Effect of Thiourea on Microsomal Oxidation of Alcohols and Associated Microsomal Functions†

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ABSTRACT: Thiourea and diethylthiourea, two compounds which react with hydroxyl radicals, inhibited NADPH-dependent microsomal oxidation of ethanol and 1-butanol. Inhibition by both compounds was more effective in the presence of the catalase inhibitor, azide. Inhibition by thiourea was noncompetitive with respect to ethanol in the absence of azide but was competitive in the presence of azide. Urea, a compound which does not react with hydroxyl radicals or H_2O_2, was without effect. Thiourea had no effect on NADH- and NADPH-cytochrome *c* reductase, NADPH oxidase, and NADH- and NADPH-dependent oxygen uptake. Thiourea inhibited the activities of aniline hydroxylase and aminopyrine demethylase. Thiourea, but not other hydroxyl radical scavengers, e.g., dimethyl sulfoxide, mannitol, and benzoate, reacted directly with H_2O_2 and decreased H_2O_2 accumulation in the presence of azide. Therefore the actions of thiourea are complex because it can react with both hydroxyl radicals and H_2O_2. Differences between the actions of thiourea and those previously reported for dimethyl sulfoxide, mannitol, and benzoate, e.g., effects on drug metabolism, effectiveness of inhibition in the absence of azide, or kinetics of the inhibition, probably reflect the fact that thiourea reacts directly with H_2O_2 whereas the other agents do not. The current results remain consistent with the concept that microsomal oxidation of alcohols involves interactions of the alcohols with hydroxyl radicals generated from microsomal electron transfer.

Recent experiments (Cederbaum et al., 1977, 1978) have implicated hydroxyl radicals (·OH) in the liver microsomal system for oxidizing alcohols. Primary aliphatic alcohols are well-known scavengers for hydroxyl radicals (Anbar & Neta, 1967; Dorfman & Adams, 1973), yielding corresponding aldehydes as products (e.g., Swallow, 1953).

The liver microsomal ethanol oxidizing system requires NADPH and appears to involve a cytochrome P-450 pathway (Ohnishi & Lieber, 1977). The oxidation of NADPH by cytochrome P-450 generates hydrogen peroxide (Gillette et al., 1957; Nordblom & Coon, 1977). Microsomes are often contaminated with catalase which can utilize H_2O_2 to oxidize ethanol in a peroxidatic reaction (Keilin & Hartree, 1945; Chance, 1950). In a preliminary study (Cederbaum et al., 1977), we utilized azide to inhibit catalase and observed that four strong hydroxyl radical scavengers (namely, thiourea, mannitol, benzoate, and dimethyl sulfoxide) inhibited the oxidation of ethanol by liver microsomes. More detailed studies indicated that mannitol, benzoate, and dimethyl sulfoxide were more effective inhibitors in the presence of azide than in its absence (Cederbaum et al., 1978). When the P-450 pathway was bypassed by generating H_2O_2 with xanthine plus xanthine oxidase in the absence of azide, these scavengers did not block the peroxidatic activity of catalase. The scavengers also failed to block partial reactions involved in microsomal electron transfer, nor did they block the metabolism of drugs such as aminopyrine and aniline.

The current communication concerns the actions of thiourea on microsomal oxidation of ethanol and 1-butanol, on microsomal oxidation of drugs (aniline and aminopyrine) and on associated microsomal electron-transfer pathways. In contrast to the other scavengers, thiourea was effective in inhibiting ethanol oxidation in the absence of azide, and it also suppressed drug metabolism. Differences between thiourea and the other scavengers appear to reflect the capacity of thiourea to react directly with H_2O_2 as well as with hydroxyl radicals.

Methods

Liver microsomes were prepared from male Sprague–Dawley rats as previously described (Cederbaum et al., 1976), washed once, and suspended in 125 mM KCl. Protein was determined by the method of Lowry et al. (1951).

