Bull. Environ. Contam. Toxicol. (1992) 48:869–876

Environmental Contamination and Toxicology

Differential Effects of Long-Term Exposure to Aroclor 1254 on Lipid Secretion by Primary Cultures of Adult Rat Hepatocytes

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PCBs produce hepatic triglyceride (TG) accumulation (fatty liver) in experimental animals and humans exposed accidentally and occupationally (Kimbrough et al., 1972; Schecter et al., 1985; Stehr-Green et al., 1986). It has been suggested that this effect could be due to a block in TG secretion (Hinton et al.. 1978; Sandberg and Glauman, 1980). On the other hand, increased levels of plasmatic TG and cholesterol have been described in rats after dietary exposure to Aroclor 1254 (Aro) and other PCBs (Allen et al., 1976; Garthoff et al., 1977; Kato et al., 1978; Quazi et al., 1984); hypertriglyceridemia and hypertension have been also described in humans exposed for long periods to low concentrations of PCBs (Kries et al., 1981; Schecter et al., 1985; Smith et al., 1982; Stehr-Green et al., 1986).

Since the study of hepatic lipid metabolism and its alteration by toxic chemicals is complicated in the whole animal, short term cultures of adult rat hepatocytes have been used (Gravela et al., 1977; Forte, 1984). We have described a system for the long term culture of adult rat hepatocytes which for several weeks maintain differentiated functions, like fatty acid and TG synthesis and their export to the culture medium (Kuri-Harcuch and Mendoza-Figueroa, 1989; Mendoza-Figueroa et al., 1988). In this paper we used this culture system to study the effect of long-term exposure to micromolar concentrations of Aro on the secretion of lipids by cultured hepatocytes.

MATERIALS AND METHODS

Hepatocytes were isolated from male Wistar rats (180-200 g) by the collagenase-perfusion method (Berry and Friend, 1969) as described earlier (Mendoza-Figueroa et al., 1988). Cells were suspended in Eagle's medium modified by Dulbecco-Vogt, supplemented with 7% calf serum (HyClone, Logan, UT), 5 μg/mL insulin and 10^{-7} M d-biotin

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(basal medium), seeded at 35,000 cells/cm^2 on a feeder layer of 3T3 cells lethally treated with Mytomicin C (a gift from Bristol-Myers Co., Syracuse, NY) (Mendoza-Figueroa et al., 1988) and placed in a 37° C humidified incubator gassed with 90% air 10% CO_2. After 30 min incubation, dishes were rinsed with serum free medium and refed with basal medium supplemented with 10 µg/mL hydrocortisone. Aro (Analabs, Inc., New Haven, CON) was dissolved in dimethyl sulfoxide (DMSO) (10 mg/mL) and added to the culture dishes at the indicated concentrations 24 h after cell seeding; the concentration of DMSO in the culture medium was 1.0% and control cultures received the same concentration of DMSO. Culture media were changed daily.

The cultures were treated for two weeks with the indicated concentrations of Aro. Then 0.2 µCi of [^{14}C] acetate (55 mCi/mmol; New England Nuclear, Boston, MA) were added per mL of culture medium to hepatocytes cultured in 35-mm dishes. After 24 h incubation the cells were scraped and disrupted by sonication; the released and cellular lipids were extracted from culture medium and sonicated cells, respectively, as described by Cham and Knowles (1976) and analyzed by thin layer chromatography (TLC). The extracted lipids were evaporated under a nitrogen stream, dissolved in 30 µL of chloroform and analyzed by TLC with heptane:di-isopropyl ether:acetic acid (60:40:4) (Kates, 1972). The radioactivity that comigrated with purified standards of phospholipids (PL), cholesterol (C), TG and cholesterol esters (CE) was determined by liquid scintillation counting in a Packard spectrophotometer model Tri-Carb 460 using Aquasol (New England Nuclear, Boston, MA).

The results are expressed as the means ± standard deviation of duplicate determinations from four culture dishes in a typical experiment; similar results were obtained in two independent experiments. Statistical comparison between control and treated cultures was made with Student's t-test.

