Effect of antimitotic agent colchicine on carbon tetrachloride toxicity

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Received 21 January 1993/Accepted 3 March 1993

Abstract. A single administration of a subtoxic dose of CCl4 (100 μl/kg, i.p.) is known to induce hepatocellular regeneration and tissue repair at 6 and 48 h in rats, permitting prompt recovery from the limited liver injury associated with that dose of CCl4. Substantial evidence has accumulated to indicate that the early-phase hepatocellular regeneration and tissue repair are critical for recovery from halomethane hepatotoxicity. The objective of these studies was to test this concept in an experimental framework, wherein a selective ablation of the early-phase cell division should result in prolongation of liver injury followed by recovery. The studies were designed to evaluate the influence of the antimitotic agent colchicine (1 mg/kg, i.p. in saline) on CCl4 toxicity. Colchicine was administered 2 h prior to CCl4 or corn oil injection. Toxicological end points and markers of hepatocellular regeneration were assessed at various time points (2, 6, 12, 24, 48 and 72 h) after the injection of CCl4 to male Sprague-Dawley rats. Hepatocellular injury was assessed through elevations of serum alanine and aspartate aminotransferase and by histopathological examination of the liver. Incorporation of 3H-thymidine in hepatocellular nuclear DNA and mitotic index were used as indices of hepatocellular regeneration. Hepatocellular regeneration stimulated by CCl4 at 2-6 h was blocked by colchicine as evidenced by the decreased 3H-thymidine incorporation and mitotic index, without any significant effect on the second phase of cell division at 48 h. Ablation of this early phase of tissue repair resulted in prolongation of CCl4 hepatotoxicity. Rats treated with CCl4 alone recovered promptly within 24 h, whereas, colchicine pretreated rats recovered from liver injury after 48 h. Morphometric analysis of hepatocellular necrosis revealed that liver injury at 6 and 12 h after CCl4 was similar in rats regardless of colchicine pretreatment, indicating that prolongation of liver injury was due to delayed liver tissue healing mechanisms. The possibility that prolongation of hepatotoxicity is due to colchicine-induced enhancement of CCl4 metabolism was further investigated in vivo. 14CCl4-derived 14CO2 exhalation, covalent binding of 14CCl4 and 14CCl4-derived total radiolabel in the liver and lipid peroxidation were unaltered by colchicine pretreatment. These findings suggest the pivotal importance of the early- as well as the late-phase stimulation of hepatocellular regeneration and tissue healing processes in determining the final outcome of CCl4-induced liver injury.

Key words: Colchicine – Carbon tetrachloride – Hepatocellular regeneration – Potentiation of liver injury

Introduction

CCl4 is a hepatotoxic halomethane, capable of producing hepatocellular fatty degeneration and centrilobular necrosis (Smuckler 1976; Cheeseman et al. 1985; Bruckner et al. 1986). Increased bioactivation by cytochrome P-450 is widely accepted as the mechanism by which xenobiotics potentiate CCl4 hepatotoxicity (Reynolds et al. 1981; Reynolds et al. 1985). The magnitude of increase in CCl4 toxicity would therefore depend upon the amount of cytochrome P-450 induction and accordingly increased bioactivation of CCl4. However, phenobarbital (PB) doubles the cytochrome P-450 levels and potentiates CCl4 toxicity 1.6-fold, whereas chlordecone (CD) increases the cytochrome P-450 levels 1.5-fold and potentiates CCl4 toxicity 67-fold (Klingensmith and Mehendale 1982). These observations indicate that the eventual potentiation of CCl4 hepatotoxicity is not directly associated with the extent of bioactivation. Whereas the hepatotoxic injury observed 24 h after CCl4 administration to PB or CD treated rats in quite similar (Klingensmith et al. 1983), the rats treated with PB + CCl4 recover, while those treated with CD + CCl4 do not

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; CLC, colchicine; CD, chlordecone; 3H-T, 3H-thymidine; PB, phenobarbital.