Microsomal oxidation of ethanol or 1-butanol was assayed as previously described (Cederbaum et al., 1977), with the use of flasks containing 0.6 mL of 15 mM semicarbazide in the center well. In most experiments, the final concentration of ethanol or butanol was 51 mM. The reaction was initiated with the NADPH-generating system (see below) and was terminated after 15 min at 37 °C by the addition of trichloroacetic acid (final concentration, 5%). After an overnight diffusion period, the optical density of the semicarbazone complex was determined at 224 nm and compared with standards. Blanks included zero-time controls and flasks without microsomes. Peroxide generation was assayed by the ferrithiocyanate method (Thurman et al., 1972), using a reaction mixture of 100 mM potassium phosphate, pH 7.4, 10 mM $MgCl_2$, 0.3 mM $NADP^+$, 10 mM glucose 6-phosphate, 7 units of glucose-6-phosphate dehydrogenase, 5 mM azide, and about 5 mg of microsomal protein in a volume of 3.0 mL. After 20 min at 37 °C, 1 mL of 20% Cl_3CCOOH was added, the samples were centrifuged, and aliquots of the supernatant were reacted with 0.1 mL of 2.5 M potassium thiocyanate plus 0.2 mL of 10 mM ferrous ammonium sulfate. After 3–5 min, the absorbance of the ferrithiocyanate complex was determined at a wavelength of 480 nm. Aniline hydroxylase (Kato & Gillette, 1965), aminopyrine demethylase (Cochin & Axelrod, 1959), NADPH and NADH-cytochrome *c* reductase activities (Phillips & Langdon, 1962), and microsomal oxygen consumption were assayed as previously described (Cederbaum et al., 1977, 1978).

All values refer to mean ± SEM. Statistical analysis was performed by Student's *t* test. The number of experiments

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Table I: Effect of Thiourea and Diethylthiourea on Microsomal Oxidation of Ethanol and 1-Butanol in the Absence and Presence of Azide[a]

			−azide		+azide	
alcohol	addition	concn (mM)	alcohol oxidn (nmol min^{-1} (mg of protein)$^{-1}$)	effect (%)	alcohol oxidn (nmol min^{-1} (mg of protein)$^{-1}$)	effect (%)
ethanol	none		9.85 ± 0.59 (16)		8.00 ± 0.92 (8)	
	thiourea	3.3	8.38 ± 0.63 (12)	−15[b]	6.69 ± 0.95 (7)	−16
		10	8.15 ± 0.69 (14)	−17[b]	5.03 ± 0.76 (7)	−37[b]
		33	7.22 ± 0.54 (16)	−27[c]	2.67 ± 0.32 (8)	−67[d]
		67	6.19 ± 0.52 (15)	−37[d]	1.38 ± 0.17 (8)	−83[d]
		100	5.51 ± 0.51 (14)	−44[d]	0.87 ± 0.13 (5)	−89[d]
ethanol	none		10.11 ± 0.92 (3)		7.25 (2)	
	diethylthiourea	3.3	8.29 ± 0.16 (3)	−18[b]	5.49 (2)	−24
		10	7.13 ± 0.75 (3)	−29[c]	4.19 (2)	−42
		33	6.15 ± 0.79 (3)	−39[c]	2.97 (2)	−59
		67	5.30 ± 1.41 (3)	−48[c]	2.12 (2)	−71
butanol	none		3.39 ± 0.59 (4)		7.98 ± 0.97 (4)	
	thiourea	10	1.97 ± 0.64 (4)	−42[b]	5.28 ± 0.92 (4)	−34[b]
		33	1.12 ± 0.51 (4)	−67[e]	3.50 ± 0.41 (4)	−56[d]
		67	0.63 ± 0.32 (4)	−81[d]	1.96 ± 0.49 (4)	−75[d]

[a] NADPH-dependent microsomal oxidation of 51 mM ethanol or 51 mM 1-butanol to acetaldehyde or butyraldehyde was assayed in the absence or presence of 1 mM azide, and the indicated concentrations of thiourea or diethylthiourea. [b] $p < 0.05$. [c] $p < 0.01$. [d] $p < 0.001$. [e] $p < 0.005$.

