ± 1 (13)	+ 50	N.S.
3	10 ± 1 (15)	12 ± 1 (10)	+ 20	N.S.

* Sodium diethyldithiocarbamate, 400 mg/kg (1·78 m-moles/kg) dissolved in saline and given i.p. to mice at the time indicated prior to exposure to six atmospheres of 100% oxygen. Number of animals in parentheses. N.S. = not significant.

TABLE 4. EFFECT OF DISULFIRAM TREATMENT ON POST-EXPOSURE SURVIVAL*

Treatment	Disulfiram dosage (mg/kg)	Time (hr) between disulfiram administration and oxygen exposure	Per cent of animals surviving (days)				
			0	1	3	7	14
Vehicle	0		100 (10)	100	100	100	100
Disulfiram	200		100 (10)	100	90	90	90
Disulfiram	400		100 (10)	80	60	60	60
Disulfiram and oxygen	200	1	100 (14)	64	43	36	36
Disulfiram and oxygen	200	4	100 (8)	75	50	37	37
Disulfiram and oxygen	400	1	100 (8)	88	88	88	88
Disulfiram and oxygen	400	4	100 (16)	69	56	25	19

* Disulfiram was suspended in 1% methyl cellulose and administered to mice i.p. at the various times indicated before exposure for 6 hr to four atmospheres of 100% oxygen. Number of animals in parentheses.

Post-exposure survival of disulfiram-treated mice was good with mice surviving for as long as 2 weeks after OHP (Table 4). Control animals exposed simultaneously with disulfiram-treated animals never survived the exposure period.

In addition to studies with disulfiram and DDTC, other dopamine-β-hydroxylase inhibitors such as 1,1-dimethyl-3-phenyl-2-thiourea (DPT), 1,1-dimethyl-3-(4′-methyl phenyl) thiourea (DMPT) and 1-imidazyl-3-phenyl-thiourea (IPT) were not found to be effective oxygen protectants (Table 5).

TABLE 5. EFFECT OF INHIBITORS OF DOPAMINE-β-HYDROXYLASE ON OXYGEN CONVULSIONS*

Treatment	Dose (mg/kg)	Convulsive time $CT_{50} \pm$ S.E. (min)	
		Control	Treated
Disulfiram	200	12 ± 1 (14)	47 ± 5 (16)†
IPT‡	200	12 ± 1 (18)	9 ± 0·1 (17)
DPT§	200	12 ± 1 (18)	11 ± 0·1 (18)
DMPT‖	200	12 ± 1 (18)	10 ± 0·1 (17)

* All drugs dissolved in 1% methyl cellulose and administered to mice i.p. 4 hr before exposure to six atmospheres of 100% oxygen. Number of animals in parentheses. Dosage of IPT, DTT and DMPT based upon studies of Johnson *et al.*[6]

† $P < 0{\cdot}002$.

‡ IPT—1-imidazyl-3-phenyl-thiourea.

§ DPT—1,1-dimethyl-3-phenyl-2-thiourea.

‖ DMPT—1,1-dimethyl-3-(4′-methyl phenyl) thiourea.

Histopathological examination of lung tissue from mice pretreated with disulfiram prior to OHP exposure did not show the typical emphysema, atelectasis and edema found in animals exposed to OHP (Figs. 1–3). No statistically significant differences existed between lung tissue from control animals and animals treated with disulfiram prior to oxygen exposure (Table 6).

TABLE 6. PROTECTION WITH DISULFIRAM AGAINST LUNG DAMAGE*

Treatment	Average rating ± S.E.	Significance (Compared to control)
Control (12)	1·70 ± 0·14	
Disulfiram (8)	1·87 ± 0·14	N.S.
Oxygen (4)	2·62 ± 0·22	$0{\cdot}02 < P < 0{\cdot}05$
Disulfiram and oxygen (8)	1·87 ± 0·14	N.S.

* Disulfiram, 200 mg/kg (0·68 m-moles/kg) suspended in 1% methyl cellulose and administered i.p. to mice 4 hr before exposure to three atmospheres of 100% oxygen for 2 hr. Lung tissue was arbitrarily graded on a scale from one to three, with the larger number representing a greater degree of damage. Number in parentheses is the number of lungs used to obtain the average value. N.S. = not significant.

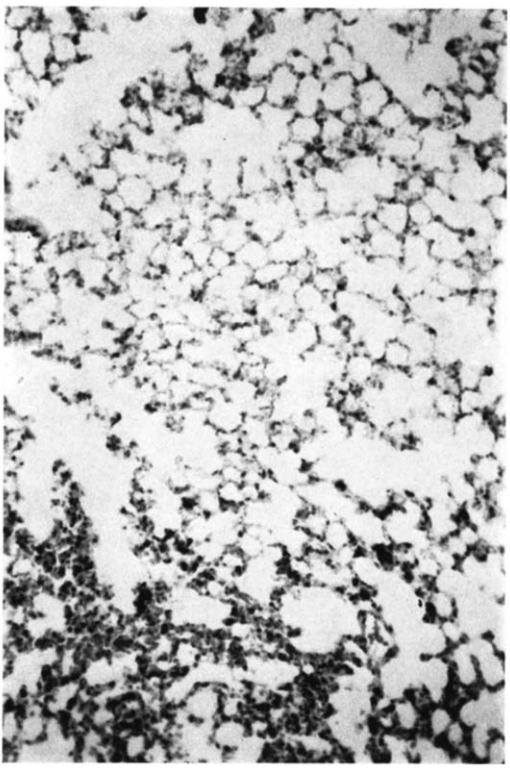

FIG. 1. Lung section from control mouse receiving vehicle only.

