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SPECIES AND STRAIN DIFFERENCES IN TARGET ORGAN ALKYLATION AND TOXICITY BY 4-IPOMEANOL

PREDICTIVE VALUE OF COVALENT BINDING IN STUDIES OF TARGET ORGAN TOXICITIES BY REACTIVE METABOLITES* †

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Abstract—The organ specificities of the *in vivo* covalent binding of 4-ipomeanol were closely correlated with the patterns of organ-specific damage by 4-ipomeanol in several different animal species and strains. In all species tested, the lung was a major target for 4-ipomeanol covalent binding and toxicity. In the hamster and the mouse, 4-ipomeanol caused liver necrosis and kidney necrosis, respectively, in addition to pulmonary damage. Correspondingly, high levels of covalent binding of 4-ipomeanol occurred in these target organs in these species. These *in vivo* results, in addition to studies of the *in vitro* covalent binding of 4-ipomeanol in microsome preparations from the various target tissues, were consistent with the view that the organ-specific toxicities of 4-ipomeanol were caused by a highly reactive 4-ipomeanol metabolite(s) primarily produced *in situ* in the respective target tissues. The present results suggest that studies of both the *in vivo* and the *in vitro* covalent binding of 4-ipomeanol may have some utility in predicting the target organ specificity of 4-ipomeanol toxicity in other species. The present investigations also have identified some relevant new *in vivo* toxicity models for future studies of the relationships between the metabolism and the toxicity of 4-ipomeanol.

4-Ipomeanol (1-[3-furyl]-4-hydroxypentanone) is a naturally occurring pulmonary toxin [1]. Previous investigations demonstrated that, when toxic doses of radiolabeled 4-ipomeanol were administered to rats by intraperitoneal injection, large amounts of radioactive material became covalently bound preferentially to the lungs [2, 3]. It also was shown that the covalent binding of 4-ipomeanol to livers and lungs of rats *in vivo*, and in incubation mixtures with rat liver or lung microsomes *in vitro*, was mediated by cytochrome P-450-dependent mixed-function oxidases present in these target tissues [3, 4]. Without prior metabolism, 4-ipomeanol is not sufficiently reactive to alkylate biological macromolecules. Therefore, the covalent binding of 4-ipomeanol has proven to be a valuable indicator of the *in vivo* formation and fate of a highly reactive metabolite(s) of the parent compound which appears to be responsible for causing lung damage in rats. The toxic metabolite(s) is so reactive that most of it becomes covalently bound to nucleophilic cellular constituents immediately in the vicinity of its formation [5].

Although a role for *in situ* metabolic activation in the pulmonary toxicity of 4-ipomeanol is established, the underlying basis for the organ-selective metabolism and toxicity of the compound remains to be fully elucidated. The selectivity does not appear to be related to preferential uptake or concentration of the parent compound in target tissue [3, 5], but instead most likely has a metabolic basis. Indeed, the target organ selectivity of the covalent binding and toxicity of 4-ipomeanol can be altered by changes in the metabolic activities of potential target tissues. For example, in rats pretreated with 3-methylcholanthrene, the major site of covalent binding and organ damage is shifted from the lung to the liver [3, 6].

The present investigation arose from the need to identify and characterize additional animal models useful for comparative studies on the metabolic basis of the organ-specific damage by 4-ipomeanol. We report here a study of species and strain differences in target organ alkylation and toxicity which reveals some striking species-related differences in organ-selective damage by 4-ipomeanol that are closely correlated with corresponding differences in the organ-specific covalent binding of the compound.

MATERIALS AND METHODS

Animals. New Zealand White rabbits (500–600 g) and Hartley guinea pigs (200–220 g) were obtained from the Dutchland Laboratories (Denver, PA). Mice (25–30 g) of the BALB/cJ, C3H/HeJ, DBA/2J, C57BL/6J, and A/J strains were obtained from The Jackson Laboratory (Bar Harbor, ME). Swiss–Webster mice (25–30 g), rats (200–250 g) of the Lewis, Sprague–Dawley and Fisher-344 strains, and

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Golden Syrian hamsters (65–70 g) were obtained from the National Institutes of Health (Bethesda, MD) animal stock. Male animals were used in all studies.