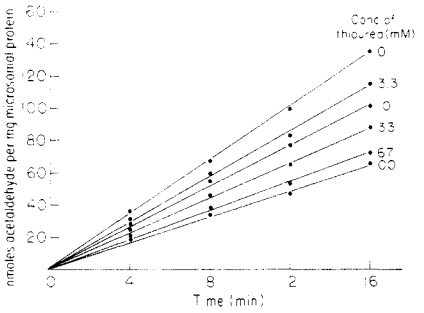

FIGURE 1: Effect of reaction time on the inhibition of NADPH-dependent microsomal oxidation of ethanol by thiourea in the absence of azide. Results are the averages from two different microsomal preparations.

is stated in the legends or indicated in Tables I–III.

Results

Inhibition of NADPH-Dependent, Microsomal Alcohol Oxidation by Thiourea and Diethylthiourea. The biomolecular rate constants for the reactions of the various substances studied with hydroxyl radicals (M^{-1} s^{-1}) are as follows: ethanol, 0.7–1.1 × 10^9; 1-butanol, 2.2 × 10^9; thiourea, 4.7 × 10^9; and urea, 7 × 10^5 (Dorfman & Adams, 1973; Anbar & Neta, 1967). Diethylthiourea appears closely similar to thiourea in its reactivity with hydroxyl radicals (Cohen et al., 1976).

The oxidation of ethanol by liver microsomes in the presence of an NADPH-generating system resulted in the generation of acetaldehyde at a rate which was linear with time (Figure 1). Increasing concentrations of thiourea or diethylthiourea led to progressive inhibition of acetaldehyde production (Figure 1 and Table I). Thiourea inhibited ethanol oxidation at all time periods tested, i.e., there was no lag in the inhibition (Figure 1). By contrast, urea, which is not an effective hydroxyl radical scavenging agent, had no effect on microsomal ethanol oxidation, e.g., rates of ethanol oxidation (nmol min^{-1} (mg of microsomal protein)$^{-1}$) in the absence of azide were 10.08 ± 1.20 for the control and 10.33 ± 0.59, 10.09 ± 0.54, and 9.77 ± 0.49 in the presence of 33, 67, and 100 mM urea, respectively (n = 4).

Sodium azide (1 mM) added to the system to inhibit catalase led to some diminution in the rate of ethanol oxidation; e.g., in experiments with thiourea (Table I), the diminution was 19%. Both thiourea and diethylthiourea were more effective inhibitors in the presence of azide (Table I). Previously, a greater effectiveness of three other hydroxyl radical scavengers had been noted in the presence of azide rather than its absence (Cederbaum et al., 1978). In the presence of azide, urea, at concentrations of 10, 33, 67, and 100 mM, inhibited the microsomal oxidation of ethanol by only 4, 5, 9, and 16%, respectively (n = 5).

1-Butanol is a substrate for the microsomal alcohol oxidizing system, whereas the peroxidatic activity of catalase with 1-butanol is negligible (Keilin & Hartree, 1945; Lieber, 1975; Cederbaum et al., 1978). Azide doubled the rate of 1-butanol oxidation (Table I) in confirmation of previous observations (Cederbaum et al., 1978). Thiourea decreased the rate of 1-butanol oxidation to similar extents in either the absence or presence of azide (Table I). Thus, the oxidatiton of 1-butanol differed from ethanol with regard to sensitivity to azide as well as the effect of azide on the subsequent action of thiourea.

Control experiments indicated that thiourea had no effect on the NADPH-generating system (that is, at thiourea concentration of 33, 67, and 100 mM, the activity of glucose-6-phosphate dehydrogenase was affected +4, −4, and −7%, respectively). Thiourea inhibited ethanol oxidation when 3 mM NADPH was used instead of the NADPH-generating system.

