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Effects of Dichloroacetate and Glyoxylate on Low Density Lipoprotein Uptake and on Growth of Cultured Fibroblasts (41814)

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Abstract. The effects of dichloroacetate, a known hypocholesterolemic agent, were studied in cultured growing and confluent human fibroblast cells. Microscopic examination showed no visible adverse effects of dichloroacetate on confluent cells during exposure to concentrations as high as 5 m*M* for 96 hr. Higher concentrations resulted in cell death after varying periods of incubation. There were no viable cells after 24 hr of exposure to 100 m*M* dichloroacetate. In contrast, much lower concentrations proved lethal to growing cells; cell growth, as determined by cell numbers at specified times after splitting, was suppressed by 1 m*M* dichloroacetate and 5 m*M* concentrations resulted in cell death. Similar effects were noted with glyoxylate. The hypocholesterolemic effect of dichloroacetate is probably not due to any effect on the low density lipoprotein pathway, since concentrations of up to 1 m*M* dichloroacetate did not affect the cellular binding and uptake of ^{125}I-labeled low density lipoprotein. It is concluded that growing and rapidly metabolizing cells are much more sensitive to the toxic effects of dichloroacetate and glyoxylate than confluent cells.

Recent evidence has indicated that the drug sodium dichloroacetate may be effective in the treatment of lactic acidosis and in reducing plasma cholesterol levels in hypercholesterolemia. The effect of this compound in reducing lactic acidemia is thought to be due to the increase in pyruvate dehydrogenase activity which would in turn allow the conversion of more lactate to pyruvate (1–4). The mechanism by which it lowers plasma cholesterol levels in humans (2, 5) is unknown, although Stacpoole *et al.* (6) have reported the *in vitro* inhibition of hepatic cholesterol biosynthesis by sodium dichloroacetate and its metabolite, glyoxylate. No toxicity has been reported in patients with lactic acidosis treated with dichloroacetate (7), but its continuous use as a hypercholesterolemic agent cannot be recommended because of the development of a polyneuropathy in one of the two patients on long-term treatment (5).

One of the metabolic anomalies associated with familial hypercholesterolemia is a defect in the cell membrane receptor mechanism, which is needed for uptake of low density lipoprotein (LDL). This results in decreased cellular uptake of LDL and loss of feedback regulation of cholesterol biosynthesis (8). LDL is the major transport lipoprotein for cholesterol; in familial hypercholesterolemia, LDL levels are greatly elevated. It is known that certain pharmacological agents, such as ethinyl estradiol, increase the catabolism of LDL by affecting its binding to cell receptors (9). This is presumably responsible for lowering the plasma cholesterol level *in vivo*. Inhibition of 3-hydroxy-3-methylglutaryl-CoA (HMGCoA) reductase, the rate-limiting enzyme in cholesterol synthesis, results in an increase in the number of LDL receptors in cultured fibroblasts (10) and a subsequent increase in the amount of LDL uptake. Since dichloroacetate and glyoxylate reportedly inhibit this enzyme (6), it seemed possible that these agents might increase the specific binding and uptake of LDL *in vitro*.

The present study was undertaken (1) to determine whether the hypocholesterolemic effect of dichloroacetate might be due, in part, to increased cellular uptake of LDL and (2) to determine the toxic concentrations of dichloroacetate and glyoxylate on growing and confluent cultured human fibroblasts.

Materials and Methods. Plasma lipoproteins were fractioned and isolated by differential density ultracentrifugation (11) in a Sorval OTD-2 ultracentrifuge (Sorvall Co., Newtown, Conn.) and a T865 rotor. After dialysis, the apoprotein of LDL was radiolabeled by the iodine monochloride technique (12). Free ^{125}I was removed by extensive dialysis against phosphate-buffered saline (PBS), pH

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7.2. Labeled LDL was sterilized by filtration through a filter (Amicon Corp., Lexington, Mass.) of 0.45-μm pore size.

Normal skin fibroblasts, line 969 (NIGMS Human Genetic Mutant Cell Repository, Camden, N.J.) and skin fibroblasts from a patient with familial hypercholesterolemia, line 1915 (NIGMS Human Genetic Mutant Cell Repository) were maintained in culture in Eagle's minimum essential medium supplemented with 10% fetal calf serum (Gibco, Grand Island, N.Y.) and used at passages 18–22.

