Biochemical Pharmacology, Vol. 31, No. 11, pp. 2031–2037, 1982.
Printed in Great Britain.

0006-2952/82/112031-07 $03.00/0

A CORRELATION BETWEEN HYDROXYL RADICAL GENERATION AND ETHANOL OXIDATION BY LIVER, LUNG AND KIDNEY MICROSOMES*

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(*Received* 7 *August* 1981; *accepted* 1 *December* 1981)

Abstract—A comparison of the abilities of microsomes from liver, kidney and lung to oxidize ethanol and to generate hydroxyl radicals was conducted to determine if these two variables correlated with one another. The oxidation of 2-keto-4-thiomethylbutyric acid (KTBA) to ethylene, and the production of formaldehyde from dimethylsulfoxide (Me_2SO), served as chemical probes for the detection of the production of hydroxyl radicals by the microsomes. Liver microsomes oxidized ethanol at rates several-fold greater than those found with lung and kidney microsomes. This greater rate of ethanol oxidation by liver microsomes correlated with a greater rate of oxidation of the hydroxyl radical scavengers by the liver microsomes (liver > lung ≈ kidney). In all tissues, the addition of azide, an inhibitor of catalase, augmented the rate of oxidation of Me_2SO and KTBA. The addition of iron–EDTA (a ·OH-potentiating agent) increased the rates of oxidation of ethanol by the microsomes from the three tissues. This increase again correlated with an increase in the oxidation of Me_2SO and KTBA. The greater rate of oxidation of ethanol and the hydroxyl radical scavengers by liver microsomes may reflect the relative specific content of cytochrome P-450 (6- to 12-fold greater) and specific activity of NADPH-cytochrome *c* reductase (4-fold greater) in liver as compared to lung and kidney microsomes. Relative turnover numbers (units per nmole cytochrome P-450) demonstrated equivalent activities for liver and kidney, whereas lung had a higher turnover number for ethanol oxidation and hydroxyl radical generation. These data support the hypothesis that the oxidation of ethanol by microsomes may be mediated by the relative capacity of the microsomes to generate hydroxyl radicals during microsomal electron transport, which in turn may be related to the relative content and/or activities of the components of the electron transport chain.

Recent experiments have implicated a role for hydroxyl radicals‡ or a species with the oxidizing power of ·OH in the NADPH-dependent oxidation of alcohols by liver microsomes [1, 2]. The oxidation of ethanol was blocked by a series of ·OH scavengers in a manner that was competitive with respect to ethanol and was related to the ability of the scavengers to react with ·OH [1, 2]. The scavengers had no effect on microsomal drug metabolism [1, 2]. During NADPH-dependent electron transport, liver microsomes were found to oxidize typical scavengers of ·OH, e.g. Me_2SO, KTBA and methional [3] and *t*-butyl alcohol [4]. The metabolism of the scavengers was increased in the presence of azide, which was used to inhibit the activity of catalase, present as a contaminant in the microsomes [1, 3]; thus, H_2O_2 may be serving as a precursor of ·OH. Taken as a whole, these results suggested that the oxidation of alcohols by liver microsomes represented, in part, a system potentially dependent upon the interaction of the alcohols with ·OH generated by the microsomes.

Studies on the action spectrum of inhibition of ethanol oxidation by carbon monoxide implicated a role for cytochrome P-450 in the oxidation of ethanol by liver microsomes [5]. The oxidation of ethanol in reconstituted systems containing cytochrome P-450 purified from chronic ethanol-fed rats [6] or phenobarbital-treated rats [7] was inhibited by several ·OH scavengers. Ethylene production from KTBA was observed in the presence of NADPH and purified cytochrome P-450 [7]. These results suggest a role for cytochrome P-450 in generating ·OH and in oxidizing ·OH scavengers, including ethanol. There are several references which show that certain types of xenobiotics are metabolized preferentially by the cytochrome P-450 mediated drug-metabolizing system of tissues other than liver [8]. Lung tissue is very effective in metabolizing certain xenobiotics in both microsomes [8] and in purified reconstituted systems [9, 10]. This phenomenon has led to the suggestion that various tissues contain different kinds (or forms) of cytochrome P-450 [8–11]. Also, it has been well established that the content of cytochrome P-450 varies in different tissues [8, 12].

