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EFFECT OF GLUCOCORTICOIDS ON THE PRODUCTION OF LACTATE DEHYDROGENASE, MALATE DEHYDROGENASE AND ALPHA-FETOPROTEIN BY MORRIS HEPATOMA 7777 IN VITRO[1]

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ABSTRACT

Non-AFP-producing Morris hepatoma 7777 were treated with glucocorticoids in order to compare the responses for AFP production and for lactate and malate dehydrogenases. Steroid hormone treatment did not affect the production of AFP. However, there was an approximate tripling of levels of both LDH and MDH (cytosolic plus mitochondrial).

INTRODUCTION: It was reported recently that glucocorticoids affect the alpha-fetoprotein production by an established cell line from Morris hepatoma 8994 (1). Glucocorticoids at concentrations equal to or higher than 10^{-7} lead to an increase of AFP production by an established cell line from Morris hepatoma 8994. These cells also secreted alpha M-fetoprotein into the culture medium, but only after addition of at least 4×10^{-7}M hydrocortisone or 5×10^{-8}M dexamethasone (1). Lactate dehydrogenase and mitochondrial malate dehydrogenase have been isolated in Morris hepatoma (2,3,4,5,6,7,10,11,12). It is the purpose of this paper to compare the responses of non-AFP-producing 7777 tissue culture derived (TC), to

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glucocorticoid treatment with respect to their production of AFP and the enzymes, lactate (LDH) and malate dehydrogenases (MDH).

MATERIALS AND METHODS: Cell lines: Methods used to culture these cell lines have been well described. The 7777 TC cells were derived from the Morris hepatoma 7777 obtained from Dr. H.T. Wepsic and maintained in our laboratory. These tissues were minced and cultured in plastic tissue culture flasks at $37^{o}C$ without agitation in an atmosphere of 5% CO_2 and 95% air. The media used were M-199 and Lewis medium.

Measurement of AFP: Monospecific rabbit anti-rat AFP antiserum was prepared, and Mancini's radial immunodiffusion method was used to determine the AFP concentration in the culture media. A standard curve was constructed with the AFP standard supplied by Dr. J.S. Thompson, Department of Medicine, University of Iowa. The 2.5 µg/ml is the lower limit of our assay without concentration.

Treatment with Glucocorticoid: Sterile solutions of hydrocortisone ("solucortet" Upjohn Company, Kalamazoo, Michigan) were made in M-199 and added to the cultures at the zero time, and these were cultured for two to seven days. All experiments were done in duplicate.

On day 1, 1×10^6 viable cells in 5 ml of M-199 with 10% FCS, antibiotics (pen-strept-fungi-neomycin) were seeded into 60 mm diameter tissue culture dishes. On day 2, the supernatants were removed, the dishes washed 2x, and 5 ml of M-199-10% FCS + PSF-N containing 10^{-4}, 10^{-5}, or 10^{-6}M hydrocortisone was added.

At day 3, 24-hour plates were processed by pipetting off cells using the medium they were grown in. The cells were counted. At day 4, 48-hour plates were processed in the same way as were the day 3 samples. Four-day incubation of hepatoma cells was studied as above.

On day 5, cells for the 4-day incubation study were removed and processed.

Enzyme Assays: Enzymes were assayed by the methods described (4,7,9) in duplicate. Sonicated cell suspensions were used to yield values for total extractable LDH and MDH (cytosolic plus mitochondrial MDH). The average values for duplicates are shown.

Malate Dehydrogenase Assay: In brief, the malate dehydrogenase assay procedure was as follows: Enzymatic activity was determined from the malate side of the equilibrium by monitoring the increase in optical density at 340 nm caused by the reduction of NAD^+ to NADH in a Cary Model 15 spectrophotometer at 25^oC. The assay was performed in a quartz cuvette containing 0.7 ml of 0.27 M EDTA, pH 10.0; 0.7 ml 0.4 M sodium L-malate, pH 10.0; 0.7 ml 5.97×10^{-4}M NAD^+, and 0.3 ml of 0.5 M NaCl, 0.019 M $NaHCO_3$. The reaction was initiated by injecting 0.1 ml of enzyme into the cuvette with an Eppendorf pipette.

Lactate Dehydrogenase Assay: Lactate dehydrogenase was assayed in 1.0 cm quartz cuvettes. Each cuvette contained 0.7 ml of 0.954 M Tris, pH 8.6, 0.7 ml of 0.989 M lithium L-lactate, 0.7 ml 7.2×10^{-4} M NAD^+, and 0.35 ml of 0.5 M NaCl-0.018 M $NaHCO_3$. The reaction was initiated by injecting 0.05 ml of enzyme into the cuvette with an Eppendorf pipette.

RESULTS AND DISCUSSION: Table 1 shows the results of the experiments.

Table 1: When 7777 TC cells were treated with 10^{-4}, 10^{-5}, and 10^{-6}M hydrocortisone for 24 and 48 hours, there are no changes in regard to AFP production. Hydrocortisone failed to induce AFP production. However, the mitochondrial plus cytosolic enzymes, m-MDH and c-MDH, and the cytosolic enzyme, LDH, both increased with hydrocortisone treatment during periods of 24 and 48 hours. The increments of increase were very similar.

With four days hydrocortisone treatment, 7777 TC cells showed almost double increments of LDH production and tripled to quadrupled increments of MDH, but these treatments again failed to increase the AFP production.

TABLE 1

EFFECTS OF HYDROCORTISONE ON 7777 TC CELLS

Concentration	LDH[a] I.U./10^6 cells	MDH[a] I.U./10^6 cells	AFP level[b] μg/10^6 cells
24 hours incubation:			
0 (control)	0.278	0.0842	0
1×10^{-4}M	0.338	0.1160	0
1×10^{-5}M	0.350	0.0838	0
1×10^{-6}M	0.336	0.1000	0
48 hours incubation:			
0 (control)	0.353	0.0944	0
1×10^{-4}M	0.447	0.1460	0
1×10^{-5}M	0.480	0.1500	0
1×10^{-6}M	0.405	0.1260	0
4 days incubation:			
0 (control)	0.504	0.122	0
1×10^{-4}M	1.200	0.431	0
1×10^{-5}M	1.000	0.332	0

a: Average value for duplicate samples.

b: AFP was undetected even after 13 fold concentration. The lower limit of this assay is 2.5 μg/ml without concentration.

AFP production by the MH 8994 cell line was doubled by 4×10^{-7}M hydrocortisone treatment (1). Dexamethasone treatment was reported to have no effect on AH 66 cells (10). De Nechaud, et al. postulated that the glugocorticoid effect in AFP production is the change of differentiation of this tumor cell (1).

Our data demonstrate that the response to hydrocortisone is heterogenous our 7777 cell line produced less AFP in contrast to the previous finding with MH 8894. If AFP production is due to the presence of a submetacentric marker composed of No. 7 chromosomes and short arm (11), it is less likely that a short term treatment with steroid will induce enhanced production of AFP (1).

Since there were no apparent changes in cell size observed with light microscopy, the several-fold increase in enzymatic activities is interesting. The effect of this and other hormone (thyroid) on the synthesis of these enzymes are underway at present to delineate the mechanism of increased enzyme activities.

There are several possibilities for enzyme activity increase due to hormone treatment. An increase in tyrosine aminotransferase (TAT) activity after steroid treatment was associated with increased cytoplasmic levels of functional TAT mRNA (12). It could be either due to an increase of de novo synthesis of these enzymes, or due to the elimination of inhibitory factors by the steroid, or due to hydrocortisone inhibition of the breakdown of the enzyme.

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