Under conditions of a "head-gas" method, thiourea has been reported to suppress to a limited extent the recovery of acetaldehyde (Sippel, 1973; Eriksson et al., 1975). To determine whether or not the inhibition by thiourea in our system was due to interference with the collection of acetaldehyde as a semicarbazone, recovery experiments with acetaldehyde and n-butyraldehyde were carried out by incubating known amounts of the aldehyde with thiourea at 37 °C in center well flasks containing semicarbazide. After 15 min, 1 mL of trichloroacetic acid was added (final concentration of 5%) and, after an overnight diffusion period, the semicarbazone in the center well was measured. The amounts of aldehyde added were based on those expected to be formed in typical experiments. Neither thiourea nor urea, in concentrations up to 100 mM, lowered the recoveries of 250, 500, or 1500 nmol of acetaldehyde or of 550 nmol of butyraldehyde. Under

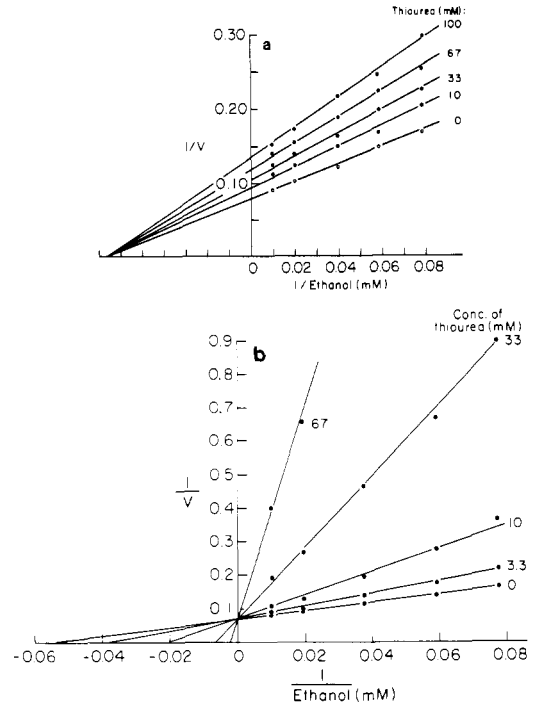

FIGURE 2: Double-reciprocal plot showing the kinetics of the inhibition of microsomal oxidation of ethanol by thiourea. Microsomal oxidation of ethanol was assayed as described in Methods in the absence or presence of 1.0 mM azide. Results are from five preparations. (a) The absence of azide. Control rates of ethanol oxidation (nmol of acetaldehyde per min per mg of microsomal protein) were 5.90 ± 0.39, 7.12 ± 0.46, 8.63 ± 0.52, 10.95 ± 1.05, and 12.28 ± 0.98 in the presence of 12.8, 17.1, 25.5, 51, and 102 mM ethanol, respectively. (b) The presence of azide. Control rates of ethanol oxidation were 5.73 ± 0.78, 6.71 ± 0.70, 8.19 ± 0.63, 9.57 ± 0.91, and 10.96 ± 1.04 in the presence of 12.8, 17.1, 25.5, 51, and 102 mM ethanol, respectively.

identical condition, 5 mM bisulfite strikingly lowered the recoveries of acetaldehyde and butyraldehyde. It was apparent that thiourea did not interfere with recoveries of acetaldehyde in our experiments.

Kinetics of Inhibition by Thiourea in the Absence and Presence of Azide. The concentration of ethanol was varied from 12.8 to 102 mM to study the kinetics of the inhibition by thiourea of microsomal ethanol oxidation in the absence and presence of azide. The amount of acetaldehyde produced increased as the concentration of ethanol was increased (legend to Figures 2a and 2b). In the absence of azide, the apparent K_m for ethanol was about 14 mM, while V_{max} was 13–14 nmol of acetaldehyde per min per mg of microsomal protein (Figure 2a). Thiourea did not change the K_m of ethanol oxidation, but the maximum velocity was lowered (Figure 2a), indicating that, in the absence of azide, thiourea was a noncompetitive inhibitor.

