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Chlordecone potentiates hepatic fibrosis in chronic liver injury induced by carbon tetrachloride in mice

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HIGHLIGHTS

- Hepatic impact of chronic co-exposure to chlordecone and CCl4 investigated in mouse model.
- Chlordecone enhances the liver damage in CCl4 murine chronic hepatitis.
- Chlordecone enhances the progression of liver fibrosis in mice.

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ABSTRACT

Chronic liver damage due to viral or chemical agents leads to a repair process resulting in hepatic fibrosis. Fibrosis may lead to cirrhosis, which may progress to liver cancer or a loss of liver function, with an associated risk of liver failure and death. Chlordecone is a chlorinated pesticide used in the 1990s. It is not itself hepatotoxic, but its metabolism in the liver triggers hepatomegaly and potentiates hepatotoxic agents. Chlordecone is now banned, but it persists in soil and water, resulting in an ongoing public health problem in the Caribbean area. We assessed the probable impact of chlordecone on the progression of liver fibrosis in the population of contaminated areas, by developing a mouse model of chronic co-exposure to chlordecone and a hepatotoxic agent, carbon tetrachloride (CCl_4). After repeated administrations of chlordecone and CCl_4 by gavage over a 12-week period, we checked for liver damage in the exposed mice, by determining serum liver transaminase (AST, ALT) levels, histological examinations of the liver and measuring the expression of genes encoding extracellular matrix components. The co-exposure of mice to CCl_4 and chlordecone resulted in significant increases in ALT and AST levels. Chlordecone also increased expression of the Col1A2, MMP-2, TIMP-1 and PAI-1 genes in CCl_4-treated mice. Finally, we demonstrated, by quantifying areas of collagen deposition and alpha-SMA gene expression, that chlordecone potentiated the hepatic fibrosis induced by CCl_4. In conclusion, our data suggest that chlordecone potentiates hepatic fibrosis in mice with CCl_4-induced chronic liver injury.

1. Introduction

Fibrogenesis is a wound-healing response to repeated liver injury. It is triggered in response to chronic liver damage, regardless of the cause of the damage: viral infections, alcohol abuse, drug exposure, metabolic dysfunctions or autoimmune disorders. It manifests as a progressive replacement of the liver parenchyma with scar tissue. This process takes place over many

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; CD, chlordecone; CCl_4, carbon tetrachloride; α-SMA, α-smooth muscle actin; ECM, extracellular matrix.

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years and can lead to other complications, such as cirrhosis, leading to liver failure or an increase in the risk of hepatocellular carcinoma. Hepatic fibrosis is characterized by the excessive accumulation of extracellular matrix (ECM) (Friedman, 2003). At advanced stages of the disease, there may be six times more ECM in fibrotic livers than in normal livers (Benyon and Iredale, 2000). This accumulation results from an imbalance between the synthesis and degradation of ECM components (Arthur, 2000), such as fibrillar collagen, essentially collagen type I (Tsukada et al., 2006), matrix metalloproteinases (MMPs) (Takahara et al., 1995) and their tissue inhibitors (TIMPs) (Benyon and Iredale, 2000).

Humans are exposed, on a daily basis, to various toxic agents, chemicals and drugs in the environment. The liver is the major site of detoxification for these compounds in the body. This organ may therefore be considered a target of their toxicity. In humans, hepatotoxicity due to exposure to alcohol, drugs such as acetaminophen, imidazole fungicidal agents and other chemicals has also been studied in a context of exposure to mixtures of these agents. It has been shown that exposure to alcohol + acetaminophen, or alcohol + imidazole fungicidal agent mixtures results in worse damage to the human liver than exposure to any of these agents alone (Zimmerman and Maddrey, 1995).

Many animal models for studying the molecular processes underlying the pathophysiology of the liver during exposure to hepatotoxic compounds administered alone or in combination have been developed, to improve our understanding of the molecular mechanisms of action of these hepatotoxic compounds and the host response, and to identify biological markers of liver damage. As in humans, mixtures of chemical agents were found to aggravate liver damage in most of the animal models studied (Brautbar and Williams, 2002).

In animals, diverse causes of fibrogenesis have been studied, but the principal *in vivo* model of fibrosis is based on the repeated administration of carbon tetrachloride (CCl_4) over a period of several weeks (Brautbar and Williams, 2002; Chang et al., 2005). CCl_4 is a hepatotoxic agent that can induce liver fibrosis, and even cirrhosis, after long-term exposure. CCl_4 is known to trigger centrilobular hepatic necrosis and the first event in its hepatotoxicity is its metabolic activation in the liver (Weber et al., 2003). CCl_4 is increased by other agents, such as alcohol, in situations of co-exposure in animals (Benyon et al., 1996; Chobert et al., 2012; McLean and McLean, 1966). In particular, the hepatotoxicity of this compound has been shown to be increased in models of co-exposure to pesticides, such as mirex and chlordecone.