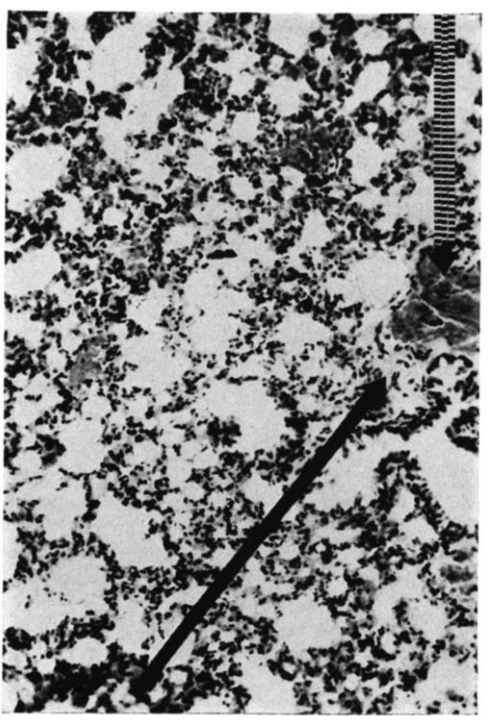

FIG. 2. Lung section from mouse exposed to three atmospheres of 100% oxygen for 2 hr. Numerous areas of edema (solid arrow), hemorrhage, (stippled arrow), broken cells and atelectasis.

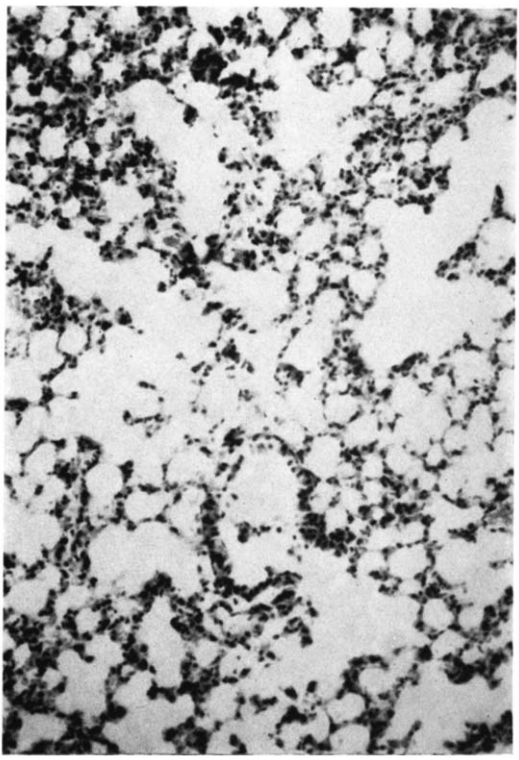

FIG. 3. Lung section from mouse pretreated with 200 mg/kg (0·68 m-moles/kg) of disulfiram 4 hr before exposure to three atmospheres of 100% oxygen for 2 hr. Cells generally intact although some are broken. Edema and hemorrhage are not present.

DISCUSSION

Although a number of agents have been found to partially protect against oxygen toxicity, the degree of protection obtained with each of these agents has varied considerably.[7] Disulfiram, however, appears to offer better protection from oxygen convulsions and lung damage than any agent reported to date. Optimum protection from oxygen toxicity was found when disulfiram was administered 4 hr before oxygen exposure. This delayed action, along with the dose–effect relationship observed (Table 1), may be due to disulfiram's insolubility and slowed absorption and distribution. This suggestion is based upon the studies of Moore[8] who found disulfiram remained as a depot in the peritoneal cavity after i.p. administration. This may explain why Jamieson *et al.*[9] failed to find any protection when they administered 120 mg/kg of disulfiram to mice 45 min before oxygen exposure whereas we observed protection at longer pretreatment time intervals.

According to Strömme,[10] disulfiram is converted to its corresponding thiol DDTC immediately upon absorption, and the thiol subsequently further metabolized. One would therefore expect DDTC to not only protect animals against oxygen toxicity, but for the pretreatment time to be less for DDTC than for disulfiram. This was found to be the case (Table 3), with optimal protection occurring when DDTC was administered to mice 1 hr before their exposure to oxygen. No protection was found at longer pretreatment times. Of interest, however, was the finding that at the pretreatment time giving optimal protection, DDTC (Table 3) failed to protect as well as disulfiram (Table 2), although larger molar concentrations of DDTC were administered. The reason for this difference is not clear at this time. It is possible that the thiol actually may be ineffective as an oxygen protectant. The reason some protection was observed may be due to the formation of disulfiram from DDTC, since DDTC has been shown to be slightly oxidized *in vivo* to the corresponding disulfide, disulfiram.[11] Protection from oxygen toxicity could therefore be due to the action of the small concentration of the newly formed disulfide and not to the thiol, thus accounting for the lesser degree of protection obtained with DDTC.