Chemicals. Unlabeled 4-ipomeanol, [5-^{14}C]-4-ipomeanol, and [^{3}H-G]-4-ipomeanol were prepared and purified as described previously [1, 5, 7]. Preliminary experiments indicated that similar results were obtained using either isotope. The products were verified to be radiochemically and chemically homogeneous prior to studies. Dose solutions for *in vivo* studies were prepared in 25% propylene glycol/water in concentrations such that the administration of a volume of 1 ml solution/100 g animal weight yielded the desired dose. Solutions of tritiated 4-ipomeanol for *in vitro* studies were prepared in absolute methanol. The specific activity of radiolabeled 4-ipomeanol was adjusted with unlabeled compound to 0.05 mCi/mmole for the *in vivo* studies (^{14}C-labeled compound), and 3 mCi/mmole for the *in vitro* studies (^{3}H-labeled compound).

Glucose-6-phosphate dehydrogenase, glucose-6-phosphate, nicotinamide adenine dinucleotide phosphate ($NADP^+$), and cytochrome *c* were obtained from the Sigma Chemical Co. (St. Louis, MO).

Toxicity studies. In all *in vivo* studies, 4-ipomeanol was administered by intraperitoneal injection. The LD_{50} (36 hr) determinations were conducted according to the procedures described by Balazs [8]; LD_{50} values (± S.E.) were calculated by the method of Litchfield and Wilcoxon [9]. Tissues for histological studies were removed immediately from animals killed (200 mg/kg of phenobarbital, i.p.) at the end of the observation period. Specimens were fixed in a 10% buffered formalin solution, dehydrated, and embedded in paraffin; section of 5 μm thickness were cut, mounted on glass slides, and stained with hematoxylin and eosin. In assessing pulmonary toxicity, no attempt was made to differentiate between damage to nonciliated and ciliated bronchiolar cells; paraffin-embedded sections generally do not provide sufficient resolution to permit this differentiation.

In vivo *covalent binding studies.* The amounts of covalently bound 4-ipomeanol metabolite(s) present in tissues after administration of the radiolabeled compound were assayed by procedures described elsewhere [3]. Tissues for these assays were removed from animals 4 hr after the administration of 4-ipomeanol; preliminary studies indicated that maximal levels of covalent binding were achieved within 1–2 hr, and remained relatively constant for several hours thereafter. Data are expressed as nmoles of covalently bound 4-ipomeanol metabolite(s) per mg of tissue protein; total tissue protein values were unchanged in the tissues studied within the 4-hr period after the administration of 4-ipomeanol.

In vitro *covalent binding studies.* With procedures described previously [4], microsomes were prepared from the various tissues, and incubations were carried out with radiolabeled 4-ipomeanol in the presence and absence of an NADPH-generating system (5 μmoles $NADP^+$, 15 μmoles glucose-6-phosphate, and 1 unit of glucose-6-phosphate dehydrogenase). The total incubation volume was 2.0 ml, and contained 2.0 mg of microsomal protein. Incubations (run in triplicate) were started by the addition of 4-ipomeanol (in 10 μl of methanol) and were terminated after 5 min by the addition of 5 ml of ice-cold methanol. The incubation concentrations of 4-ipomeanol used (1.0 mM for liver microsomes, and 0.5 mM for lung and kidney microsomes) were shown in preliminary studies to yield maximal rates of

Table 1. Lethality and target organ toxicity of 4-ipomeanol in various animal species and strains

