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Long term phenobarbital administration does not promote the multiplication of hepatocytes replicating after single cyproterone acetate administration

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Abstract

Cyproterone acetate (CPA) is a synthetic antiandrogenic compound which is widely used in clinic but suspected to be hepatocarcinogenic. CPA is also mitogenic in rat liver. Using genetic labeling of dividing cells, we examined whether hepatocytes dividing in response to acute CPA administration could give rise to preneoplastic foci after administration of a tumor promoter: phenobarbital. CPA was administered orally to rats and dividing hepatocytes were genetically labeled using retroviral vectors carrying the β-galactosidase gene. After labeling rats were given phenobarbital for 10 months and sacrificed. The presence of β-galactosidase labeled hepatocytes as well as preneoplastic hepatocytes was assessed by immunohistochemistry. Genetic labeling of hepatocytes was obtained in all animals. At the end of phenobarbital administration, no hepatic tumors were observed. Preneoplastic foci were not increased in treated animals as compared to control rats. Moreover β-galactosidase positive hepatocytes were never detected in preneoplastic foci. Finally, the size of the β-galactosidase postive clusters was smaller in treated animals as compared to control rats. We conclude that acute CPA administration is not carcinogenic in rat liver and does not initiate preneoplastic hepatocytes capable to give rise to foci after phenobarbital promotion. Therefore the mitogenic property of CPA is distinct from its putative carcinogenic activity. Finally, analysis of the size of β-galactosidase positive cells clusters demonstrate that phenobarbital does not induce hepatocyte proliferation in rats.

Keywords: Cell lineage; Genetic labeling; Retrovirus; Carcinogenesis; Phenobarbital

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Introduction

Cyproterone acetate (CPA) is a synthetic progestin with antiandrogenic activity which is widely used in clinic as an oral contraceptive as well as for the treatment of prostate cancer and hirsutism. However, CPA is mitogenic and carcinogenic in the adult rat liver and these effects deserve further attention. When administered to rat, CPA induces rapidly DNA synthesis in hepatocytes resulting in liver hyperplasia (Schulte-Hermann et al., 1980). After CPA administration has stopped, the liver returns to a normal weight through an increase in hepatocyte apoptosis (Bursch et al., 1984; Roberts et al., 1995). Long term studies to evaluate the toxic potential of CPA revealed that this compound was a liver carcinogen when administered to rats at supra therapeutic doses for prolonged periods of time (Schuppler and Gunzel, 1979). It was then proposed that CPA behaved as a tumor promoter, capable of promoting the growth of initiated cells via its mitogenic activity, but devoid of tumor-initiating activity (Schulte-Hermann et al., 1980; Schulte-Hermann et al., 1983a; Schuppler et al., 1983). However, it was subsequently demonstrated that CPA was genotoxic in vivo and in cultured hepatocytes and could initiate preneoplastic lesions in the liver (Deml et al., 1993; Martelli et al., 1995; Neumann et al., 1992). Therefore CPA is now considered as a complete carcinogen. Recently, we used genetic labeling of hepatocytes to decipher the relationship between the immediate mitogenic and tumor initiating activities of CPA administered acutely. We demonstrated that the initiating and mitogenic activities of CPA were directed towards different hepatocyte populations (Auvigne et al., 2002). In that study, we used onco-retroviral vectors carrying the β-galactosidase marker gene to selectively label the hepatocytes dividing upon the effect of acute CPA administration. After labeling, rats were treated with 2-acetyl aminofluorene (2-AAF) to promote the growth of putative CPA-initiated cells. 2-AAF is a carcinogen promoter that is widely used in the initiation/promotion model of hepatocarcinogenesis (Solt et al., 1977). In that previous study, 2-AAF treatment resulted in the appearance of preneoplastic lesions expressing the placental form of glutathion-S-transferase (GST-p). This enzyme is a classical marker of preneoplastic, enzyme-altered hepatocytes (Satoh et al., 1985). We did not observe a preferential appearance of preneoplastic foci in the population of β-galactosidase labeled hepatocytes. However, 2-AAF is a complete carcinogen which can induce liver preneoplastic and neoplastic lesions in rats when given in the diet for long periods of time (Farber, 1956; Reuber, 1964). Therefore, in our previous study a number of preneoplastic foci could have resulted from direct effect of 2-AAF treatment and not from administration of CPA. To resolve this issue and to gain further insights into the carcinogenic potency of CPA in rats, we devised another protocol using phenobarbital as a tumor promoter. Phenobarbital is a tumor promoter compound devoid of complete carcinogenic activity (Hagiwara et al., 1999). Therefore, phenobarbital is able to amplify cells that have been initiated in response to a carcinogen leading to tumors (Peraino et al., 1971; Peraino et al., 1973). In contrast, phenobarbital alone does not cause liver tumors in rats (Anon, 2000; Gould et al., 2001). In the present study, rats were fed CPA for two consecutive days and injected with retroviral vectors containing a β-galactosidase gene to specifically label the population of hepatocytes stimulated by the mitogenic activity of CPA. Moreover, to avoid disappearance of labeled cells due to immune response against the β-galactosidase marker protein, we used the same transgenic β-galactosidase tolerant rats that were used in our previous study.