In the presence of azide, the inhibition by thiourea varied inversely with the concentration of ethanol. Thiourea concentrations as low as 3.3 mM produced significant inhibition of ethanol oxidation at the lower ethanol concentrations, viz., 12.8, 17.1, and 25.5 mM. The apparent K_m for ethanol in the presence of azide was about 18 mM; V_{max} was about 14 nmol of acetaldehyde per min per mg of microsomal protein (Figure 2b). Thiourea increased the K_m for ethanol without changing the V_{max} (Figure 2b), indicating that, in the presence of azide, thiourea was a competitive inhibitor of microsomal ethanol oxidation. The K_i for thiourea in the presence of azide was about 6 mM. A Dixon plot ($1/v$ vs. concentration of thiourea at the different ethanol concentrations) also indicated competitive inhibition by thiourea with a K_i of 7.5 mM.

Xanthine Oxidase Dependent Oxidation of Ethanol by Microsomes. The microsomal oxidation of ethanol in the presence of xanthine plus xanthine oxidase (replacing the NADPH-generating system) appears to represent the peroxidatic activity of catalase. In our experiments, this activity was inhibited by greater than 95% by the addition of 1 mM azide. A study of the effect of thiourea in the absence of azide showed inhibition of 14, 28, 44, and 59% by 10, 33, 67, and 100 mM thiourea, respectively, for an initial rate of 13.1 ± 1.9 nmol of acetaldehyde produced per min per mg of microsomal protein. Comparable concentrations of urea were without any effect. However, thiourea also inhibited the activity of xanthine oxidase itself (15, 30, 50, and 70%, respectively, at the same concentrations quoted above) as measured spectrophotometrically by following the conversion of 2 mM xanthine to uric acid at 293 nm. Therefore, inhibition of ethanol oxidation in this system can be attributed mainly to decreased production of H_2O_2. It should be noted that other hydroxyl radical scavengers (mannitol, benzoate, and dimethyl sulfoxide) had no effect on the xanthine oxidase dependent oxidation of ethanol and no effect on xanthine oxidase activity itself (Cederbaum et al., 1978).

Microsomal Drug Oxidation and Associated Reactions. The NADPH-supported oxidation of ethanol by hepatic microsomes is believed to involve, at least in part, some of the components of the microsomal drug metabolizing system. Both systems require oxygen, NADPH, and cytochrome P-450, and both are inhibited by carbon monoxide (Lieber & DeCarli, 1970). Therefore, the effects of thiourea on reactions associated with microsomal drug oxidation were evaluated. Neither NADPH nor NADH-cytochrome *c* reductase activity was affected by concentrations of thiourea which inhibited microsomal oxidation of ethanol. Similarly, thiourea had no effect on NADPH-ferricyanide reductase activity. Comparable levels of urea also had no effect on any of these reactions.

Microsomal oxygen uptake, in the presence of NADH or NADPH as substrates, may be indicative of the complete microsomal oxidative system, i.e., the transfer of electrons from reduced pyridine nucleotide to oxygen. Thiourea had no effect on oxygen consumption with either NADPH (control rate of 21.9 ± 2.3 natoms of oxygen consumed min^{-1} (mg of microsomal protein)$^{-1}$) or NADH (control rate of 13.3 ± 1.7 natoms of oxygen consumed min^{-1} (mg of microsomal protein)$^{-1}$) as substrate. Similar results were obtained when NADPH oxidase activity was assayed spectrophotometrically by measuring the disappearance of NADPH at 340 nm.

The effects of thiourea on the overall oxidation of representative type 1 and type 2 drugs were studied. The activities of aniline hydroxylase and aminopyrine demethylase were inhibited by thiourea (Table II). Control experiments indicated that thiourea did not affect the recovery of standard amounts of formaldehyde (265 and 675 nmol) incubated for 20 min and then assayed with the Nash reagent. Urea, which had no effect on microsomal oxidation of ethanol, failed to affect aniline hydroxylase activity. However, aminopyrine demethylase activity was inhibited by urea (Table II).

