

Perturbation of Calcium Homeostasis by CCl₄ in Rats Pretreated with Chlordecone and Phenobarbital

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Male Sprague-Dawley rats were maintained on normal powdered diet or on the same diet containing 10 ppm chlordecone (CD) or 225 ppm phenobarbital (PB) for 15 days. On day 15, they received a single IP injection of a subtoxic dose of CCl₄. Induction of cytochrome P-450 was greater with phenobarbital treatment than with chlordecone, but the CCl₄-induced destruction of P-450 was similar in both groups and was progressive with the dose of CCl₄ and with time after CCl₄ administration. CCl₄ given to animals on normal diet in a dose range of 25 to 200 μ L/kg did not significantly alter the P-450 levels. These findings are consistent with greater bioactivation of CCl₄ after the above two pretreatments. There was a massive accumulation of Ca²⁺ in CD- and PB-pretreated animals after CCl₄ administration, CD being more effective in this regard. Elevation of cytosolic Ca²⁺ was progressive despite the mitochondrial and microsomal sequestration of cytosolic Ca²⁺ at elevated levels. This perturbation of hepatocellular Ca²⁺ homeostasis which occurs 3 to 6 hr after CCl₄ may prevent hepatocellular repair and renovation in CD-treated animals, leading to progressive hepatic lesion, hepatic failure and animal death by 36 to 48 hr at nontoxic doses of CCl₄. Neither CD nor PB nor CCl₄ alone affected hepatic Ca²⁺. These findings suggest that excessive Ca²⁺ accumulation may be related to the progression of hepatotoxic response to CCl₄ in CD-treated animals.

Introduction

Various chemical toxins that initiate toxic events leading to liver cell death exhibit marked alterations in intracellular Ca²⁺ homeostasis with excessive accumulation of Ca²⁺ (1,2). The intracellular Ca²⁺ sequestration has been implicated as a potential mediator of toxic events which lead to hepatic cell death (3,4). Previous work from this laboratory has established the remarkable potentiation of CCl₄ hepatotoxicity and lethality by chlordecone (chlorinated insecticide, Kepone, CD) pretreatment in male (5,6) and female rats (7). Although an enhanced bioactivation of CCl₄ in CD-pretreated rats was reported (8), the quantum of increased bioactivation was considered insufficient to explain the 70-fold increase in lethality in these animals as compared to phenobarbital pretreated rats which exhibited only 2-fold increase in lethality (6).

With this background, the changes in hepatocellular Ca²⁺ homeostasis associated with potentiation of CCl₄ toxicity by CD were investigated. Also, in view of the earlier findings indicating stimulated bioactivation of CCl₄ in CD-treated animals (8), it was important to determine if enhanced bioactivation of CCl₄ by CD pretreatment resulted in greater destruction of cytochrome P-450. PB pretreatment was used as a positive control for the potentiation of CCl₄ hepatotoxicity.

Methods

Male Sprague-Dawley rats weighing 200 to 225 g (Charles River Breeding Laboratories, Wilmington, MA) were housed in a 12-hr photoperiod on a corn cob bedding untreated with any known inducers. The animals were maintained on normal commercial powdered rat chow (Ralston Purina Rat Chow Co., St. Louis, MO) or the diet containing 10 ppm CD or 225 ppm PB prepared as described previously (5) for 15 days. On day 15 a group of rats received a single IP injection of 100 μ L CCl₄/kg in corn oil vehicle (1 mL/kg) and sacrificed at 0, 0.5, 2, 6, 12, 24 and 36 hr. Hepatic microsomal cytochrome P-450 was determined by the method of Omura and Sato (9). Other groups of rats received a single IP injection of 25 to 200 μ L CCl₄/kg and sacrificed 12 hr later. Control animals received only the vehicle. Ca²⁺ levels in the whole liver, mitochondria, microsomes and cytosolic fraction were determined in nitric acid-digested samples by using atomic absorption spectrophotometry.

Results and Discussion

Hepatic microsomal cytochrome P-450 levels were determined at the time the animals would have received CCl₄ or at various time points after CCl₄ administration. CD treatment increased the hepatic microsomal P-450 by about 60%, whereas PB almost doubled P-450 levels (Fig. 1). CCl₄ administration (100 μ L/kg) to these

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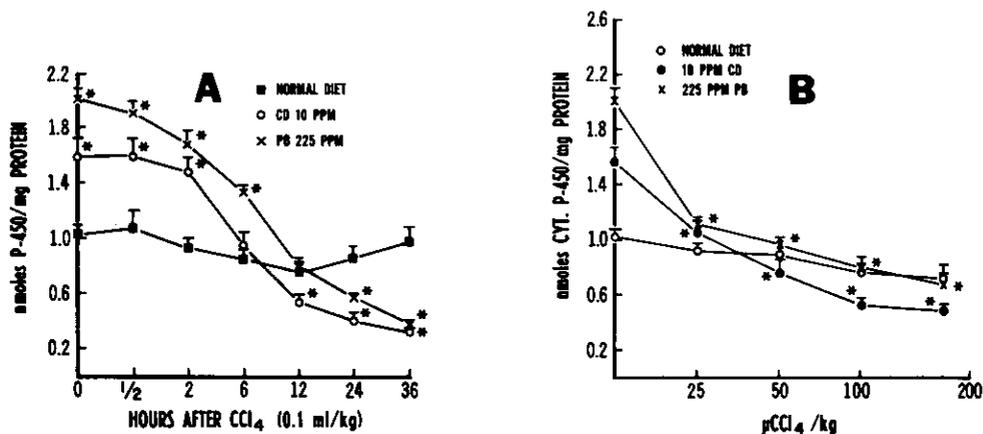