Species	Strain	LD_{50} (mg/kg, i.p.)	Target organ toxicity*		
			Liver	Kidney	Lung
Rat	Lewis	42 ± 5	−	−	+
	Sprague–Dawley	24 ± 4	−	−	+
	Fisher-344	12 ± 3	−	−	+
Guinea pig	Hartley	30 ± 5	−	−	+
Rabbit	New Zealand White	40 ± 5	−	−	+
Hamster	Golden Syrian	140 ± 10	+	(−)†	+
Mouse	BALB/cJ	60 ± 5	−	+	+
	C3H/HeJ	50 ± 5	−	+	+
	NIH-Swiss	42 ± 3	−	+	+
	DBA/2J	25 ± 3	−	+	+
	C57BL/6J	25 ± 5	−	+	+
	A/J	20 ± 3	−	+	+

* Lung damage consisted primarily of bronchiolar necrosis, sometimes accompanied by variable amounts of pulmonary edema and pleural effusion. Centrilobular hepatic necrosis was observed only in hamsters receiving high doses of 4-ipomeanol (at or near the LD_{50} value); lower doses caused only pulmonary damage. Renal cortical necrosis was observed only in mice.

† Occasionally there appeared to be some damage in the corticomedullary zones of hamster kidneys after high doses of 4-ipomeanol; it is possible that these relatively infrequently occurring lesions are unrelated to the covalent binding of 4-ipomeanol; other studies [13] indicated that, in mice, cells of the outer and middle renal cortex were most active in mediating the covalent binding of the toxin.

NADPH-mediated covalent binding. The covalently bound 4-ipomeanol was assayed in the precipitates as described elsewhere [4].

Other assays. Microsomal cytochrome P-450 levels were measured by the method of Omura and Sato [10], and cytochrome b_5 as described by Omura and Tabusue [11]. NADPH-cytochrome *c* reductase was assayed according to the method of Williams and Kamin [12].

RESULTS

Toxicity of 4-ipomeanol in various animal species and strains. Table 1 summarizes the results of the toxicity studies. In all three rat strains studied, the lung was the primary target organ for 4-ipomeanol toxicity. The lethality of the toxin was greatest in Fisher rats, lowest in Lewis rats, and intermediate in Sprague–Dawley rats. Likewise, the lung was the major site of damage by 4-ipomeanol in rabbits and guinea pigs, and the LD_{50} values in these species were similar to those of rats. In contrast, both the liver and the lungs of hamsters were damaged by 4-ipomeanol. Moreover, the hamster was the most resistant to 4-ipomeanol lethality when compared to all other species studied. The mouse was the only species studied in which lung damage consistently was accompanied by striking renal toxicity. All six mouse strains studied showed renal cortical necrosis, in addition to pulmonary bronchiolar necrosis. With respect to lethality, the mouse strains appeared to be separable into two distinct groups; the DBA/2J, C57BL/6J and the A/J strains were the most sensitive to 4-ipomeanol, while the BALB/cJ, Swiss and C3H/HeJ strains were the most resistant.

In vivo *covalent binding of 4-ipomeanol: Target organ specificity.* Figure 1 shows results of a study of the *in vivo* covalent binding of 4-ipomeanol in which a single dose (20 mg/kg) of the radiolabeled compound was administered (i.p.) to various animal species and strains. In rabbits, guinea pigs, and three strains of rats, the target organ selectivity of the covalent binding was similar. The highest relative levels of binding were found in the lungs, while the lowest levels were seen in the kidneys; an intermediate level of binding was found in the livers. In contrast, in six different strains of mice, very high levels of binding were seen in the kidneys, intermediate levels were found in the lungs, and the