Effect of Thiourea on Microsomal Generation of Peroxide. Although considerably more reactive with hydroxyl radicals, thiourea is also capable of interacting with H_2O_2 (Heikkila et al., 1976). The effect of thiourea, dimethyl sulfoxide, mannitol, and benzoate on the accumulation of H_2O_2 by microsomes incubated with a NADPH-generating system in the presence of azide (to inhibit catalase) was evaluated (Table III). Thiourea diminished the accumulation of H_2O_2, whereas

Table II: Effect of Thiourea and Urea on the Activities of Aminopyrine Demethylase and Aniline Hydroxylase[a]

addition	concn (mM)	aniline hydroxylase: nmol min^{-1} (mg of protein)$^{-1}$	aniline hydroxylase: effect (%)	aniline hydroxylase: P	aminopyrine demethylase: nmol min^{-1} (mg of protein)$^{-1}$	aminopyrine demethylase: effect (%)	aminopyrine demethylase: P
none		0.68 ± 0.15			9.48 ± 2.10		
thiourea	33	0.42 ± 0.13	−38	<0.05	6.03 ± 0.80	−36	<0.05
	67	0.39 ± 0.14	−43	<0.05	4.68 ± 0.75	−51	<0.02
	100	0.37 ± 0.09	−46	<0.02	3.98 ± 0.88	−58	<0.01
urea	33	0.62 ± 0.08	−9	NS	6.75 ± 1.22	−29	<0.05
	67	0.59 ± 0.11	−13	NS	5.05 ± 1.08	−47	<0.02
	100	0.54 ± 0.12	−21	$0.10 > p > 0.05$	4.03 ± 1.08	−57	<0.02

[a] The microsomal drug metabolism activities were assayed as described in Methods. Activity refers to nmol of formaldehyde (aminopyrine demethylase) or *p*-aminophenol (aniline hydroxylase) formed per min per mg of microsomal protein. Results are from four different preparations.

Table III: Effect of Thiourea, Dimethyl Sulfoxide, Mannitol, and Benzoate on Microsomal Generation of H_2O_2[a]

addition	concn (mM)	H_2O_2 generation (nmol min^{-1} (mg of protein)$^{-1}$)	effect (%)
none		4.60 ± 0.56	
thiourea	3.3	3.20 ± 0.92	−30
	10	2.64 ± 0.68	−43[c]
	33	2.24 ± 0.68	−51[d]
	67	1.72 ± 0.40	−63[d]
	100	1.80 ± 0.48	−61[d]
none		4.95 ± 0.71	
Me_2SO	23.5	6.65 ± 1.08	+34
	47	6.95 ± 1.10	+40
	94	8.20 ± 0.88	+65[e]
mannitol	33	5.52 ± 0.74	+11
	67	5.70 ± 0.67	+15
	100	5.46 ± 0.70	+10
benzoate	10	7.00 ± 0.98	+41[b]
	33	8.25 ± 1.63	+66[b]
	67	8.23 ± 0.83	+66[e]

[a] Microsomal production of peroxide was assayed as described in Methods in the presence of 5 mM azide. The reaction was initiated with $NADP^+$ plus glucose 6-phosphate. The experiments are the average from four to seven preparations. Blanks included flasks with scavengers, but without $NADP^+$ or without microsomes. [b] $0.10 > p > 0.05$. [c] $p < 0.05$. [d] $p < 0.01$. [e] $p < 0.02$.

the other scavengers did not.[1] Actually, significant increases in the accumulation of H_2O_2 were observed at the higher concentration of dimethyl sulfoxide and benzoate.

