Toxicology, 5 (1975) 69—77

POTENTIATION OF CARBON TETRACHLORIDE HEPATOTOXICITY IN RATS BY PRETREATMENT WITH POLYCHLORINATED BIPHENYLS

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(Received March 16th, 1975)
(Accepted May 6th, 1975)

SUMMARY

Pretreatment of male rats with Aroclor 1254 at a dose of 25 mg/kg i.p. for 6 days resulted in potentiation of the hepatotoxicity of inhaled carbon tetrachloride (CCl_4) as evidenced by a decrease in liver glucose-6-phosphatase and elevations of serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), isocitrate dehydrogenase, and sorbitol dehydrogenase. Aroclor 1254 alone did not demonstrate hepatotoxicity. Aroclor 1254 administration resulted in large increases in cytochrome *c* reductase, cytochrome P-450 (448) and *p*-nitroanisole demethylation. Subsequent exposure to CCl_4 vapor resulted in over 70% decreases in the latter two parameters. The potentiation was dose-dependent with a dose of 5 mg/kg or higher being effective. Aroclor 1260 administration gave results similar to those of Aroclor 1254, but Aroclor 1221 enhanced CCl_4 toxicity to a lesser extent.

INTRODUCTION

Since McLean and McLean [1] first demonstrated that the enzyme-inducing agent DDT was capable of potentiating the toxicity of CCl_4, many investigators have found this to be true of other enzyme-inducing agents such as phenobarbital [2—4]. In addition to phenobarbital and DDT, other investigators have looked at the influence of the polycyclic hydrocarbon type inducers which differ in the microsomal carbon monoxide binding pigment induced, i.e. P-448 rather than P-450, and in the spectrum of enzymes induced [5] Pitchumoni et al. [6] demonstrated that benzo[a]pyrene was similar to phenobarbital in enhancing the toxicity of CCl_4. However, Suarez

Abbreviations: DDT, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane; PCB, polychlorinated biphenyl; SGOT, serum glutamic oxalacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

et al. [3] demonstrated that 3-methylcholanthrene protected against the hepatotoxicity of CCl_4. It thus appears that enhancement versus protection is not simply a matter of cytochrome P-450 versus P-448.

Recently Alvares and coworkers [7] have shown that the polychlorinated biphenyls are unique as enzyme-inducing agents in that they induce the formation of cytochrome P-448 as does 3-methylcholanthrene, but they resemble phenobarbital in that they bring about a more general type of induction. In view of this uniqueness, it was of interest to ascertain what effects these compounds have on CCl_4 toxicity.

Although the PCB's as commercially used both in the past and in the present are complex mixtures, they are numbered according to the percent chlorine by weight, i.e., Aroclor 1254 contains 54% chlorine. Since the chlorine content appears to have an influence on the inducing potential of the PCB's [8,9], it was also important to determine if percent chlorine would be a factor in alteration of CCl_4 toxicity.

METHODS

Adult male albino rats (Charles River Breeding Laboratories) were used. They were allowed food and water ad lib. and were housed in temperature- and light-controlled rooms. They were injected i.p. with Aroclor 1254 dissolved in corn oil at a dose of 25 mg/kg unless otherwise indicated. Controls received corn oil alone. Injections were made daily for 6 days and 24 h after the last dose the rats were exposed to CCl_4 vapor in a dynamic inhalation chamber [10] for a period of 2 h. Chamber concentrations were determined using a Packard gas chromatograph with a flame ionization detector. 22 h after exposure the rats were lightly anesthetized with ether, liver and tail vein blood samples taken and various parameters of hepatotoxicity were assessed.

Liver and body weights were recorded. Liver glucose-6-phosphatase was measured using the procedure of Harper [11] with maleate buffer, pH 6.25. *p*-Nitroanisole demethylation was determined according to the method of Netter and Seidel [12] as modified by Kinoshita et al. [13]. SGOT and SGPT transaminases were measured utilizing the method of Reitman and Frankel [14]. Isocitrate dehydrogenase was determined according to the procedure of Ellis and Goldberg [15] and sorbitol dehydrogenase by the method of Gerlach [16].