In view of the above, it follows that a correlation should exist between the abilities of a microsomal

* These studies were supported by USPHS Grants AA03312 and AA04413 and by Research Career Development Award 5K02-AA-00003 from the National Institute on Alcohol Abuse and Alcoholism.

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‡ Abbreviations used: KTBA, 2-keto-4-thiomethylbutyric acid; Me_2SO, dimethylsulfoxide; ·OH, hydroxyl radical or a species with the oxidizing power of the hydroxyl radical.

(or cytochrome P-450) fraction to oxidize ethanol and to oxidize other ·OH scavengers, i.e. a microsomal fraction capable of generating ·OH should be capable of oxidizing ethanol and vice versa. Kidney microsomes were reported to be ineffective in oxidizing ethanol [13]. This suggests that kidney microsomes should be ineffective in generating ·OH and oxidizing ·OH scavengers. Because lung, kidney, and liver microsomes vary in cytochrome P-450 content, as well as display differential drug-metabolizing capacities, it was of interest to determine whether differences in the NADPH-dependent generation of ·OH (as reflected by the metabolism of ·OH scavengers) existed and, if differences could be established, determine how they correlate with the respective NADPH-dependent oxidation of ethanol.

MATERIALS AND METHODS

Isolation of microsomes. Male Sprague–Dawley rats were decapitated and bled thoroughly by holding vertically for 30 sec. This step effectively prevented the aspiration of blood into the lungs. The lungs, kidney, and liver were removed rapidly, placed into ice-cold buffer (0.25 M sucrose, 10.0 mM Tris, pH 7.4, 1.0 mM EDTA), and washed three times until essentially blood-free. The tissues were then diced with stainless steel scissors and washed two more times. The thoroughly washed tissues were homogenized in the same buffer by three passes with a Potter–Elvehjem homogenizer. The microsomal pellet was obtained by differential centrifugation, washed once with 125 mM KCl, and resuspended in 125 mM KCl [1–3].

Microsomal oxidation of ethanol. This was assayed as previously described [1]. Center-well flasks containing 0.6 ml of 15 mM semicarbazide HCl in 160 mM phosphate buffer, pH 7.4, were used. Essentially, the assay was conducted at 37° in a final reaction volume of 3 ml containing 83 mM potassium phosphate, pH 7.4, 10 mM sodium pyrophosphate, 0.1 mM EDTA, 10 mM $MgCl_2$, 0.4 mM $NADP^+$, 10 mM glucose-6-phosphate, 7 units of glucose-6-phosphate dehydrogenase, 51 mM ethanol, and about 5 mg of microsomal protein. The water used in preparing all buffers and reagents was passed through a Chelex-100 (Biorad) resin. The buffers thus prepared were again Chelexed before use to assure removal of any contaminating iron. The reaction was initiated by the addition of the NADPH-generating system and terminated with trichloroacetic acid (TCA, final concentration of 4.5%). Following a 24 hr diffusion period, an aliquot of the center-well contents was removed and assayed spectrophotometrically for the formation of the semicarbazone complex at 224 nm, using the extinction coefficient of 9.41 mM^{-1} cm^{-1}. Zero-time controls contained the acid added before the NADPH-generating system. In studies involving the addition of iron, Fe–EDTA complex was prepared by dissolving 5 mM ferrous ammonium sulfate in 10 mM tetrasodium EDTA.