To further demonstrate that thiourea could react directly with H_2O_2, recovery experiments of known amounts of H_2O_2 were carried out. H_2O_2 (100, 200, or 400 nmol) was added to flasks with or without thiourea and incubated for 20 min at 37 °C. Cl_3CCOOH was added and aliquots were removed and treated with thiocyanate and ferrous ammonium sulfate. Thiourea strikingly lowered the recovery of H_2O_2 at all concentrations of H_2O_2 tested; e.g., with 100 nmol of H_2O_2, absorbancy values of aliquots at 480 nm were 0.252 in the absence of thiourea and 0.090, 0.028, 0.004, and 0.002 in the presence of 3.3, 10, 33, and 67 mM thiourea, respectively. By contrast, similar concentrations of mannitol or dimethyl sulfoxide had no effect on recovery of H_2O_2. Urea did not affect the recovery of known amounts of H_2O_2 nor did urea inhibit NADPH-dependent microsomal generation of H_2O_2.

[1] If thiourea was added to samples in which color development already occurred, i.e., after the addition of thiocyanate, a time-dependent decrease in absorbance at 480 nm was found, suggesting that thiourea could interfere with color development and stability of the complex. No changes in absorbance were found for the first 10–15 min after the addition of thiourea to the ferrithiocyanate complex. Therefore, all samples were read within 3–5 min after the addition of thiocyanate.

Discussion

Previous results from our laboratory showed that mannitol, benzoate, and dimethyl sulfoxide, three compounds that react rapidly with hydroxyl radicals, inhibited the microsomal oxidation of ethanol and 1-butanol (Cederbaum et al., 1977, 1978). Strong inhibition was observed only in the presence of the catalase inhibitor, azide. The inhibition was competitive with respect to ethanol and was specific in the sense that these agents did not affect (a) catalase-dependent (xanthine oxidase dependent) oxidation of ethanol, (b) microsomal electron transfer or oxygen uptake, and (c) microsomal drug metabolism (Cederbaum et al., 1977, 1978).

Thiourea is an effective hydroxyl radical scavenging agent, but urea is not (Anbar & Neta, 1967; Heikkila et al., 1976; Cohen et al., 1976). Thiourea, but not urea, inhibited microsomal oxidation of ethanol and butanol. However, thiourea differed in several respects from the other hydroxyl radical scavengers: (a) thiourea was an effective inhibitor both in the presence and absence of azide; (b) inhibition by thiourea was noncompetitive with respect to ethanol in the absence of azide; (c) thiourea inhibited aminopyrine demethylation and aniline hydroxylation; and (d) thiourea reacted directly with H_2O_2. Like the other scavengers, thiourea was a competitive inhibitor of microsomal ethanol oxidation when azide was present and thiourea produced the same percentage inhibition of 1-butanol oxidation in the presence or absence of azide. Like the other scavengers, thiourea did not affect microsomal electron transfer reactions.

A series of reactions that can explain aspects of the data are

$$\text{catalase} + H_2O_2 + \text{ethanol} \rightarrow \text{acetaldehyde} \quad (1)$$

$$\cdot OH + \text{ethanol or 1-butanol} \rightarrow \text{acetaldehyde or butyraldehyde} \quad (2)$$

$$H_2O_2 + Fe^{2+} \xrightarrow{\text{Fenton reaction}} \cdot OH + Fe^{3+} + OH^- \quad (3)$$

$$H_2O_2 + O_2^- \xrightarrow[\text{Haber–Weiss reaction}]{} \cdot OH + O_2 + OH^- \quad (4)$$

Ethanol can be oxidized either by catalase and H_2O_2 (eq 1, only in the absence of azide) or by hydroxyl radicals (eq 2, in the presence or absence of azide). 1-Butanol is a poor substrate for catalase, but can be oxidized by hydroxyl radicals (eq 2). H_2O_2 plays a pivotal role in both systems in that it serves either directly as a reactant (eq 1), or indirectly as a source of hydroxyl radicals (eq 3 or 4) (see Cederbaum et al., 1978, for further discussion of these reactions).