In the microsomal cytochrome experiments, the livers were perfused with cold isotonic KCl, removed, and homogenized in cold isotonic KCl. The homogenate was centrifuged at 9000 *g* for 20 min in a Sorvall Model RC2B refrigerated centrifuge and the supernatant further centrifuged at 105 000 *g* for 1 h in an International Model B-60 Ultracentrifuge. Cytochrome *c* reductase activity and cytochrome P-450 content in the resulting microsomal fraction were measured according to the methods of Dallner [17]. Protein concentrations of the microsomes were determined by the method of Lowry et al. [18].

The value expressed is the mean ± standard error. Student's t test was used to compare means at a chosen level of significance of $P = 0.05$.

RESULTS

The effects of exposing the rats to 3600 ppm of CCl_4 for 2 h on 3 parameters are presented in Table I. Pretreatment with Aroclor 1254 resulted in an increase in the liver to body weight ratio. This increase was still greater in those animals exposed to CCl_4, although CCl_4 exposure by itself did not alter the ratio. At this level of exposure there was a small decrease in liver glucose-6-phosphatase activity due to the CCl_4 alone. However, in those animals pretreated with Aroclor 1254 there was a large decrease in activity to one-third of the air-exposed level. As a further indication of damage to the functioning of the liver, drug metabolism was measured since this is known to be decreased by CCl_4 and the effect potentiated by phenobarbital but protected against by 3-methylcholanthrene [4]. As indicated in Table I, a decrease in activity was seen with CCl_4 inhalation. Aroclor 1254 administration resulted in an 8-fold increase in activity which decreased dramatically following CCl_4 inhalation.

To further study the potentiation by Aroclor 1254 on the decrease in microsomal enzyme activity due to CCl_4, animals were exposed to 4200 ppm of CCl_4 and cytochrome c reductase activity and cytochrome P-450 content were measured. As indicated in Table II, cytochrome c reductase activity was elevated 2-fold by Aroclor 1254. In neither the controls nor the Aroclor-pretreated rats was the activity altered by CCl_4. In the case of the P-450 (P-448) content, Aroclor 1254 pretreatment resulted in a 3-fold increase in this cytochrome. CCl_4 inhalation resulted in a decrease in both the

TABLE I

EFFECT OF AROCLOR 1254 AND CCl_4 ON LIVER WEIGHT/BODY WEIGHT, GLUCOSE-6-PHOSPHATASE, AND p-NITROANISOLE DEMETHYLATION

Treatment	N[a]	$\frac{\text{Liver wt.}}{\text{Body wt.}} \times 100$	Glucose-6-phosphatase[b]	p-Nitroanisole demethylation[c]
Corn oil—Air	5	4.28 ± 0.07	12.0 ± 0.66	5.1 ± 0.39
Corn oil—CCl_4[d]	5	4.02 ± 0.15	10.2 ± 0.58[f]	2.9 ± 0.17[f]
Aroclor 1254[e]—air	5	4.96 ± 0.26[g]	10.2 ± 0.34	43.1 ± 0.42[g]
Aroclor 1254—CCl_4	5	5.91 ± 0.13[f,g]	3.6 ± 0.37[f,g]	12.0 ± 1.57[f,g]

[a] Number of rats
[b] μmoles PO_4/g/min.
[c] μg/50 mg/30 min.
[d] 3600 ppm for 2 h.
[e] 25 mg/kg i.p. for 6 days.
[f] Significantly different ($P < 0.05$) from group receiving same pretreatment.
[g] Significantly different ($P < 0.05$) from group receiving same exposure.