Microsomal oxidation of hydroxyl radical scavenging agents. Two probes were used for the determination of the ability of microsomes to oxidize hydroxyl radical scavenging agents during microsomal electron transport. These were, respectively, the production of formaldehyde from Me_2SO [14] and the production of ethylene from KTBA [3]. Reactions were carred out in 25 ml Erlenmeyer flasks at 37°, using the same incubation mixture as that described above except that either 33 mM Me_2SO or 10 mM KTBA replaced the ethanol. Reactions were initiated with the NADPH-generating system and were terminated by the addition of TCA. In the Me_2SO experiments, aliquots were centrifuged and formaldehyde was determined using the clear supernatant fluid by the method of Nash [15]. In the KTBA experiments, the flasks were stoppered with gas-sealed rubber caps, and ethylene was determined by gas chromatography on aliquots of the head space [3].

Protein concentrations were determined according to the method of Lowry *et al.* [16]. Cytochrome P-450 content was determined by the method of Omura and Sato [17] with a Perkin–Elmer model 554-dual beam spectrophotometer using an extinction coefficient of 91 mM^{-1} cm^{-1}. NADPH–cytochrome *c* reductase was measured according to Phillips and Langdon [18] on a Carey 14 u.v. spectrophotometer.

Statistical analysis was performed by Student's *t*-test.

RESULTS

The NADPH-dependent oxidation of ethanol by liver, lung, and kidney microsomes was compared over the time course of 0 to 30 min (Fig. 1). Azide (1.0 mM) was included in these experiments to block the activity of catalase. It was observed that liver microsomes displayed a much greater capacity to oxidize ethanol than did lung or kidney microsomes. Essentially no difference was observed in the relative

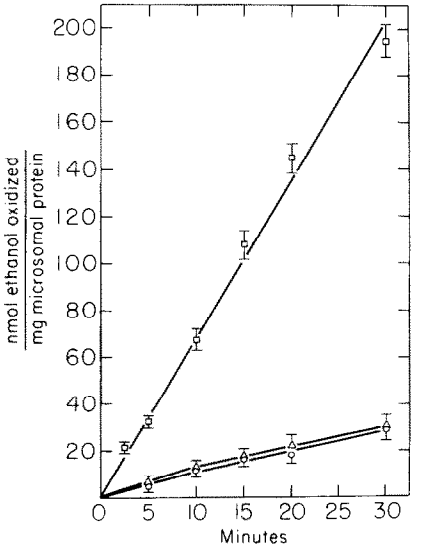

Fig. 1. Time courses for the oxidation of ethanol by microsomes from liver, lung, kidney. Ethanol oxidation was assayed as described in Materials and Methods in the presence of 1.0 mM azide. Results are from eight to twelve determinations with four to six microsomal preparations. Key: (□) liver microsomes, (△) lung microsomes, and (○) kidney microsomes.

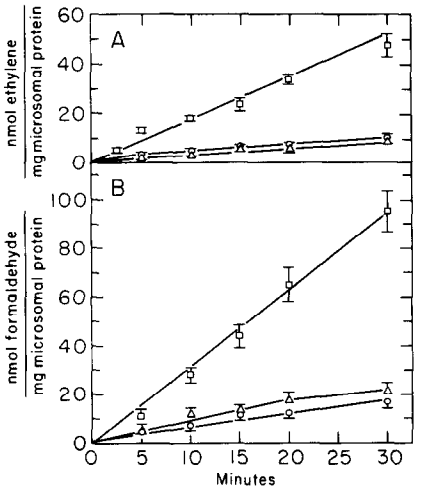

Fig. 2. Time course for the oxidation of KTBA (A) or of Me_2SO (B) by microsomes from liver, lung and kidney. The oxidation of KTBA to ethylene, or the oxidation of Me_2SO to formaldehyde, was assayed as described in Materials and Methods in the presence of 1.0 mM azide. Results are from eight to twelve determinations with four to six microsomal preparations. Key: (□) liver microsomes, (△) lung microsomes, and (○) kidney microsomes.

rates of ethanol oxidation between the lung and kidney microsomal system.