Chlordecone, also known as kepone, is an organochlorine pesticide that was produced essentially from 1958 through 1975 in the United States, for export to Latin America, Africa and Europe for use as an ant and cockroach poison. Kepone was also used to control banana root borer, primarily in the Caribbean area. Kepone production was banned in 1976 after it was discovered that the Life Science Products Company factory in Hopewell, Virginia, which was responsible for producing this chemical, had caused serious contamination of the environment and many of its workers were found to have medical problems. All workers at this factory presented symptoms of chlordecone poisoning, including headaches, anxiety, irritability and memory disturbance, abnormal hepatic function and defective spermatogenesis. Studies of chlordecone toxicity in humans and animal models implicated this agent in a broad array of syndromes, affecting the nervous and reproductive systems in particular. Chlordecone is known to accumulate mostly in the liver (Egle et al., 1978) triggering hepatomegaly (Guzelian, 1985). Chlordecone is not itself hepatotoxic, but several studies have shown that it can strongly potentiate the hepatotoxic effects of CCl_4 in models of acute co-exposure (Curtis et al., 1979; Klingensmith et al., 1983; Mehendale, 1989).

Chlordecone is no longer used, but it persists in the soils and water of the Caribbean area (Cabidoche et al., 2009; Clostre et al., 2015), therefore the Caribbean population is continually exposed to it. In fact, the main source of people contamination with chlordecone in these areas is the food consumption (Cabidoche et al., 2009; Clostre et al., 2015). Various types of food products can contain amounts of chlordecone which lead to a daily exposure of the population to this molecule. Epidemiological studies have shown that the average of daily chlordecone ingestion in these areas is around 0.11 μg/Kg (Guldner et al., 2010). It thus remains a public health problem, due to its potential contribution to many chronic diseases, including liver diseases.

In this study, we developed a mouse model of chronic co-exposure to chlordecone and CCl_4, to assess the impact of chlordecone on the development and progression of liver fibrosis. We evaluated the liver damage caused by the interaction between chlordecone and CCl_4 with repeated exposure, the expression of genes involved in matrix remodeling and the amount of collagen deposited in mouse livers.

2. Materials and methods

2.1. Animals and experimental protocol

Eight-week-old male C57Bl/6 mice were purchased from Janvier (Le Genest-sur-Isle, France) and reared in specific pathogen-free (SPF) conditions in the local animal house, in accordance with French laws and institutional guidelines for the handling and care of experimental animals (accreditation of M. Samson #3596).

Animals were assigned to four groups at random, with four mice in each. In the first group, the mice received olive oil daily, by gavage, as a control treatment. In the second group, mice were treated, once weekly, with CCl_4 (Sigma-Aldrich, St. Louis, MO, USA) at a dose of 100 mg/kg. In the third group, the mice received chlordecone (Santa Cruz Biotechnology) daily, at a dose of 5 mg/kg. In the fourth group, the mice received chlordecone at a dose of 5 mg/kg daily for two weeks and then 100 mg/kg CCl_4 once weekly, in addition to their daily dose of chlordecone. CCl_4 and chlordecone were diluted in olive oil and administered by oral gavage. During the 12 weeks of the study, the mice were observed and their body weight was measured once weekly. The doses of the chemicals used were well tolerated and no deaths occurred. Blood was collected from the mice 48 h and 72 h after the administration of CCl_4 in weeks 5, 7, 9 and 12 of the study. The mice were killed 72 h after the administration of the last dose of CCl_4. The liver was removed and fragments of this organ were fixed in 4% paraformaldehyde and embedded in paraffin or frozen in liquid nitrogen in the presence of the cryoprotectant isopentane. Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined according to IFCC primary reference procedures, with an Olympus AU2700 Autoanalyser® (Olympus Optical Optical Co., Ltd., Tokyo, Japan).

2.2. RNA isolation and RT-qPCR

We homogenized liver tissue samples weighing approximated 100 mg and extracted total RNA in Trizol reagent (Invitrogen). The first-strand cDNA was produced with SuperScriptTM II Reverse Transcriptase (Invitrogen). The cDNA was then amplified, with the GAPDH housekeeping gene as a control. Quantitative PCR was performed with the fluorescent dye SYBR Green and the double strand-specific SYBR® Green system (Applied Biosystems), with an ABI 7900HT Prism sequence analyzer (Applied Biosystems). Total cDNA (30 ng) was used as a template for amplification, with the specific primer pair used at a final concentration of 300 nM. Each measurement was performed in triplicate. We determined 18S