The sodium dichloroacetate was the generous gift of Dr. Peter Stacpoole (University of Florida); its purity had been tested in his laboratory (7). Glyoxylic acid was purchased from Fisher Scientific Co. (Fairlawn, N.J.). The addition of glyoxylic acid to medium altered its pH; it was therefore necessary to adjust the pH of glyoxylate to 7.2 with NaOH. The pH of all other media was monitored before and after incubation with the cells and it was not necessary to adjust the pH of medium containing dichloroacetate. All media were filter-sterilized after addition of the reagents.

The acute lethal concentrations of dichloroacetate and glyoxylate were determined by incubating confluent cells in 60 × 15-mm dishes with 2 ml medium containing the indicated concentrations for 96 hr at 37°C in a humidified atmosphere containing 5% CO_2. Cells were examined microscopically at 1, 2, 4, 8, 24, 30, 48, and 96 hr. Control studies were performed using the same concentrations of added sodium chloride (1–100 m*M*) to determine whether the toxic effects noted were due to an osmolar effect. Results were recorded at each time period simply as: no dead cells present, a mixture of dead and viable cells, or no viable cells present.

The effects of various concentrations on growing cells were determined as follows: Cells from several T75 tissue culture flasks were trypsinized, combined and dispersed into petri dishes with 4 ml medium per dish. After 24 hr the medium was replaced with medium containing the indicated concentrations of sodium dichloroacetate or glyoxylate, incubated for the specified time intervals, and then harvested for counting. Medium containing the same concentrations of sodium chloride was used in the control group. Triplicate samples were taken at each time period for each concentration. Dishes were washed twice with PBS, 0.5 ml trypsin was added to each dish and incubated until the cells began to detach. Saline (1.5 ml) was added and the contents of each dish were aspirated into a Pasteur pipet several times to disperse clumps. The contents were then transferred to a test tube and cell counts were made in a counting chamber. The counts were made at least five different times per tube, averaged, and counts per plate were calculated. The average of each triplicate set was then calculated and the results were plotted as number of cells per plate vs number of hours of incubation. The experiments were performed twice on each cell line.

The cellular uptake of LDL was determined in a standard cell binding assay, as previously described (8, 13). Cells were grown to confluency in media containing the indicated concentrations of dichloroacetate or glyoxylate. The uptake of ^{125}I-LDL by the cells was measured as a function of concentration of LDL in the medium during 2 hr of incubaton at 37°C. Student's *t* test was used to determine whether a statistically significant difference occurred between treated and control cells.

Results. Dichloroacetate and glyoxylate treatments produced similar results in each type of experiment. Exposure of confluent monolayers of normal skin fibroblasts to 1, 2, or 5 m*M* concentrations of dichloroacetate for 96 hr resulted in no microscopically visible effect (Fig. 1). Confluent cells at a concentration of 10 m*M* showed no visible effects within 48 hr, but dead or dying cells, as judged by trypan-blue exclusion, were noted in the medium at 72–96 hr. At concentrations of 50 and 100 m*M*, there were no visible changes during the first 8 hr, but by 24 hr, dead cells were present in the medium. There were no viable cells after incubation for 72 hr in 50 m*M* dichloroacetate and after 48 hr in 100 m*M*. Identical results were obtained when FH cells were used. The toxic effects of high concentrations of these compound was not due to hyperosmolarity, as there were no effects on confluent cells of added sodium cloride up to 100 m*M*.

The data in Fig. 2 indicate no effect of 0.1 and 0.5 m*M* dichloroacetate on the number of growing normal cells over the 72-hr ob-

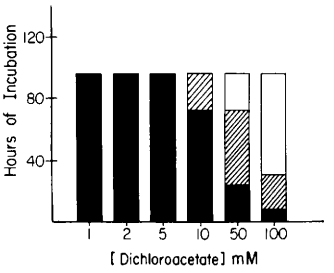

FIG. 1. Viability of confluent cells in various concentrations of dichloroacetate. (■) No dead cells, (▨) dead cells floating in the medium, (□) no viable cells.