If the microsomal ethanol-oxidizing system reflects, in part, the production of ·OH from NADPH via microsomal electron transport, then liver microsomes should generate proportionately more ·OH than lung and kidney microsomes over the time course studied. Further, since no difference was observed in the relative rates of microsomal ethanol oxidation by the lung with respect to the kidney, it should follow that these tissues would display no respective differences in their capacity to generate hydroxyl radicals. The microsomal mediated metabolism of two ·OH scavengers was exploited as chemical probes for the relative ·OH generating capacity of liver, lung and kidney microsomes; these were the formation of ethylene from KTBA and formaldehyde from Me_2SO. Figure 2 shows the relative rates of NADPH-dependent metabolism of KTBA and Me_2SO by liver, lung, and kidney microsomes in the presence of 1.0 mM azide. It was noted that with both probes for hydroxyl radical production the microsomes from the respective tissues showed the same relative pattern as that observed for ethanol oxidation over the same time course, i.e. the activity of the liver microsomes was approximately 4- to 5-fold greater than that of lung and kidney microsomes. No differences were observed between lung and kidney microsomes with regard to oxidizing the ·OH scavengers, consistent with the similar rates of ethanol oxidation by microsomes from these two tissues.

Two pathways commonly considered to be sources of ·OH in biological systems are the ferrous iron catalyzed decomposition of hydrogen peroxide (H_2O_2) (Fenton Reaction) [19]:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\cdot}OH + OH^- \quad (1)$$

or the iron-catalyzed Haber–Weiss reaction [20, 21]:

$$Fe^{3+} - \text{chelate} + O_2^- \rightarrow Fe^{2+} - \text{chelate} + O_2 \quad (2)$$

$$Fe^{2+} - \text{chelate} + H_2O_2 \rightarrow Fe^{3+} - \text{chelate} + {}^{\cdot}OH + OH^- \quad (3)$$

With both pathways, H_2O_2 serves as a precursor of ·OH. Because microsomes are contaminated with catalase, the above experiments were carried out in the presence of azide. Under these conditions, H_2O_2 accumulates; consequently, azide should augment the metabolism of ·OH scavengers because of an increase in ·OH production via pathways 1 or 2 plus 3. To verify this, experiments were carried out in the absence and presence of azide. To verify that azide inhibited catalase, a titration curve of the ability of azide to inhibit H_2O_2-dependent (catalase-dependent) oxidation of ethanol was carried out. At azide concentrations of 0.1, 0.5 or 1.0 mM, liver catalase was inhibited 36, 54 and 82–95% respectively; lung catalase was inhibited by 41, 79 and 95% respectively; kidney catalase was inhibited by 58, 81 and 89% respectively. Therefore, experiments were conducted in the presence of 1.0 mM azide. The production of formaldehyde from Me_2SO and of ethylene from KTBA by all three microsomal preparations was augmented by the addition of azide to the assay mixture (Table 1). Thus, the data are consistent with the idea that H_2O_2 may be serving as an intermediate for ·OH production in each of the respective tissues.

Results with ethanol in the absence and presence of azide are more difficult to interpret. Ethanol, unlike Me_2SO or KTBA, serves as a substrate for the peroxidatic activity of catalase. Hence, azide should decrease one pathway of microsomal ethanol oxidation (catalase) while increasing another pathway (·OH). The net effect of azide would depend on rates of H_2O_2 production, content of catalase, content of iron (see below), and the substrate. These considerations have been discussed previously [1–3]. Under our conditions, the addition of azide resulted in 25, 39 and 53% inhibition of the microsomal ethanol-oxidizing system for liver, lung, and kidney respectively (Table 2). Again, the mean differences between the lung and kidney were not statistically significant, in either the presence or absence of azide.