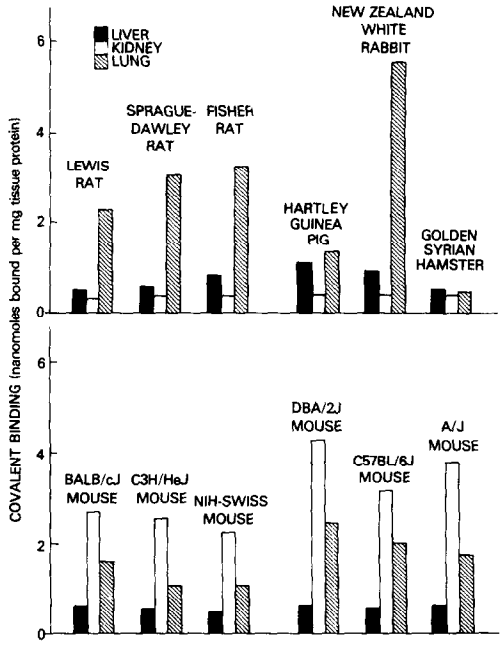

Fig. 1. Organ-specificity of covalent binding of 4-ipomeanol in various animal species and strains. All animals received a 20 mg/kg dose of [5-^{14}C]-4-ipomeanol, intraperitoneally, 4 hr prior to the assays. Values plotted are the means of determinations on at least three animals each; standard errors (not shown) averaged less than 6 per cent of the respective mean values.

Table 2. NADPH-cytochrome *c* reductase activities, cytochromes P-450 and b_5, and NADPH-dependent covalent binding of [^{3}H-G]-4-ipomeanol in microsomes of livers, kidneys and lungs of various species*

Species	Tissue	NADPH-cytochrome *c* Reductase (nmoles/min/mg)	Cytochrome b_5 (nmoles/mg)	Cytochrome P-450 (nmoles/mg)	Covalent binding of 4-ipomeanol (nmoles/mg/5 min)
Rat	Liver	132.3 ± 15.6	0.56 ± 0.02	0.88 ± 0.01	2.50 ± 0.31
(Sprague–Dawley)	Kidney	32.8 ± 0.4	0.14 ± 0.01	0.28 ± 0.02	0.00 ± 0.03
	Lung	53.5 ± 14.3	0.07 ± 0.00	0.06 ± 0.00	3.36 ± 0.32
Guinea pig	Liver	144.3 ± 9.4	0.68 ± 0.03	1.08 ± 0.08	6.10 ± 0.51
(Hartley)	Kidney	32.0 ± 1.5	0.21 ± 0.01	0.22 ± 0.04	0.23 ± 0.15
	Lung	65.2 ± 3.6	0.12 ± 0.01	0.19 ± 0.01	7.38 ± 1.11
Rabbit	Liver	151.0 ± 11.6	1.04 ± 0.04	1.81 ± 0.16	4.04 ± 0.65
(New Zealand	Kidney	25.3 ± 2.3	0.26 ± 0.01	0.25 ± 0.03	0.07 ± 0.04
White)	Lung	88.3 ± 13.5	0.13 ± 0.10	0.33 ± 0.02	12.42 ± 1.49
Hamster	Liver	153.3 ± 4.4	0.15 ± 0.12	1.35 ± 0.10	5.09 ± 0.68
(Golden Syrian)	Kidney	38.0 ± 1.4	0.20 ± 0.00	0.32 ± 0.01	0.34 ± 0.10
	Lung	71.7 ± 5.4	0.10 ± 0.00	0.13 ± 0.02	1.59 ± 0.20
Mouse	Liver	120.0 ± 6.4	0.44 ± 0.04	1.07 ± 0.08	3.08 ± 0.39
(Swiss)	Kidney	74.5 ± 7.3	0.27 ± 0.04	0.37 ± 0.10	4.85 ± 0.61
	Lung	101.4 ± 11.5	0.15 ± 0.01	0.14 ± 0.02	2.91 ± 0.50

* Values shown are means ±S.E. of determinations for three replications of the experiment.