TABLE II

EFFECT OF AROCLOR 1254 AND CCl_4 ON SERUM TRANSAMINASES AND MICROSOMAL CYTOCHROME *c* REDUCTASE AND CYTOCHROME P-450

Treatment	N[a]	Cytochrome *c* reductase[b]	Cytochrome P-450[c]	SGPT[d]	SGOT[d]
Corn oil—air	5	76 ± 4.9	161 ± 13.3	13 ± 2.3[g]	40 ± 5.6[g]
Corn oil—CCl_4[e]	5	78 ± 8.1	95 ± 18.7[h]	338 ± 43.1[g,h]	446 ± 140.2[h]
Aroclor 1254[f]—air	5	144 ± 11.4[i]	522 ± 37.3[i]	11 ± 1.4	33 ± 2.3
Aroclor 1254—CCl_4	5	132 ± 7.2[i]	63 ± 20.2[h]	2213 ± 193.4[g,h,i]	2316 ± 297.7[h,i]

[a] Number of rats.
[b] nmoles of cytochrome *c* reduced/mg protein/min.
[c] Difference in absorbance between 450 and 500 nm/mg protein × 10^4.
[d] Reitman-Frankel units
[e] 4200 ppm for 2 h.
[f] 25 mg/kg i.p. for 6 days.
[g] 4 animals in this group.
[h] Significantly different ($P < 0.05$) from group receiving same pretreatment.
[i] Significantly different ($P < 0.05$) from group receiving same exposure.

controls and in the Aroclor-pretreated groups although the magnitude of the change in the latter case (88%) was much greater than that seen in the controls (41%).

Serum enzyme measurements were utilized as additional indicators of increased CCl_4 toxicity. As indicated in Table II, CCl_4 exposure increased SGPT and SGOT 26-fold and 11-fold, respectively. However, in the Aroclor-pretreated rats these transaminases were elevated 200-fold and 70-fold, respectively. Aroclor 1254 itself was without effect.

To test the influence of the chlorine content on hepatotoxicity, Aroclor 1254 was compared with Aroclor 1221 and Aroclor 1260 in separate experiments. Two other serum enzymes, sorbitol dehydrogenase and isocitrate dehydrogenase were used in hopes of increasing the sensitivity. Preliminary experiments indicated no alteration of these enzymes due to Aroclor 1254 alone. Therefore, Aroclor-pretreated rats exposed to air were not included in these studies. As indicated in Table III, exposure to 2900 ppm of CCl_4 for 2 h did not result in a significant change in glucose-6-phosphatase activity in the corn oil-pretreated rats. Activity was decreased by 35% in the Aroclor 1221-pretreated animals. In the animals pretreated with Aroclor 1254 the decrease in the enzyme activity due to CCl_4 was 70%. In the case of the sorbitol dehydrogenase, no activity was seen in the air controls, but, mea-

TABLE III

COMPARISON OF EFFECTS OF AROCLORS 1221, 1254 AND 1260 ON CCl_4 HEPATOTOXICITY

Treatment	N[a]	Glucose-6-phosphatase[b]	Sorbitol dehydrogenase[c]	Isocitrate dehydrogenase[d]
Corn oil—air	5	15.9 ± 0.75	0	0
Corn oil—CCl_4[e]	5	13.5 ± 0.90	55 ± 22.3[i]	9 ± 0.7[h,i]
1221[f]—CCl_4[e]	5	10.4 ± 0.78[i,j]	62 ± 10.7[i]	15 ± 1.6[i,j]
1254[f]—CCl_4[e]	5	4.7 ± 0.40[i,j,k]	495 ± 85.5[i,j,k]	1324 ± 465.1[i,j,k]
Corn oil—air	5	19.4 ± 0.91	0	0
Corn oil—CCl_4[g]	5	15.0 ± 0.83[i]	40 ± 1.1[i]	13 ± 1.0[i]
1254[f]—CCl_4[g]	5	7.1 ± 0.99[i,j]	309 ± 36.3[i,j]	570 ± 220.3[i,j]
1260[f]—CCl_4[g]	5	5.7 ± 0.90[i,j]	424 ± 66.7[i,j]	1110 ± 349.5[i,j]

[a] Number of rats.
[b] μmoles PO_4/g/min.
[c] $\Delta E^{60\ sec}_{366\ nm} \times 10^3$/0.2 ml serum.
[d] IU/l.
[e] 2900 ppm for 2 h.
[f] 25 mg/kg for 6 days.
[g] 2900 ppm for 2 h.
[h] 4 rats in this group.
[i] Significantly different ($P < 0.05$) from corn oil—air group.
[j] Significantly different ($P < 0.05$) from corn oil—CCl_4 group.
[k] Significantly different ($P < 0.05$) from other Aroclor pretreated group.

surcable levels were found after CCl_4 inhalation. These were not significantly altered by Aroclor 1221 pretreatment but were elevated 9-fold by Aroclor 1254 pretreatment. Similar findings were seen with the isocitrate dehydrogenase. Only slight activity was seen with CCl_4 exposure in the corn oil controls. This was increased to a small degree (66%) in the Aroclor 1221 pretreated rats but much elevated in the Aroclor 1254-pretreated animals (147-fold).