As shown in equations (1)–(3), ·OH can be generated via a ferrous iron catalyzed decomposition of H_2O_2. It has been shown previously that the liver microsomal ethanol-oxidizing system was significantly stimulated by the addition of iron–ethylenediaminetetraacetic acid (Fe–EDTA) [22]. Table 2 shows that Fe–EDTA (in the presence of azide) also increases the microsomal ethanol-oxidizing system of lung and kidney over the range of 3.4 to 50.0 μM. The extent of increase of ethanol oxidation was the same with kidney and lung microsomes. Stimulation of ethanol oxidation by Fe–EDTA should be accompanied by an increase in formaldehyde and ethylene generation from Me_2SO and KTBA respectively. Table 2 shows that Fe–EDTA increased the oxidation of Me_2SO and KTBA in all three microsomal preparations. Thus, by merely introducing a ·OH potentiating component (50 μM Fe–EDTA) to the system it was observed that lung and kidney

Table 1. Effect of azide on the oxidation of ethanol, Me_2SO and KTBA by microsomes from liver, lung and kidney*

Tissue	Scavenger	Activity [nmoles·min^{-1}·(mg microsomal protein)$^{-1}$] −Azide	Activity [nmoles·min^{-1}·(mg microsomal protein)$^{-1}$] +Azide	Azide effect (%)
Liver	Ethanol	8.81 ± 0.40	6.50 ± 0.30†	−25
	Me_2SO	1.07 ± 0.08	2.70 ± 0.62‡	+152
	KTBA	0.55 ± 0.10	1.48 ± 0.09‡	+169
Lung	Ethanol	3.11 ± 0.13	1.92 ± 0.05†	−39
	Me_2SO	0.64 ± 0.17	1.15 ± 0.23†	+74
	KTBA	0.20 ± 0.01	0.36 ± 0.05†	+80
Kidney	Ethanol	3.42 ± 0.29	1.63 ± 0.16‡	−53
	Me_2SO	0.28 ± 0.05	0.81 ± 0.11‡	+187
	KTBA	0.22 ± 0.06	0.47 ± 0.10‡	+110

* Experiments were conducted in the absence or presence of 1.0 mM sodium azide. Activity is expressed as nmoles of product produced per min per mg protein. Each result is the mean ± S.E.M. of duplicate or triplicate experiments with three to four microsomal preparations. The concentrations of ethanol, Me_2SO and KTBA employed in all experiments were 51, 33 and 10 mM respectively.

† Significant azide effect ($P < 0.05$).

‡ Significant azide effect ($P < 0.01$).

microsomes could be induced to oxidize ethanol at rates that were approximately one-half or one-third, respectively, that of normal control liver microsomes that did not contain added iron.

Because ethanol oxidation as well as ·OH generation can occur during microsomal electron transport without the addition of exogenous iron-chelates, a possible role for a Fenton-type, or modified Fenton-type, reaction involving the iron porphyrin of cytochrome P-450 may be possible. Alternatively, cytochrome P-450 may play a crucial role in the generation of H_2O_2, the precursor of ·OH. Consequently, the relative capacity of microsomes from liver, lung and kidney to generate ·OH and thus oxidize ethanol may be a reflection of the cytochrome P-450 content and/or the NADPH–cytochrome P-450 reductase activity of these microsomal preparations.

Table 3 shows that liver microsomes had a 4-fold greater activity of NADPH-cytochrome P-450 reductase per mg of microsomal protein compared to lung and kidney microsomes. Liver microsomes had a 5.8- and 12.9-fold greater specific content of cytochrome P-450 than kidney and lung respectively (Table 3). Kidney microsomal preparations showed a 2.2-fold higher specific content of cytochrome P-450 with respect to lung. Thus, the observed differences in the rates of NADPH-dependent oxidation of ethanol, KTBA and Me_2SO might possibly be related to differences in either the activity of the reductase and/or the content of cytochrome P-450 of the respective tissues. When the rates of oxidation