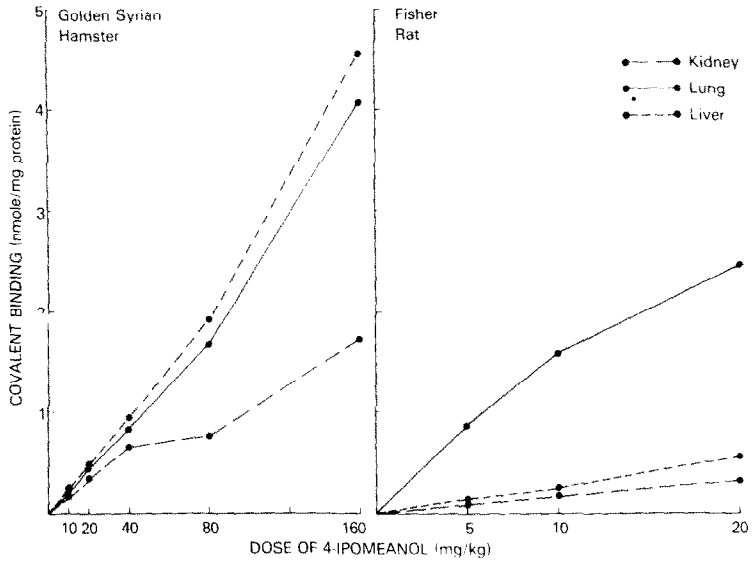

Fig. 2. Dose-dependence of covalent binding of 4-ipomeanol in tissues of hamsters and rats. Values plotted are means of determinations on at least three animals per point; standard errors (not shown) averaged less than 6 per cent of the respective mean values.

lowest binding was measured in the livers. In the hamster, the levels of covalent binding in the liver equaled or exceeded those measured in the lungs and kidneys.

Other studies indicated that these characteristic patterns of organ selective covalent binding of 4-ipomeanol were not dependent upon the dose of the compound. For example, Fig. 2 shows that the relative levels of covalent binding of 4-ipomeanol in the livers, kidneys and lungs of hamsters and rats were similar over a wide range of doses (up to and including lethal doses). Figure 3 similarly illustrates this dose-independent consistency in the organ-selective covalent binding of 4-ipomeanol in the six different strains of mice studied.

In vitro *covalent binding of 4-ipomeanol.* Table 2 shows the levels of cytochromes P-450 and b_5, NADPH-cytochrome *c* reductase, and NADPH-dependent covalent binding of 4-ipomeanol in the various microsome preparations studied. All of the lung and liver preparations were active in mediating the covalent binding of 4-ipomeanol. Mouse kidney microsomes gave high levels of NADPH-mediated covalent binding of 4-ipomeanol; only very small amounts of binding were seen with kidney microsomes of other species.

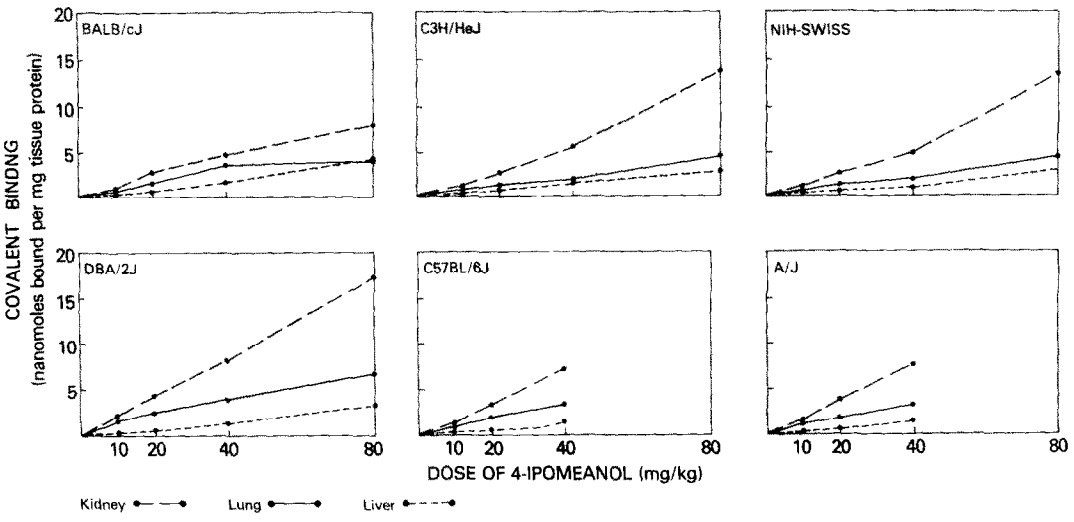

Fig. 3. Dose-dependence of covalent binding of 4-ipomeanol in tissues of different strains of mice. Values plotted are means of determinations on at least three animals per point; standard errors (not shown) averaged less than 6 per cent of the respective mean values.