servation period. After 24 hr, growth was suppressed at 1.0 m*M* and higher concentrations. At the two highest concentrations (5 and 10 m*M*) there was no cell growth at all; rather, cell death occurred at these concentrations. In other experiments using both normal and FH cells grown in lower concentrations of dichloroacetate (0., 0.05, 0.1, 0.25, 0.5, and 1.0 m*M*), little or no difference in cell numbers was noted between the FH and normal cells. Also, no differences occurred in groups at different concentrations, except in the 1.0 m*M* group. As in the experiments mentioned above, at 1.0 m*M* dichloroacetate, a decline in cell number occurred between 0 and 96 hr (data not shown). Growth curves varied slightly depending on the number of cells initially plated into each dish. Growth of fibroblasts in various concentrations of glyoxylate indicated that concentrations of 3 m*M* and above always reduced the cell numbers at 24, 48, and 72 hr.

The cellular binding and uptake of LDL were not affected when confluent cells were preincubated in the presence of 1.0 m*M* dichloroacetate (Fig. 3). Also, the presence of this chemical in the ^{125}I-LDL incubation medium did not affect the uptake of LDL. Similar results were obtained when cells were preincubated in glyoxylate rather than dichloroacetate (data not shown).

Discussion. This study indicates that confluent cells can tolerate much greater concentrations of dichloroacetate and glyoxylate than growing cells. Dichloroacetate has several metabolic effects (2, 4, 6, 7, 14), but the mechanism for its toxicity is not presently known. These *in vitro* findings may not necessarily reflect *in vivo* effects, since Moore *et al.* reported polyneuropathy but no abnormal clinical findings involving cells that have a high turnover rate (5).

The lowering of plasma cholesterol levels by dichloroacetate is probably not mediated by any effect on binding and uptake of LDL,

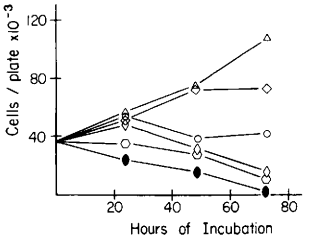

FIG. 2. Cell numbers during growth in various concentrations of dichloroacetate. Normal fibroblasts were seeded into petri dishes. After 24 hr, cells were removed from three plates, counted separately, and averaged for the 0 time point. Complete medium containing 0 (□), 0.1 (△), 0.5, 1.0 (○), 2.0 (◇), 5.0 (o), or 10 m*M* (⧫) dichloroacetate was added to other dishes and allowed to incubate at 37°C. An average variation of 7% occurred in counting cells from triplicate plates at each time point. The 0.5 m*M* values are not shown separately here, as they were virtually identical to those for the 0 concentration. Similar results were obtained with familial hypercholesterolemic cells.

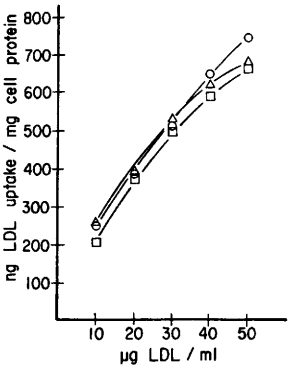

FIG. 3. Uptake of ^{125}I-LDL by confluent normal cells preincubated in the presence and absence of DCA. (○) Cells preincubated in medium only, incubated with ^{125}I-LDL and medium; (△) cells preincubated in medium only, incubated with [^{125}I]LDL in the presence of 1.0, dichloroacetate; (□) cells preincubated for 48 hr in 1.0 m*M* dichloroacetate and medium, incubated with ^{125}I-LDL and 1.0 m*M* dichloroacetate.

as indicated by the *in vitro* studies here. Stacpoole *et al.* (6) have recently reported that both dichloroacetate and glyoxylate inhibit the activity of hydroxymethyl glutaryl coenzyme A reductase in hepatic cells *in vitro,* thereby reducing hepatic cholesterol biosynthesis, which may entirely account for its hypocholesterolemic action. Since dichloroacetate inhibits the kinases which catalyze the phosphorylation of pyruvate dehydrogenase and succinyl-CoA synthetase, its action on cholesterol biosynthesis may be due to an effect on reductase kinase. It remains to be determined which effects or which combination of effects are responsible for the toxicity of the compound to growing and rapidly metabolizing cultured cells.

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