Table 2. Effect of Fe–EDTA on the oxidation of ethanol, Me_2SO and KTBA by microsomes from liver, lung and kidney*

Tissue	Scavenger	Activity [nmoles·min^{-1}·(mg microsomal protein)$^{-1}$] Concentration of Fe–EDTA (μM) 0	3.4	8.5	17	50
Liver	Ethanol†	9.62 ± 0.60	14.57 ± 0.65	17.53 ± 0.74	20.07 ± 0.95	23.15 ± 0.96
	Me_2SO	2.70 ± 0.48	4.26 ± 0.63		7.50 ± 0.91	8.58 ± 0.68
	KTBA	1.48 ± 0.09	2.06 ± 0.06	2.99 ± 0.56	3.39 ± 0.26	6.96 ± 0.98
Lung	Ethanol	1.17 ± 0.21		2.88 ± 0.43	3.73 ± 0.14	4.54 ± 0.55
	Me_2SO	1.13 ± 0.21		2.63 ± 0.22	3.74 ± 0.33	5.46 ± 0.11
	KTBA	0.36 ± 0.02		1.36 ± 0.06	1.93 ± 0.19	3.11 ± 0.19
Kidney	Ethanol	0.92 ± 0.20	1.59 ± 0.15	2.14 ± 0.25	2.67 ± 0.29	3.23 ± 0.47
	Me_2SO	0.64 ± 0.07	1.17 ± 0.08	1.70 ± 0.08	2.03 ± 0.13	2.51 ± 0.11
	KTBA	0.41 ± 0.05	0.94 ± 0.02	1.28 ± 0.12	2.10 ± 0.02	2.56 ± 0.05

* Experiments were carried out in the presence of the indicated concentrations of Fe–EDTA. Activity refers to nmoles of acetaldehyde or formaldehyde or ethylene produced per min/mg protein. Each result is the mean ± S.E.M. of six to nine determinations with three microsomal preparations. The concentrations of ethanol, Me_2SO and KTBA were 51, 33 and 10 mM respectively.

† Results with liver microsomes and ethanol are from Ref. 22.

Table 3. Cytochrome P-450 content and NADPH–cytochrome *c* reductase activity in liver, lung and kidney microsomes*

Tissue	Cytochrome P-450 specific content (nmoles/mg microsomal protein)	NADPH–cytochrome *c* reductase activity [nmoles · min^{-1} · (mg microsomal protein)$^{-1}$]
Liver	0.93 ± 0.13	102.8 ± 3.5
Lung	0.07 ± 0.01†	26.1 ± 1.2†
Kidney	0.16 ± 0.02†‡	22.5 ± 2.7†

* Assay conditions were as described in Materials and Methods. Liver data represent mean ± S.E.M. of duplicate experiments with three microsomal preparations. Lung and kidney data represent mean ± S.E.M. of triplicate experiments with five microsomal preparations.

† Significant from liver ($P < 0.001$).

‡ Significant from lung ($P < 0.01$).

of ethanol, KTBA and Me_2SO were calculated on a per nmole of cytochrome P-450 basis (Table 4), some interesting patterns emerged. First, liver and kidney preparations showed identical rates of ethanol oxidation. These identical rates of ethanol oxidation correlated with similar rates of ·OH generation, as reflected by the oxidation of KTBA and Me_2SO (Table 4). However, the lung microsomes displayed approximately two times the rate of ethanol oxidation with respect to either liver or kidney microsomes. This augmented rate of ethanol oxidation was concomitant with augmented rates in ·OH generation of a similar magnitude, i.e. the oxidation of both probes by lung microsomes was approximately two to four times greater than by kidney and liver microsomes when expressed on a per nmole of cytochrome P-450 basis (Table 4).