DISCUSSION

These studies demonstrate strong correlations between the organ specificities of the *in vivo* covalent binding and toxicity of 4-ipomeanol, and the results are consistent with the view that the tissue-specific toxicities of the compound are caused by a highly reactive metabolite(s) produced *in situ* within the respective target organs. In rats, rabbits and guinea pigs, the covalent binding of 4-ipomeanol occurred preferentially in the lungs, and the lungs were the major site of damage produced by the compound. In hamsters, the administration of toxic doses of 4-ipomeanol resulted in relatively high levels of covalently bound 4-ipomeanol metabolite(s) in both the lungs and the livers; median lethal doses of 4-ipomeanol frequently caused both hepatic and pulmonary necrosis in hamsters. Similarly, in mice, relatively high levels of covalent binding of 4-ipomeanol occurred in both the kidneys and the lungs, and median lethal doses of 4-ipomeanol caused necrosis in both of these organs.

Whereas these results illustrate some striking interspecies differences in the patterns of target organ alkylation and toxicity of 4-ipomeanol, the intraspecies organ specificities seem to be highly consistent. For example, although there were differences in the actual amounts of covalently bound material in the tissues of different strains of rats or mice, the relative amounts of material covalently bound in the lungs, livers and kidneys showed a highly characteristic pattern among the different strains of the individual species.

These studies suggest that the readily determined index of *in vivo* covalent binding may have some utility as a predictor of the organ specificity of 4-ipomeanol toxicity in other species. Moreover, the finding that the patterns of tissue alkylation were consistent over a wide range of doses indicates that a single-dose study of the *in vivo* covalent binding of 4-ipomeanol may be sufficient to suggest its potential target organ toxicity, as long as the dose studied is at or near a toxic level. It should be emphasized that the simple assay used to measure covalently bound 4-ipomeanol in these studies does not indicate whether the bound material is highly localized within target structures or cells within a particular tissue. Thus, autoradiographic studies may be an important adjunct, particularly with potential target organs of relatively heterogeneous cellular composition such as the lung and the kidney. For example, autoradiography has shown that the covalently bound 4-ipomeanol in lungs of rats, mice and hamsters was located primarily in the nonciliated bronchiolar epithelium, a major site of 4-ipomeanol-induced damage in the lungs of these species [5]. Similarly, the covalently bound 4-ipomeanol in kidneys of mice was located in the renal cortical tubules, and these structures were the major site of damage by 4-ipomeanol in the mouse [13].

Especially among different strains of a given species, the actual magnitude of the covalent binding in target organs appears also to have some predictive value in terms of the acute lethality of 4-ipomeanol and/or its potency in causing tissue damage. For example, the Lewis rat was the most resistant rat strain studied, and the *in vivo* covalent binding values obtained with the Lewis rat lungs (Fig. 1) were significantly lower ($P < 0.01$) than for either the Sprague–Dawley rat or the Fisher rat. Similarly, as a group, the DBA/2J, C57BL/6J and the A/J mouse strains were the least resistant to 4-ipomeanol, and as a group, the *in vivo* covalent binding values for the lungs and kidneys were significantly higher ($P < 0.01$) than for the more resistant mouse strains.

The correlation between covalent binding and the potency of 4-ipomeanol is less consistent in interspecies comparisons, but in extreme cases a correlation does seem apparent. For example, compared to the other species tested, the hamster was highly resistant to 4-ipomeanol, and in single-dose studies (e.g. Fig. 1), the covalent binding values obtained for hamster lungs invariably were very low in relation to the lungs of other species. On the other hand, the highest *in vivo* covalent binding values were obtained for rabbit lungs (e.g. Fig. 1), but the potency of 4-ipomeanol in this species was similar to that of several of the other species tested. 4-Ipomeanol also seemed roughly equipotent in guinea pigs when compared to several other species, although the covalent binding values obtained in guinea pig lungs were considerably less than those for all of the other species tested except the hamster.

