Biochemical Pharmacology, Vol. 46, No. 7, pp. 1307–1310, 1993.
Printed in Great Britain.

0006-2952/93 $6.00 + 0.00

The effects of long-term administration of 3-thia fatty acid, a peroxisome proliferator, to Morris 7800 C1 hepatoma cells

(*Received* 1 *March* 1993; *accepted* 8 *June* 1993)

Abstract—Morris 7800 C1 hepatoma cells were grown in the presence of 80 μM tetradecylthioacetic acid (TTA), a peroxisome proliferator, for 1 year (long-term-treated cells). The growth of the Morris 7800 C1 hepatoma cells was inhibited in cells treated with TTA for up to 8 days. Treatment of the cells with TTA for 1 year did not reduce growth further. The growth inhibition was easily reversed by insulin (0.4 μM). Peroxisomal acyl-CoA oxidase (ACO) (EC 1.3.99.3) activity was increased 5.5 times in cells treated with TTA for 3 days. In the cells treated with TTA for 1 year the ACO activity was increased only two times. A similar ACO mRNA half-life (two times the control) was found in cells treated with TTA for 1 year and for 3 days. This implies a loss of effect of TTA on the transcription rate of the ACO gene in long-term-treated cells.

When administered to rats, tetradecylthioacetic acid (TTA*) induces peroxisomal proliferation and hypolipemia [1]. When TTA is present in the growth medium of Morris 7800 C1 hepatoma cells, it induces peroxisomal β-oxidation activity in the cells [2]. This effect of TTA on hepatoma cells is strongly potentiated by dexamethasone and counteracted by insulin [3]. We have shown previously that the effects of TTA, dexamethasone and insulin on acyl-CoA oxidase (ACO) activity is matched by similar effects on the ACO mRNA level in these cells [4]. TTA also inhibits the growth rate of hepatoma cells; dexamethasone potentiates this effect, while insulin suppresses it and normalizes the growth rate [3].

The object of the present study was to investigate whether long-term adaption to TTA occurred regarding growth rate, ACO activity and mRNA level in the hepatoma cells. We show that the growth inhibition remained unchanged and easily reversible by insulin in cells treated with TTA for 1 year. The effects of TTA on ACO mRNA level and enzyme activity were, however, markedly reduced with time.

Materials and Methods

Materials. Ham's F-10 medium, horse serum and calf serum were from Flow Laboratories (Irvine, U.K.). Anti-pleuropneumonia-like organism (PPLO), fungizone, penicillin and streptomycin were from Gibco (Grand Island, NY, U.S.A). TTA was synthesized as described previously [2]. Guanidinium isothiocyanate was obtained from Merck (Hohenbrunn, München, Germany). Agarose was purchased from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Other chemicals were obtained from the Sigma Chemical Co. (St Louis, MO, U.S.A.). Amersham International Radiolabelled [γ^{32}P]ATP was from Amersham (U.K.). Bio Trans nylon filter was from ICN (Irvine, U.S.A.).

Cell culture. The establishment, cloning and cell propagation of Morris hepatoma 7800 C1 cells has been described previously [5]. The cells were cultivated as described [3]. They were grown in (145 × 20 mm) plastic culture dishes. The cell medium was Ham's F-10 medium supplemented with 10% horse serum, 3% calf serum, penicillin (50 U/mL), streptomycin (50 μg/mL), fungizone (2.5 μg/mL) and anti-I-PPLO (60 μg/mL). The cells were incubated at 37° in a humidified atmosphere of 95% O_2 and 5% CO_2, and were grown in the same medium for 5 days after plating. The medium was subsequently changed every second day until the cultures reached plateau phase. The medium was then supplemented with either TTA (80 μM), dexamethasone (0.25 μM) and/or insulin (0.4 μM). The cells were treated for 3 days at which time there was a steady response of the cells to these compounds. The long-term-treated cells were, in addition, grown with 80 μM TTA in the medium for 1 year. Prior to this, the concentration of TTA was gradually increased by adding 5 μM TTA every 3 weeks, starting with 5 μM and ending at 80 μM.