Aroclor 1260 pretreatment was very similar to Aroclor 1254 (Table III). In none of the three parameters was there a statistical difference between the two Aroclors although they both showed remarkable potentiation of the changes due to CCl_4.

Because of the impressive potentiating ability of Aroclor 1254 of the hepatotoxicity of CCl_4, it was of interest to determine if even lower doses of the Aroclor would enhance CCl_4 toxicity. Therefore, groups of rats were injected with 5, 10 or 25 mg/kg of Aroclor i.p. for 6 days. The results as presented in Table IV indicate that even at the dose of 5 mg/kg Aroclor 1254 potentiated the toxicity of CCl_4 measured in terms of alterations in liver glucose-6-phosphatase and the serum enzymes sorbitol dehydrogenase and isocitrate dehydrogenase although in the case of the latter enzyme, the large standard errors prevented the nearly 8-fold potentiation from being statistically significant. It should also be noted that the potentiating ability appeared to be dose-related.

Of further interest in view of the ability of Aroclor 1254 to potentiate CCl_4 hepatotoxicity was determining if it would also potentiate the toxicity

TABLE IV

DOSE RESPONSE OF AROCLOR 1254 ON HEPATOTOXICITY

Treatment	N[a]	Glucose-6-phosphatase[b]	Sorbitol dehydrogenase[c]	Isocitrate dehydrogenase[d]
Corn oil—air	8	18.6 ± 0.61	1 ± 0.7	0
Corn oil—CCl_4[e]	8	13.2 ± 0.47[g]	46 ± 9.6[g]	12 ± 3.1[g]
Aroclor 1254[f]—CCl_4				
5 mg/kg	8	9.6 ± 0.51[g,h]	132 ± 27.0[g,h]	91 ± 37.1[g]
10 mg/kg	8	8.3 ± 0.41[g,h]	246 ± 53.7[g,h]	319 ± 140.0[g,h]
25 mg/kg	8	5.8 ± 0.16[g,h,i,j]	340 ± 40.8[g,h,i]	488 ± 119.7[g,h,i]

[a] Number of rats.
[b] μmoles PO_4/g/min.
[c] $\Delta E^{60\ sec}_{366\ nm} \times 10^3/0.2$ ml serum.
[d] IU/l.
[e] 4200 ppm for 2 h.
[f] i.p. daily for 6 days.
[g] Significantly different ($P < 0.05$) from corn oil—air.
[h] Significantly different ($P < 0.05$) from corn oil—CCl_4.
[i] Significantly different ($P < 0.05$) from Aroclor 1254—5 mg/kg.
[j] Significantly different ($P < 0.05$) from Aroclor 1254—10 mg/kg.

TABLE V

INFLUENCE OF CONCENTRATION OF CCl_4 ON POTENTIATION OF HEPATOTOXICITY BY AROCLOR 1254

Pretreatment	CCl_4[a] (ppm)	N[b]	Glucose-6-phosphatase[c]	Sorbitol dehydrogenase[d]	Isocitrate dehydrogenase[e]
Corn oil	0	3	19.7 ± 1.06	0	1 ± 0.7
Corn oil	590	3	17.6 ± 0.35	0	2 ± 0.6
Aroclor 1254[f]	590	3	10.4 ± 0.24[g,h]	31 ± 3.8[g,h]	42 ± 11.1[g,h]
Corn oil	2350	3	16.1 ± 0.52[g]	15 ± 5.0[g,i]	8 ± 7.3
Aroclor 1254[f]	2350	3	7.1 ± 1.56[g,h]	148 ± 15.9[g,h,i]	288 ± 113.6[g]