As shown in Table 2, *in vitro* measurements of cytochrome P-450, cytochrome b_5, or NADPH-cytochrome *c* reductase did not reveal any consistent correlations with either the covalent binding patterns observed *in vivo*, or with the ability of the various microsome preparations to mediate the covalent binding of 4-ipomeanol *in vitro*. However, previous experiments, such as those showing strong inhibitory effects by carbon monoxide, clearly indicated the participation of a cytochrome P-450 in the NADPH-dependent covalent binding of 4-ipomeanol *in vitro* in rat [4] and mouse [13] liver and lung microsome preparations, and in mouse kidney microsomes [13]. However, multiple forms of "cytochrome P-450" are known to be present in tissues including the liver [14] and the lung [15], and several of the forms show different substrate specificities in catalyzing mixed-function oxidations. It is probable that the relative amounts of different types of cytochrome P-450 may vary considerably, not only from tissue to tissue, but also among animal species. The method for assay of cytochrome P-450 used in the present study provided only an estimate of the total cytochrome P-450 pool. However, it seems likely that only certain forms of this hemoprotein class are active in mediating the metabolism of 4-ipomeanol. Moreover, evidence was presented recently that cytochrome b_5 also played an important role in the metabolic activation of 4-ipomeanol in rat lung microsomes, probably by facilitating the transfer of reducing equivalents to cytochrome P-450 [16]. The essential role of NADPH-cytochrome *c* (P-450) reductase in the cytochrome P-450-mediated covalent binding of 4-ipomeanol in rat liver and lung microsomes likewise has been demonstrated; an antibody prepared against purified NADPH-cytochrome *c* reductase inhibited the NADPH-dependent reduction of cytochrome P-450 and the NADPH-dependent covalent

binding of 4-ipomeanol in microsomal incubations [16].

Whereas the determinations of the microsomal cytochromes P-450, cytochrome b_5, or the NADPH-cytochrome *c* reductase activities appeared to offer no obvious or potential predictive utility as to the organ-specific metabolism and covalent binding of 4-ipomeanol *in vivo*, the determinations of the *in vitro* covalent binding of the 4-ipomeanol in the various preparations did show some interesting correlations with the *in vivo* covalent binding data. For example, in microsome preparations from all species except the hamster, the *in vitro* covalent binding values obtained for the lung preparations consistently were equal to or greater than the values obtained with the corresponding liver preparations. This seems remarkable in light of the fact that the lung cytochrome P-450 values invariably were much lower (by 80–93 per cent) than the liver cytochrome P-450 values. The hamster was the only species for which the *in vitro* covalent binding values obtained with the liver microsomes far exceeded those obtained with the corresponding lung microsomes. Interestingly, the hamster was relatively resistant to 4-ipomeanol toxicity, but sufficiently large doses of the compound yielded high *in vivo* covalent binding values in both the liver and the lung, and the hamster was the only species in which 4-ipomeanol caused liver necrosis in addition to pulmonary injury. Similarly, mouse kidney microsomes were highly active in mediating the *in vitro* covalent binding of 4-ipomeanol. In contrast, kidney microsomes from all other species tested either were almost completely inactive or gave very low covalent binding values. This was of particular interest because the mouse was the only species tested in which 4-ipomeanol consistently caused renal cortical necrosis in addition to pulmonary bronchiolar necrosis.