RESULTS AND DISCUSSION

Control hepatocytes after 15 d in culture showed their typical epithelial morphology with granulous cytoplasm, central nucleus and intercellular spaces resembling biliary canaliculi (Fig. 1a). The presence of 1% DMSO did not produce alterations in the cultured hepatocytes as previously shown (Isom et al. 1985; Mendoza-Figueroa et al., 1989). The exposure for 15 d to Aro concentrations up to 1.0 µg/mL did not produce morphologic alterations (Fig. 1b), but the cultures exposed to 10-20 µg/mL showed intracytoplasmic lipid droplets (Fig. 1c) due to triglyceride accumulation, as we have shown previously (Mendoza-Figueroa et al., 1989). The tested concentrations of Aro were comparable to those observed in serum, liver and adipose tissue of exposed humans (2-521 ppm) (Acquavella et al., 1986; Chen and Wong, 1985; Emmet, 1985; Takamatsu et al.,

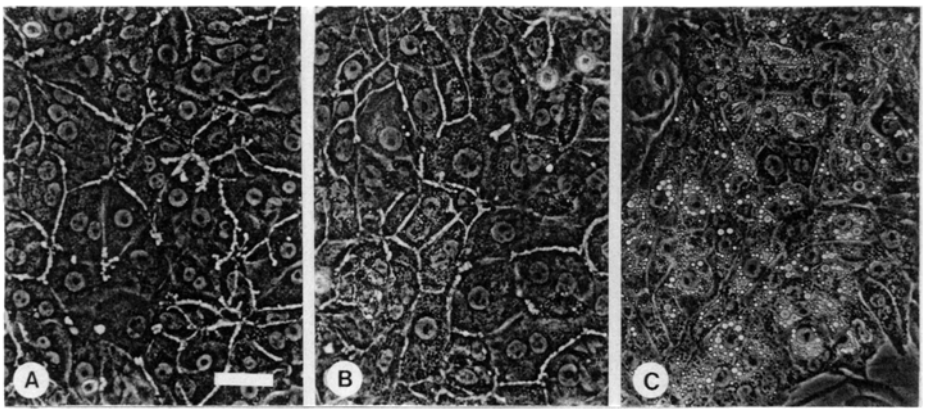

Figure 1. Effect of Aroclor 1254 exposure on cell morphology. Untreated hepatocytes (A) or treated for 2 weeks with 1.0 μg/mL (B) or 20 μg/mL of Aroclor 1254 (C). Phase contrast optics; bar, 50 μm.

1985).

The secretion of labeled lipids to the culture medium increased significantly in cultures treated with 0.1-1.0 μg/mL of Aro, with a maximum increase of 95% (Fig. 2c); on the other hand, the secretion of lipids was inhibited up to 57% at higher concentrations (10-20 μg/mL). Since the increased secretion of these lipids could be due to higher de novo synthesis by the hepatocytes, we determined also the incorporation of the radioactive precursor into cellular and total (cellular plus secreted) lipids (Fig. 2a-b) as a measure of the synthesis of lipids by the cultured hepatocytes.

The exposure to 0.01-1.0 μg/mL Aro did not produce changes in the synthesis of cellular and total lipids, but at higher concentrations (10-20 μg/mL) the incorporation of the radioactive precursor decreased significantly (Fig. 2a-b). These results show that in the hepatocytes exposed to low concentrations of Aro the increased secretion of total lipids is not paralelled by a higher rate in their synthesis whereas in those treated with high concentrations of Aro the decreased secretion is accompanied by a decreased synthesis. These results are in agreement with previous work, both in vivo and in vitro, showing a decreased synthesis of lipids at high concentrations of Aro (Dzogbefia et al., 1978; Mendoza-Figueroa et al., 1989). They also suggest that the inhibition of lipid secretion observed at high concentrations of Aroclor could be due both to a block in the lipid secretion processes and to a decreased synthesis of lipids from two carbon precursors (Hinton et al., 1978; Mendoza-Figueroa et al., 1989; Sandberg and Glauman, 1980).