A direct demonstration of the role for H_2O_2 in the cytochrome P-450 mediated pathway stems from the observation that reagent H_2O_2 added to the system stimulated the oxidation of ethanol and butanol (Cederbaum et al., 1978).

Indirect evidence is provided by the observation that azide, which prevents the decomposition of H_2O_2 by catalase, actually stimulated the oxidation of 1-butanol (Cederbaum et al., 1978, and Table I). Differences in the effects of azide on the oxidation of ethanol vs. 1-butanol, as well as on the effectiveness of the scavengers as inhibitors of ethanol oxidation, have been attributed to (1) the presence of two H_2O_2-dependent pathways for oxidizing alcohols, and (2) to the ability of azide to increase the level of H_2O_2 and thereby stimulate the pathway dependent upon hydroxyl radicals (Cederbaum et al., 1978). In essence it appears that inhibition of one of the two pathways (e.g., with azide or with hydroxyl radical scavengers) can lead to stimulation of the alternate pathway due to their mutual dependence upon H_2O_2.

Thiourea, but not the other hydroxyl radical scavenging agents, reacted directly with H_2O_2. Moreover, thiourea, but not the other scavengers, decreased H_2O_2 accumulation during NADPH oxidation by microsomes (Table III). Consequently, differences between the actions of thiourea and those of the other agents may reflect the ability of thiourea to react with H_2O_2. Interaction with H_2O_2 may be responsible for the noncompetitive inhibition found with thiourea in the absence of azide. It may also explain the sensitivity of the microsomal system to thiourea, but not the other scavengers, in the absence of azide (i.e., although dimethyl sulfoxide, mannitol, and benzoate will inhibit the hydroxyl radical pathway, ethanol can still be oxidized by the H_2O_2 plus catalase-dependent pathway). The competitive nature of the inhibition by thiourea in the presence of azide is similar to the competitive nature of the inhibition found with the other scavengers. This may reflect the fact that thiourea is much more reactive with hydroxyl radicals than with H_2O_2 (Heikkila et al., 1976). In this respect thiourea was a more effective inhibitor of NADPH-dependent microsomal oxidation of ethanol in the presence of azide than it was of the xanthine oxidase dependent (catalase-dependent) oxidation of ethanol in the absence of azide.

The inhibition of drug metabolism by thiourea, but not the other scavenging agents, may reflect the ability of thiourea to react directly with peroxide. Schemes of drug metabolism involving ternary complexes with oxygen bound to cytochrome P-450 as a peroxide intermediate have been suggested (Estabrook et al., 1971, 1975; Nordblom et al., 1976). Cytochrome P-450 can also function as a peroxidase with organic hydroperoxides (Hrycay & O'Brien, 1972, 1974).

Various thiono-sulfur-containing compounds, including thiourea, were shown to decrease cytochrome P-450 levels, but only when they were preincubated with microsomes and NADPH (Hunter & Neal, 1975). It is possible that the inhibition of microsomal oxidation of drugs and alcohols by thiourea may reflect, in part, effects on microsomal cytochrome P-450 levels. However, it should be noted that this would not explain the greater sensitivity of ethanol oxidation to thiourea in the presence of azide, nor the change from noncompetitive to competitive inhibition when azide was added. Moreover, no preincubation with thiourea was employed in our studies and thiourea did not exhibit a lag in its inhibitory action (Figure 1). Therefore, reactivity with H_2O_2 remains the most likely explanation for the actions of thiourea.

In summary, differences between the actions of thiourea and those of dimethyl sulfoxide, mannitol, and benzoate may reflect the fact that thiourea reacts with H_2O_2, whereas the other agents do not. Thiourea appears to have multiple effects on microsomal functions, while the actions of the other scavengers appear to be more specific. The current results remain consistent with the concept that the microsomal oxidation of alcohols involves the interaction of alcohols with hydroxyl radicals that are generated by microsomal electron transfer.

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