Administration of TTA to the cells. TTA was administered to the cells as the free acid dissolved in the medium. It was initially dissolved in 0.1 M NaOH at 90° to a concentration of 5 mM and then added slowly to the culture medium at 45° to a final concentration of 80 μM TTA.

Morphological characterization of cells treated with 80 μM *TTA for* 1 *year*. Cells treated with 80 μM TTA for 1 year (long-term-treated cells) grew in compact colonies of irregular shape with well-defined smooth borders. Microscopic examination showed normal appearance of these cells, except for some small spherical droplets which we have shown by Sudan red staining to be fat.

mRNA stability studies. The 7800 C1 hepatoma cells were treated with TTA (80 μM) as above. The incubation was continued in the presence of actinomycin D (2.5 μg/mL, Sigma A-4264) for a maximum 12 hr and cells were harvested at different time points during this period. The concentration of actinomycin D used in this experiment (2.5 μg/mL) inhibited incorporation of [^{3}H]uridine into RNA by more than 95% after 1/2 hr (data not shown).

Enzyme assay and DNA measurements. ACO was measured by the method of Small *et al.* [6] as described previously [4]. DNA was measured in the same sample by the method of Labarca and Paigen [7].

Synthetic oligomer used as probes. A 39-base synthetic oligonucleotide was made from the rat ACO cDNA and was chosen from exon I [8]. The oligomer was end labelled with [γ^{32}P]ATP as described [9]. Specific activities of approximately 2–6 × 10^8 cpm/μg DNA were obtained.

Northern blot analysis of RNA. Total RNA was extracted by the guianidinium thiocyanate method [10, 11] and transferred to Bio Trans nylon filter and hybridized as described [4]. Autoradiography was performed using Amersham hyperfilm MP (RPN 6) with intensifying screens at −70° for varying lengths of time. The size of the mRNAs was calculated on the basis of 18S and 28S rRNA which were visualized by ethidium bromide.

Scanning. The signal intensities were estimated by the use of a densitometer (Vitatron TLD 100) connected to an integrator (Hewlett Packard HP 3390A).

* Abbreviations: TTA, tetradecylthioacetic acid; ACO, acyl-CoA oxidase.

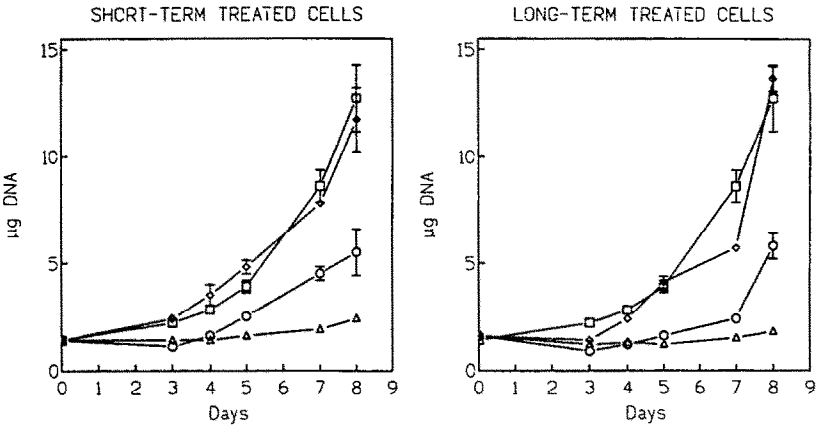

Fig. 1. The growth rate of Morris 7800 C1 hepatoma cells after short-term and long-term treatment with TTA. Morris 7800 C1 hepatoma cells were treated with 80 μM TTA, 0.25 μM dexamethasone and 0.4 μM insulin for the time period indicated. The long-term-treated cells have, in addition, been grown with 80 μM TTA in the medium for 1 year prior to treatments indicated. The controls for long-term- and short-term-treated cells are the same, i.e. cells without additions to the medium. The cells were harvested at different time points and the amount of DNA measured. The values represent means ± SEM for four dishes. Symbols: control (□), TTA (○), TTA + dexamethasone (△), TTA + insulin (◇).