DISCUSSION

The pathway of oxidation of primary aliphatic alcohols by liver microsomes to their corresponding aldehydes was suggested to involve, at least in part, the interaction of the alcohols with ·OH generated during NADPH-dependent electron transfer [1–4]. Therefore, it can be anticipated that a relationship between the ability of a microsomal fraction to oxidize ethanol and to oxidize other ·OH scavengers should exist. Experiments were carried out to confirm such a correlation. Results in Figs. 1 and 2 indicate that liver microsomes, which are more active in oxidizing ethanol than are kidney and lung microsomes, are also more active in oxidizing two scavengers of ·OH, KTBA and Me_2SO. The equivalent rate of oxidation of ethanol by kidney and lung microsomes is associated with equivalent rates of oxidation of KTBA and Me_2SO by these two microsomal preparations. Consequently, a correlation between the abilities to oxidize ethanol and to generate ·OH appears to exist. The comparable rates of oxidation of ·OH scavengers by kidney and lung microsomes can be contrasted to the 2-fold greater activity of aminopyrine demethylase by rat kidney microsomes, as compared to rat lung microsomes [23]. These results are consistent with previous data that the activities of aminopyrine demethylase and aniline hydroxylase can be dissociated from the production of ·OH [1].

A Fenton-type of reaction ($H_2O_2 + Fe^{2+} \rightarrow {}^{\cdot}OH + OH^- + Fe^{3+}$) may be a possible mechanism whereby ·OH is generated in biological systems. It has been suggested that H_2O_2 may be the precursor of ·OH generated by liver microsomes [1–3]. This notion was based partially on the observation that azide, a potent inhibitor of catalase, stimulated the oxidation of ·OH scavengers [1–4]. The present data suggest that H_2O_2 may be the precursor of ·OH generated by lung and kidney microsomes, since azide increases the oxidation of both KTBA and Me_2SO (Table 1). The ability of azide to inhibit ethanol oxidation (Table 1) reflects the ability of ethanol to act as a peroxidatic substrate with catalase.

Table 4. Turnover numbers calculated for the oxidation of ethanol, Me_2SO and KTBA by microsomes from liver, kidney and lung*

Tissue	Turnover number [nmoles · min^{-1} · (nmole cytochrome P-450)$^{-1}$]		
	Ethanol	Me_2SO	KTBA
Liver	7.74 ± 0.37	3.22 ± 0.36	1.90 ± 0.16
Kidney	6.63 ± 1.25	5.81 ± 1.25	2.19 ± 0.63
Lung	14.72 ± 2.86†‡	12.92 ± 2.90†‡	4.86 ± 1.42†‡

* Turnover numbers were calculated from the average specific activities found with microsomes (Figs. 1 and 2) divided by the content of cytochrome P-450 (Table 3). Liver values are means ± S.E.M. for six to nine determinations on three microsomal preparations. Lung and kidney values are means ± S.E.M. for ten to fifteen determinations on five microsomal preparations.

† Significant from liver ($P < 0.05$).

‡ Significant from kidney ($P < 0.05$).

When alcohols which are not substrates for the peroxidatic activity of catalase are used, e.g. 1-butanol, isopropanol and *tert*-butyl alcohol, azide produces an increase in the oxidation of these alcohols [1, 4, 24], which corresponds to the increase in ·OH generation by microsomes from liver, kidney, and lung. Previous results indicated that Fe–EDTA stimulated the oxidation of ethanol and 1-butanol by rat liver microsomes in a reaction which was sensitive to competing ·OH scavengers [22]. Ethanol oxidation by lung and kidney microsomes is also increased by Fe–EDTA (Table 2). Concomitant with this increase in microsomal ethanol oxidation by Fe–EDTA is an increase in the microsomal oxidation of KTBA and Me_2SO by Fe–EDTA (Table 2). In general, the ability of Fe–EDTA to increase ethanol oxidation parallels the increase by Fe–EDTA of ·OH production, e.g. with kidney microsomes, 8.5 μM Fe–EDTA increases the oxidation of ethanol, Me_2SO and KTBA by 133, 166 and 202% respectively.