FIGURE 1. Destruction of cytochrome P-450. Male Sprague-Dawley rats were maintained on a normal powdered diet or on a diet containing 10 ppm chlordecone or 225 ppm phenobarbital for 15 days. On day 15 (A) they received a single IP injection of 100 μ L CCl₄/kg and were sacrificed at different time points as indicated or (B) they received single IP injection of different doses of CCl₄ (25–200 μ L/kg) and sacrificed 12 hr later. Controls received only the corn oil vehicle (1 mL/kg). Microsomal cytochrome P-450 was determined in the liver and expressed as nmoles cytochrome P-450/mg protein. Asterisks denote that the values are significantly different from zero point level of rats fed normal diet, $p < 0.05$.

rats caused a progressive and time-dependent destruction of P-450 (Fig. 1A). The percent destruction remained the same in both CD- and PB-pretreated animals, despite the unequal induction of cytochrome P-450. Administration of different doses of CCl₄ (25–200 μ L/kg) caused a significant destruction of P-450 at all the doses (Fig. 1B). In the rats maintained on normal diet, these doses of CCl₄ did not affect P-450 levels. These data are suggestive of enhanced bioactivation of CCl₄ in CD and PB pretreated animals. Previous studies (8,10) have shown greater *in vivo* and *in vitro* metabolism of CCl₄. Since this enhanced metabolism of CCl₄ occurs at lesser increases in P-450 levels, these findings are consistent with induction of specific form(s) of CCl₄-bioactivating hemoprotein by CD (10). However, in view of remarkable differences in the potentiation of CCl₄ toxicity between PB and CD treatments, it is necessary to consider factors other than just bioactivation that might be playing a role in initiating or promoting hepatic cell death due to CCl₄ poisoning.

Dietary exposure to CD or PB did not influence whole liver or subcellular Ca²⁺ levels. CCl₄ administration at a dose of 200 μ L/kg to rats maintained on normal diet caused a significant rise in Ca²⁺ levels, but lower doses had no effect. Previous studies suggest that these animals recover to normal by 36 hr (11,12). A significant elevation in whole liver Ca²⁺ levels was evident after CCl₄ administration to both CD- and PB-pretreated rats at all four doses used (Fig. 2A), but the increase was much higher in CD-pretreated animals. These results are consistent with our earlier observations which indicated that animals receiving CD + 100 μ L CCl₄/kg exhibit total hepatic failure with extensive hepatocellular necrosis which progresses and leads to animal death by 36 hr. In contrast, the animals receiving normal diet or PB + CCl₄ do not show such extensive necrosis, and these animals recover later (6,10,12).

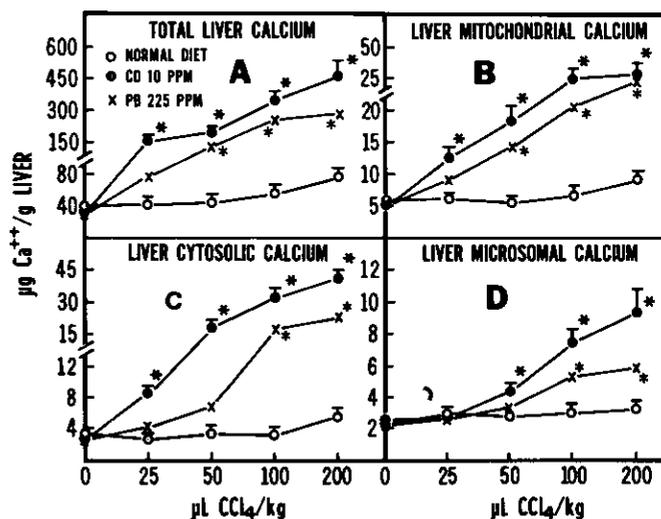


FIGURE 2. Ca²⁺ levels in subcellular fractions of the liver. Animals were maintained and treated as described in Fig. 1B. Ca²⁺ levels in (A) whole liver, (B) mitochondria, (C) cytosol and (D) microsomes were determined. The values were expressed as μ g Ca²⁺/g liver. Asterisks denote that the values are significantly different from zero point level of rats fed normal diet, $p < 0.05$.

Increased Ca²⁺ levels after CCl₄ administration were readily evident in mitochondria (Fig. 2B) due to a continuous influx of extracellular Ca²⁺ in cytosol (Fig. 2C). Microsomes also play a role in sequestering increased cytosolic Ca²⁺ levels (Fig. 2D); this was especially evident at higher doses of CCl₄. Plasma membrane changes taking place presumably due to increased lipid peroxidation or other factors consequent to CCl₄ bioactivation disrupt the permeability barrier with a consequent influx of Ca²⁺ which results in massive Ca²⁺ accumulation in the cell. Although, hepatic mitochondria and microsomes continue to regulate ever increasing cytosolic Ca²⁺ by increased sequestration,

the cytosolic Ca^{2+} levels still remain high (Fig. 2C), leading finally to cell death. Our earlier time-course histomorphometric studies (11,12) indicate that whereas animals treated with CCl_4 (100 $\mu\text{L}/\text{kg}$) recover from liver damage by virtue of hepatocellular repair and renovation, those treated with CD + CCl_4 do not. Instead, 3–4 hr after CCl_4 when hepatocellular repair would have occurred (11,12), a progressive increase in cytosolic Ca^{2+} occurs in animals receiving the CD + CCl_4 combination treatment, suggesting a cause-effect relationship. In animals receiving CCl_4 alone, Ca^{2+} homeostasis is unperturbed, allowing the hepatocellular repair, renovation and recovery.

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