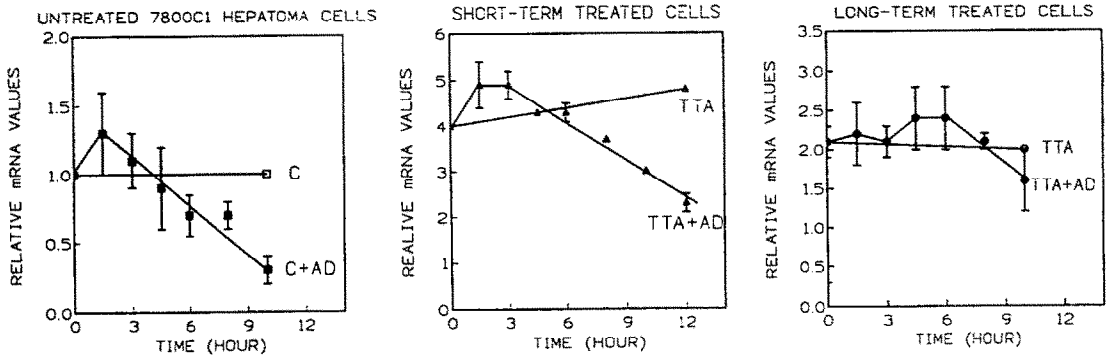

Fig. 2. The mRNA half-life of ACO after short-term and long-term treatment with TTA. Morris 7800 C1 hepatoma cells were treated with 80 μM TTA for 3 days. Then actinomycin D (AD) (2.5 μg/mL) was added for a period of 12 hr and the cells were harvested at different time points. The long-term-treated cells have, in addition, been grown with 80 μM TTA in the medium for 1 year prior to treatment. The resulting northern filters were hybridized to ^{32}P-labelled probe for ACO and subjected to autoradiography. The densities of the hybridization signals are presented relative to the control (C) level at each time point. Each time point represents three dishes and the values are means ± SEM. Symbols: C (□), C + AD (■); short-term-treated: TTA (△), TTA + AD (▲); long-term-treated: TTA (○), TTA + AD (●). The calculated mRNA half-lives were as follows: control $T_{1/2}$ = 5.9 hr ± 0.3; short-term-treated cells $T_{1/2}$ = 10.0 hr ± 0.4; long-term-treated cells $T_{1/2}$ = 9.1 hr ± 0.8.

Statistics. The enzyme measurements are given as mean values ± SEM. The Student's *t*-test was used for statistical calculations.

Results and Discussion

Effects of TTA, dexamethasone and insulin on the growth rate of long-term- and short-term-treated hepatoma cells. Figure 1 shows that the effects of TTA, dexamethasone and insulin on growth rate are the same for the short-term- and long-term-treated cells. Although the growth rate of the cells has been reduced for 1 year in the long-term-treated cells when compared to cells without any addition to the medium, insulin immediately reversed this effect of TTA (Fig. 1).

TTA is a hypolipidemic agent [1] similar to the n−3 (ω−3) series of polyunsaturated fatty acids [12, 13]. Interestingly, the n−3 polyunsaturated fatty acid, eicosapentaenoic acid, has been shown to reduce cell growth in McA-RH7777 rat hepatoma cultures [14], in other neoplasms [15, 16] and in vascular cells [17]. The mechanisms of growth inhibition are not known.