The lower rates of ethanol oxidation by kidney and lung microsomes, as compared to liver microsomes, correlate with the lower rates of oxidation of other ·OH scavengers. To determine why kidney and lung microsomes display low rates of ·OH generation, the activity of NADPH–cytochrome *c* reductase and the content of cytochrome P-450 were assayed (Table 3). It is of interest that the differences in the specific content of cytochrome P-450, as well as the microsomal rate of NADPH–cytochrome *c* reductase of lung and kidney with respect to liver, are essentially the same as the differences observed for ·OH generation and ethanol oxidation. These results suggest that differences in the rate of ·OH generation between liver and kidney or lung microsomes may reflect differences in NADPH-dependent electron transfer. Microsomal electron transfer would be required to produce H_2O_2, the likely precursor of ·OH. The rate of oxidation of ethanol by catalase–H_2O_2 which occurs in the absence of azide can be used as a measure of H_2O_2 production by the microsomes. Results of Table 1 (−azide) indicate that ethanol is oxidized by liver microsomes at rates close to 3-fold higher than with kidney or lung microsomes. This suggests that liver microsomes generate H_2O_2 at rates 3-fold greater than kidney or lung microsomes. However, this must be an underestimate since the actual level of H_2O_2 reflects the rate of H_2O_2 production minus the rate of H_2O_2 utilization. Liver microsomes utilize H_2O_2 to generate ·OH to a greater extent than kidney or lung microsomes.

A second role for microsomal electron transfer would be to reduce ferric iron to the ferrous state, the required redox state for iron to participate in a Fenton-type of reaction. The nature of the iron catalyst is not known. In view of the Chelex treatment of all solutions, adventitious iron in the microsomes or catalysis by cytochrome P-450 in kidney and lung microsomes may contribute to the lower rate of ·OH production and, subsequently, of ethanol oxidation.

Many pulmonary mixed-function oxidations are apparently more active than their hepatic counterparts when reaction rates are considered on a P-450 basis (turnover number) [8]. The present data demonstrate that turnover numbers are also higher in lung for the microsomal oxidation of ethanol and ·OH production than in liver or kidney which are essentially the same. The higher turnover found with lung microsomes may possibly reflect the nature of the predominant species of cytochrome P-450 present in lung, as compared to liver and kidney. For example, in rabbits, markedly greater turnover numbers (with respect to cytochrome P-450) have been reported for NADPH–cytochrome P-450 reductase [25] and NADPH–cytochrome *c* reductase [25] in lung compared to liver. Either of these activities may be rate-limiting in terms of ethanol oxidation or ·OH generation. Chronic consumption of ethanol is known to result in the production of a species of cytochrome P-450 which has a greater turnover number with respect to ethanol oxidation than control cytochrome P-450 [26–28]. In view of the fact that lung microsomes displayed higher turnover numbers for ethanol oxidation than the other tissues, it may be of interest to consider further the use of the lung P-450 as a model for the chronic ethanol-induced P-450.

In summary, the current data clearly demonstrate that a correlation exists between the relative effectiveness of microsomes from various tissues to mediate NADPH-dependent ethanol oxidation and their concomitant capacity to generate ·OH. This correlation is consistent with the possibility that the microsomal ethanol-oxidizing system involves, at least in part, the interaction of ethanol with ·OH generated by the microsomes. The generation of ·OH by liver, lung, and kidney microsomes may reflect the relative specific activities of NADPH–cytochrome *c* reductase and/or the specific content of cytochrome P-450 of the respective tissues. Finally, with respect to turnover numbers, the pulmonary cytochrome P-450, which has been found to exhibit a high degree of specificity towards the oxidation of simple aromatics and secondary amines [8], also exhibits a high degree of specificity for the oxidation of ethanol (and possibly other primary aliphatic alcohols) as well as the ·OH radical scavenging agents used in this study.

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