Animals treated with disulfiram in doses of 200 and 400 mg/kg showed excellent post-exposure survival (Table 4). All disulfiram-treated animals were alive and appeared in excellent condition after 6 hr of exposure to four atmospheres of 100% oxygen, whereas all controls died during the exposure period. The animals were observed for 2 weeks following oxygen exposure. During this 2-week period some deaths occurred. Since post-mortem examinations were not carried out, the deaths cannot be directly attributed to oxygen poisoning. Of interest was the observation that after the administration of 400 mg/kg of disulfiram, a larger number of animals survived when disulfiram was administered 1 hr rather than 4 hr before oxygen exposure. Since 400 mg/kg of disulfiram is close to the LD_{50}[12] the possibility that death may be due to disulfiram toxicity cannot be overlooked. One may speculate further that exposure of animals to OHP soon after disulfiram administration decreased the toxicity of disulfiram. As the time interval between disulfiram administration and oxygen exposure is lengthened, oxygen has a lesser effect on disulfiram toxicity. This may not be an unreasonable assumption since it was observed that some mice not exposed to oxygen died shortly after the administration of 400 mg/kg of disulfiram (Table 4).

Disulfiram[13] and DDTC[14] are both effective inhibitors of dopamine-β-hydroxylase;

however, protection from oxygen toxicity does not appear to be related to dopamine-β-hydroxylase inhibition. This is concluded from the observation that other agents recently shown to be effective dopamine-β-hydroxylase inhibitors[6] were found to be ineffective oxygen protectants (Table 5). It would therefore appear that disulfiram produced its protective action in some manner unrelated to dopamine-β-hydroxylase inhibition. Although inhibition of dopamine-β-hydroxylase can influence catecholamine synthesis, recent studies[2] have shown that neither brain norepinephrine, dopamine nor serotonin are implicated in oxygen convulsions.

The mechanism by which disulfiram protects against oxygen convulsions, lung damage and death is uncertain at this time. Disulfiram has been found to have many diverse actions[13,15,16] including its effect on cellular respiration[17] and electron and energy transfer in the cytochrome chain.[18] Hassinen[17,18] observed that the effects of disulfiram on mitochondria resembled those of amobarbital, and that disulfiram interfered with the first phosphorylation site of the respiratory chain. This suggests that disulfiram may exert its effect at some point in the cytochrome chain on the NAD side of ubiquinone.

Pyruvate and α-ketoglutarate, two of several key substrates supplying electrons for the cytochrome chain, are linked to thiol-disulfide oxidation–reduction reactions by way of dihydrolipoic acid, a coenzyme necessary in the oxidation of these α-oxyacids. Molecular oxygen has been shown to inhibit the oxidation of α-oxyacids *in vitro*, probably as a result of dihydrolipoic acid oxidation.[19] If this also occurred *in vivo* during oxygen exposure, inhibition of pyruvate and α-ketoglutarate oxidation could conceivably reduce the supply of electrons entering the cytochrome chain and subsequently interfere with normal electron flow. In addition, this inhibition would also cause an increased NAD/NADH ratio. This could account for the finding by Chance[20] that high oxygen pressures increased the steady state NAD/NADH ratios. If oxygen toxicity is considered to occur as a result of oxidation of dihydrolipoic acid,[19] disulfiram could provide protection by acting either as an inhibitor of oxygen-induced sulfhydryl oxidation, or by enhancing electron transfer along the cytochrome chain thus maintaining normal electron flow. This assumption is not unreasonable since disulfiram not only can act as an antioxidant as a result of reduction to its corresponding thiol, but also functions as an electron carrier by virtue of the oxidation–reduction properties of disulfides. As a result, normal electron transfer along both the forward and reversed pathways would be maintained providing protection from both lung damage and convulsions. This would be consistent with the suggestion by Chance *et al.*[21] that oxygen convulsions occurred as a result of inhibition of reversed electron transfer, a rapid event, while a slower inhibition of forward electron transfer produced lung damage. This postulated site of disulfiram's action could therefore account for its effectiveness in protecting against both oxygen convulsions and lung damage since its action at this site could influence both forward and reversed electron flow.

In conclusion, disulfiram has been found to provide excellent protection from oxygen toxicity. It is proposed that this effect may be due to disulfiram's ability to either block the oxygen-induced oxidation of key sulfhydryl groups or coenzymes, or function itself as an electron transfer agent thus maintaining normal oxidation–reduction reactions and electron flow to the cytochrome chain. Neither brain nor lung NAD, NADH, ATP, nor citric acid cycle intermediates have been determined

from oxygen-exposed animals previously treated with disulfiram. These and other studies must be carried out to more accurately define the role of disulfiram in oxygen toxicity and electron transfer. These investigations are presently in progress.

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