Effect of TTA on the ACO activity in short-term- and long-term-treated cells. Previously, TTA has been shown to increase both the mRNA steady-state level and enzyme activity for ACO in short-term-treated Morris 7800 C1 hepatoma cells [4, 18]. Dexamethasone acted synergistically and insulin antagonized these effects of TTA [4, 18]. The increased ACO mRNA level and enzyme activity after

Table 1. ACO activity in Morris 7800 C1 hepatoma cells after short-term and long-term treatment with TTA

	ACO activity (nmol/min/mg DNA)	
Control	87 ± 5 (4)	
	Short-term-treated	Long-term-treated
TTA	500 ± 27† (4)	193 ± 20* (3)
TTA + dexamethasone	905 ± 120† (4)	317 ± 16† (3)
TTA − insulin	235 ± 12† (4)	176 ± 10† (4)

Morris 7800 C1 hepatoma cells were treated with 80 μM TTA, 0.25 μM dexamethasone and 0.4 μM insulin for 3 days as described. The long-term-treated cells have, in addition, been grown with 80 μM TTA in the medium for 1 year prior to treatment. The cells were harvested and sonicated and acyl-CoA (palmitoyl-CoA) oxidase was measured with 60–70 μM palmitoyl-CoA and 0.1–0.3 μg DNA of cells as described.

The values are means ± SEM for the number of dishes in parentheses.

* $P < 0.005$ (vs control), † $P < 0.0005$ (vs control).

treatment with TTA was partly due to a doubling of the ACO mRNA half-life, but an increase in the transcription rate was also implied [4].

Figure 2 and Table 1 show that long-term treatment with TTA of hepatoma cells reduced the effects of the 3-thia acid on ACO mRNA level and enzyme activity. In the short-term-treated cells (3 days), TTA increased the ACO activity 5.5 times compared to two times in the long-term-treated cells (Table 1). Parallel results were found for the ACO mRNA level (Fig. 2). Similar results were also found for the mRNA levels of the peroxisomal bifunctional enzyme and the thiolase (not shown). To our knowledge, no other studies on long-term treatment of peroxisome proliferators have shown a similar reduction in ACO activity. On the contrary, the increase in ACO activity has been shown to be maintained after administering peroxisome proliferators to rats *in vivo* for 1 year [19–21]. The reduced effect of TTA on the long-term-treated cells was not due to a reduced uptake of the 3-thia acid (results not shown).

Figure 2 further shows that the increased mRNA half-life for ACO found in the short-term TTA-treated cells was maintained in the long-term-treated cells. Actinomycin D was more toxic to the long-term-treated cells than the short-term-treated cells and, consequently, mRNA quantitation in these cells beyond 10 hr treatment was very unreliable. However, the present results indicate that the reduced effects of TTA on the ACO activity in the long-term treated cells was chiefly a result of a reduced effect of TTA on the transcription rate. The effect of peroxisome proliferators on the transcription rate of e.g. the ACO gene is believed to be mediated through a peroxisome proliferator-activated receptor which binds to a specific response element in the 5′ flanking region of this gene [22, 23]. The ligand for this receptor has not yet been identified but it has been speculated that the peroxisome proliferators may modulate their mechanism through some indirect mechanism, e.g. perturbation of lipid metabolism or, alternatively, changes in phosphorylation of proteins including the peroxisome proliferator-activated receptor [24]. It is, therefore, tempting to speculate that the altered lipid metabolism generally found in hepatoma cells [25, 26] could explain the loss of response to TTA of gene transcription in the long-term-treated cells.

In conclusion, long-term treatment with TTA of Morris 7800 C1 hepatoma cells maintains the inhibition of growth rate. However, the induction of the ACO mRNA steady-state level and enzyme activity is significantly reduced, probably through a diminished effect of TTA with time on the transcription rate of this gene.

Acknowledgements—We want to thank Borghild M. Arntsen for skilful technical assistance. This work was supported by the Norwegian Council on Cardiovascular Diseases and the Norwegian Research Council of Science and Humanities.

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