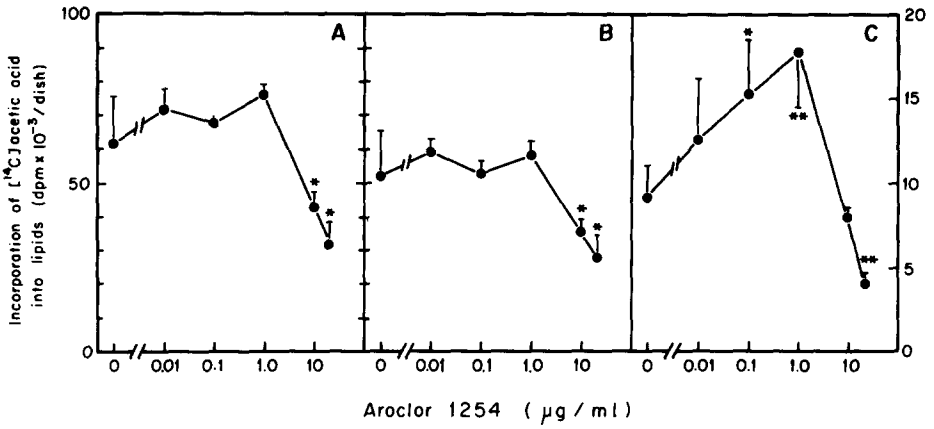

Figure 2. Effect of Aroclor 1254 exposure on the incorporation of [^{14}C] acetate into total (A), cellular (B) and released lipids (C) by cultured hepatocytes. Cultures were treated for two weeks with the indicated concentrations of Aroclor. Then 0.2 µCi of [^{14}C] acetic acid were added per mL of culture medium. After 24 h incubation the cultures were processed as described in the Methods Section. Mean + standard deviation from 4 culture dishes. Asterisks represent significantly different values from control values by the Student's t test (* $p < 0.05$, **$p < 0.005$).

To study the effect of Aro exposure on the synthesis and secretion of the different species of lipids, released and cellular lipids were analysed by TLC. The analysis of the released lipids showed a dose dependent increase in the secretion of CE and TG at Aroclor concentrations of 0.01-1.0 µg/mL with a maximum value that was 3.3 and 2.8 fold higher than the control value, respectively (Fig. 3c-d); similarly, the secretion of PL and C increased in the cultures treated with Aro concentrations of 0.1-1.0 µg/mL and 1.0 µg/mL, respectively, with a maximum value 79% and 54% higher than the control value (Fig. 3a-b). On the other hand, the secretion of radioactive lipids decreased in the cultures exposed to 20 µg/mL of Aroclor the observed values being 2.1, 3.8, 1.7 and 1.3 fold lower for FL, C, TG and CE, respectively (Fig. 3a-d).

For cellular lipids, the cultures treated with 0.1-1.0 µg/mL Aro showed an increase in the incorporation of the radioactive precursor into CE, with a maximum value 2 fold higher than the control value (Fig. 4d) whereas in the cultures treated with 20 µg/mL the observed values were 3.5 and 2.6 fold lower for C and TG, respectively (Fig. 4b-c). On the other hand, the incorporation of the radioactive precursor into total lipidic species (released plus cellular) increased for CE and C at Aro concentrations of 0.01-1.0 and 1.0 µg/mL, respectively, with a maximum value 2.4 and 1.2 fold higher than the control value whereas in the cultures treated with