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Earlier experiments (Lockard et al. 1983a, b; Bell et al. 1988; Kodavanti et al. 1989a, b, c; Soni and Mehendale 1991a, b) revealed that a low dose of CCl₄ (100 µl/kg, i.p.) induces hepatocellular regeneration and tissue repair as early as 2–6 h, which helps the animal to recover from the accompanying liver injury within 24 h (Lockard et al. 1983a, b; Kodavanti et al. 1989a, b, c; Mehendale 1990). However, chloroquine pretreated rats receiving the same dose of CCl₄ fail to show hepatocellular division at the early time points (Lockard et al. 1983a, b; Kodavanti et al. 1989a, b, c). In the absence of hepatocellular regeneration, limited toxic injury caused by CCl₄ enters the progressive phase (Curtis et al. 1979; Curtis and Mehendale 1980; Lockard et al. 1983a, b; Bell et al. 1988; Mehendale 1990) leading to hepatic failure culminating in death, starting at 36 h (Mehendale 1984, 1990). Based on these studies, it was hypothesized that suppressed hepatocellular regeneration was responsible for the amplification of CCl₄ toxicity (Mehendale 1990).

Recent studies designed to validate this concept experimentally using partially hepatectomized rats have provided supportive evidence (Bell et al. 1988; Kodavanti et al. 1989a, b, c; Young and Mehendale 1989; Mehendale 1990). In another study (Cai and Mehendale 1990), the extraordinary sensitivity of gelsb was found to be due to lack of a prompt stimulation of the early-phase hepatocellular regeneration (Cai and Mehendale 1991a, b). The administration of a toxic dose of CCl₄ to rats is known to cause stimulation of hepatocellular regeneration at 36–48 h after the treatment (Leevy et al. 1959; Smuckler 1976) and our studies indicate that the stimulation of the early- and late-phase cell division are of critical importance for recovery from CCl₄ toxicity (Mehendale 1990). Regeneration of liver following both toxic injury (Leevy et al. 1959; Smuckler 1976; Kodavanti et al. 1989b) and surgical partial hepatectomy (Higgins and Anderson 1931; Nakata et al. 1985; Kodavanti et al. 1989a, b, c) are well documented.

The present studies were designed to investigate the role of the early-phase stimulation of hepatocellular regeneration by CCl₄ in the recovery from CCl₄ toxicity. Suppression of the early phase of hepatocellular division at 6 h without affecting the regenerative response observed at 48 h would be expected to result in prolongation of CCl₄ toxicity until 48 h. Colchicine (CCl₄ in saline) is known to be non-toxic (Rao and Mehendale 1991a, b) and quite effective in blocking liver cell division, without causing any adverse effects (Reddy et al. 1969). Through preliminary experiments, we established that a single administration of CLC (1 mg/kg, i.p.) results in selective ablation of the early-phase (6 h) stimulation of hepatocellular regeneration induced by a low dose of CCl₄ without affecting the second phase (48 h) significantly. At 2, 6, 12, 24, 48 and 72 h after the last treatment, blood was collected from the dorsal aorta under diethyl ether anesthesia. The blood was used for the estimation of serum enzymes, alanine and aspartate aminotransferase (ALT and AST) by the method of Reitman and Frankel (1957) using Sigma kit# 505-OP, (Sigma Chemical Co., St Louis, MO).

In vivo incorporation of ³H-thymidine into hepatocellular rat liver nuclear DNA

The procedure used is essentially that of Chang and Looney (1965). Rats undergoing various treatments were injected with 50 µCi of ³H-T intraperitoneally, 2 h before killing. This time period falls within the linear range of ³H-T incorporation and maximal incorporation was observed in accordance with the previously reported findings (Chang and Looney 1965; Kodavanti et al. 1989a, b, c; Soni and Mehendale 1991a, b, c). Livers were removed, rinsed in saline, blotted and weighed portions were homogenized in 20 ml 2.2 M sucrose solution (d = 1.28). This and all other procedures were performed at 4°C. The homogenates were centrifuged at 4000 g for 1 h in a Beckman L-65 centrifuge. In this procedure, the nuclei form a sediment, whereas the intact cells, connective tissue, mitochondria and erythrocytes migrate to the top. The sedimented nuclei were washed twice with 15 ml 0.25 M sucrose and finally suspended in 3 ml 0.25 M sucrose (Chaveau et al. 1956; Kodavanti et al. 1989a, b, c; Soni and Mehendale 1991a, b, c).