Thus, in several respects, the *in vitro* covalent binding studies show consistencies with the *in vivo* covalent binding and toxicity studies, and, therefore, seem to be of some potential value in predicting target organ susceptibilities to 4-ipomeanol toxicity. However, this possibility needs further investigation. It also should be emphasized that the present *in vitro* covalent binding studies were carried out with saturating concentrations of 4-ipomeanol. Perhaps a complete kinetic analysis of the *in vitro* covalent binding reactions in the various tissue preparations would be of additional value in further assessing the potential utility of *in vitro* measurements in predicting the *in vivo* target organ specificity of 4-ipomeanol. One such study has been reported for rat lung and liver microsomes [4]. The K_m value for the covalent binding of 4-ipomeanol in lung microsomes was more than 10-fold lower than the corresponding value for liver microsomes, thus raising the question that the much lower K_m for the covalent binding reaction in lung microsomes may be an important determinant of the remarkable pulmonary specificity of the *in vivo* covalent binding and toxicity of 4-ipomeanol in rats [3, 4].

Finally, a major purpose of these studies was to identify new animal models useful for the further study of metabolic and dispositional factors underlying the target organ specificity of 4-ipomeanol. This goal seems to have been accomplished, as illustrated by the striking differences (as well as similarities) among the responses of the various species and strains to 4-ipomeanol. While the pulmonary toxicity of 4-ipomeanol in the various species certainly is of continuing interest, the renal toxicity of 4-ipomeanol in the mouse seems to offer an intriguing new toxicity model worthy of further investigation. Kidney microsomes of the other species tested appeared to contain the essential components for mixed-function oxidations, yet none except those of the mouse had substantial activity for mediating the covalent binding of 4-ipomeanol. It is important to note that adult male mice were used in these studies. More recent studies in our laboratory [17, 18] indicate that immature male and adult female mice are highly resistant to the renal toxicity of 4-ipomeanol, and there is very little covalent binding of 4-ipomeanol in the kidneys of these mice compared to adult male mice. Further investigations of the renal metabolism of 4-ipomeanol in various species, as well as from resistant mice and susceptible mice, may contribute to the further elucidation of the biochemical mechanisms of 4-ipomeanol activation and toxicity.

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REFERENCES

1. M. R. Boyd, L. T. Burka, T. M. Harris and B. J. Wilson, *Biochim. biophys. Acta* **337**, 184 (1974).
2. M. R. Boyd, L. T. Burka and B. J. Wilson, *Toxic. appl. Pharmac.* **32**, 147 (1975).
3. M. R. Boyd and L. T. Burka, *J. Pharmac. exp. Ther.* **207**, 687 (1978).
4. M. R. Boyd, L. T. Burka, B. J. Wilson and H. A. Sasame, *J. Pharmac. exp. Ther.* **207**, 677 (1978).
5. M. R. Boyd, *Nature, Lond.* **269**, 713 (1977).
6. M. R. Boyd, *Toxic. appl. Pharmac.* **33**, 132 (1975).
7. M. R. Boyd, T. M. Harris and B. J. Wilson, *Nature, New Biol.* **236**, 158 (1972).
8. T. Balazs, in *Methods in Toxicology* (Ed. G. E. Paget), p. 49. Blackwell, Oxford (1970).
9. J. T. Litchfield and F. Wilcoxon, *J. Pharmac. exp. Ther.* **96**, 99 (1949).
10. T. Omura and R. Sato, *J. biol. Chem.* **239**, 2370 (1964).
11. T. Omura and S. J. Tabusue, *J. Biochem., Tokyo* **67**, 249 (1970).
12. C. A. Williams and H. Kamin, *J. biol. Chem.* **237**, 587 (1962).
13. M. R. Boyd and J. S. Dutcher, *Toxic. appl. Pharmac.* **45**, 229 (1978).
14. D. A. Hangen, T. A. van der Hoeven and M. J. Coon, *J. biol. Chem.* **250**, 3567 (1975).
15. C. R. Wolf, M. M. Szułowski, L. M. Ball and R. M. Philpot, *Chem. Biol. Interacts*, **21**, 29 (1978).
16. H. A. Sasame, J. R. Gillette and M. R. Boyd, *Biochem. biophys. Res. Commun.* **84**, 389 (1978).
17. R. B. Jones, C. Allegra and M. R. Boyd, *Toxic. appl. Pharmac.* **48**, A129 (1979).
18. M. R. Boyd and N. S. Longo, *Toxicol. appl. Pharmac.* in press.