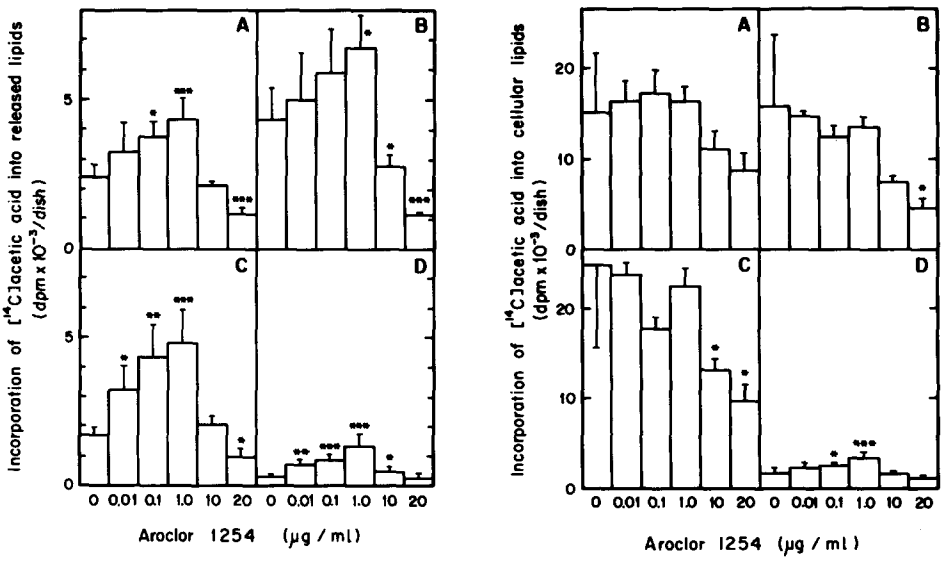

Figure 3 and 4. Effect of Aroclor 1254 exposure on the incorporation of [^{14}C] acetate into released (left) and cellular lipids (right) by cultured hepatocytes. Phospholipids (A), cholesterol (B), triglycerides (C) and cholesterol esters (D). Cultures were treated for two weeks with the indicated concentrations of Aroclor. Then 0.2 µCi of [^{14}C] acetic acid were added per mL of culture medium. After 24 h incubation the cultures were processed as described in the Methods Section. Mean + standard deviation from 4 culture dishes. Asterisks represent significantly different values from control values by the Student's t test (*$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$)

20 µg/mL the incorporation into the four species decreased 1.8, 2.9, 2.5, 1.5 fold for PL, C, TG and CE, respectively, with respect to the control values (Table 1).

These results show that the synthesis of C and CE by the hepatocytes is increased by 2 weeks exposure to low concentrations of Aro, whereas the synthesis of TG and PL is unchanged. Our results are in agreement with previous work showing that dietary exposure to PCBs increases the synthesis of cholesterol measured by [^{3}H] H_2O incorporation and the activity of 3-hydroxy-3-methyl glutaryl Co A reductase (Kato and Yoshida, 1980; Quazi et al., 1984), a key enzyme in the synthesis of cholesterol, by changing the enzyme-lipid interaction and increasing the mRNA levels of the enzyme (Jenke, 1985). Our results suggest that the increased secretion of C and CE by the hepatocytes could be due to an increased synthesis of cholesterol; they also show that long term exposure to low concentrations of Aro (0.1-1.0 µg/mL) increases the secretion of cellular lipids by cultured hepatocytes, whereas their synthesis and secretion are inhibited at higher concentrations

Table 1. Effect of Aro on the synthesis of lipids by cultured hepatocytes. Cultures were treated for two weeks with the indicated concentrations of Aroclor. Then 0.2 μCi of [^{14}C] acetic acid were added per mL of culture medium. After 24 h incubation the cultures were processed as described in the Methods Section. Mean ± stardard deviation from 4 culture dishes. Asterisks represent significantly different values from control values by the Student's t test (*$p < 0.05$, **$p < 0.005$, ***$p < 0.0005$)

Aro μg/mL	PL dpm/dish	C dpm/dish	TG dpm/dish	CE dpm/dish
0	17613±3494	16573±3922	26605±7667	1963±459
0.01	19765±2529	19636±1511	27027±1235	2996±640*
0.10	21065±1921	18279± 767	22087± 380	3393±262**
1.00	20747±2257	20226±1092*	27327±2259	4679±468***
10.00	13190±2201	10231± 958**	15213± 721	2125±424
20.00	9836±1480*	5705± 904***	10629±1586**	1319±287*

(10-20 μg/mL). Our results suggest that the in vivo observed hypertriglyceridemia and hypercholesterolemia could be due, at least partially, to an increased secretion of TG and cholesterol by the liver. Further work is necessary in order to determine the mechanism(s) by which Aro increases the synthesis and secretion of these lipids by the liver.

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Received November 1, 1991; accepted December 30, 1991.