[a] 2 h exposure.
[b] Number of rats.
[c] μmoles PO_4/g/min.
[d] $\Delta E^{60\ sec}_{366\ nm} \times 10^3$/0.2 ml serum.
[e] IU/l.
[f] 25 mg/kg for 6 days.
[g] Significantly different ($P < 0.05$) from corn oil—air.
[h] Significantly different ($P < 0.05$) from group with same exposure.
[i] Significantly different ($P < 0.05$) from group with same pretreatment.

of a lower level of CCl_4 exposure. To determine this, animals were exposed to 590 ppm CCl_4 for 2 h and compared with animals exposed to 2350 ppm. The data in Table V indicate that at this lower level of exposure, no toxicity due to CCl_4 alone was evidenced by any of the three parameters measured. However, in all three cases there were significant changes in the case of the Aroclor pretreated animals exposed to CCl_4. As expected, there were changes due to CCl_4 alone when it was inhaled at the higher concentration and these were further exacerbated in those animals pretreated with Aroclor 1254.

DISCUSSION

Although Bruckner et al. [19] reported that Aroclor 1242 administration resulted in liver toxicity as evidenced by elevated SGOT values, the present experiments indicated little if any hepatotoxicity due to the Aroclor alone. This discrepancy is probably due to the fact that Bruckner et al. used larger doses and/or longer time periods. CCl_4 alone produced toxicity similar to other studies concerned with its inhalation [14].

That Aroclor 1254 potentiates the toxicity of CCl_4 was demonstrated in all of the studies reported here. As expected [19] the administration of the Aroclor increased the liver to body weight ratio. This was further increased following CCl_4 administration. Liver glucose-6-phosphatase activity was decreased. The rises in the four serum enzymes that were measured were all greatly increased by the pretreatment with Aroclor 1254.

The effects of Aroclor 1254 on drug metabolism were as expected.

Aroclor-induced increases in cytochrome *c* reductase and cytochrome P-450 have been shown by other workers [19] as has the increase in *p*-nitroanisole demethylation [9]. As with phenobarbital [4], neither the normal level of cytochrome *c* reductase nor the elevated level following induction was altered by CCl_4 inhalation. However, similar to the case with phenobarbital, there was a dramatic decrease in P-450 content following CCl_4 exposure in those animals pretreated with the Aroclor 1254 as compared with the controls. Similar comparisons can be made with the effect on drug metabolism as evidenced by the large decrease in *p*-nitroanisole demethylation.

The comparison of the Aroclors was important in view of the findings of Chen and DuBois [9] that the ability of Aroclors 1221, 1254 and 1260 to induce *N*-demethylation and also EPN detoxification increased in proportion to the chlorine content of the Aroclor but that the *O*-demethylation of *p*-nitroanisole was induced to a greater extent by Aroclor 1254 than by Aroclor 1260. The results (Table III) indicate that the two more chlorinated mixtures increase the hepatotoxicity of CCl_4 more than the less chlorinated Aroclor 1221, although no difference was detected between the two more chlorinated ones.

An indication that Aroclor 1254 is a potent potentiator of CCl_4 toxicity was indicated by the finding that a dose of only 5 mg/kg for 6 days was enough to demonstrate potentiation. Because of the difficulty in showing statistical significance due to the large variation in response from animal to animal following CCl_4 inhalation, it did not seem worthwhile to give lower doses of the Aroclor. Another indication of its potency was the observation that even at a lower level of CCl_4 (590 ppm) than is usually employed in these studies there were significant elevations of the serum enzymes and decrease in liver glucose-6-phosphatase in the induced animals.

A general conclusion that can be drawn from the results of these experiments is that the Aroclors which induce P-448 rather than P-450 [7] are very potent enhancers of CCl_4 hepatotoxicity so that care must be taken in considering the suggestion that 3-methylcholanthrene protects against CCl_4 by virtue of the fact that it induces P-448 [20].

ACKNOWLEDGEMENTS

The author wishes to acknowledge the able technical assistance of Mrs. Barbara Schultz and Mrs. Nadylis Wood. The Aroclors were a gift of the Monsanto Company, St. Louis, Missouri. The work was supported by NIEHS Grant No. 00596.

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